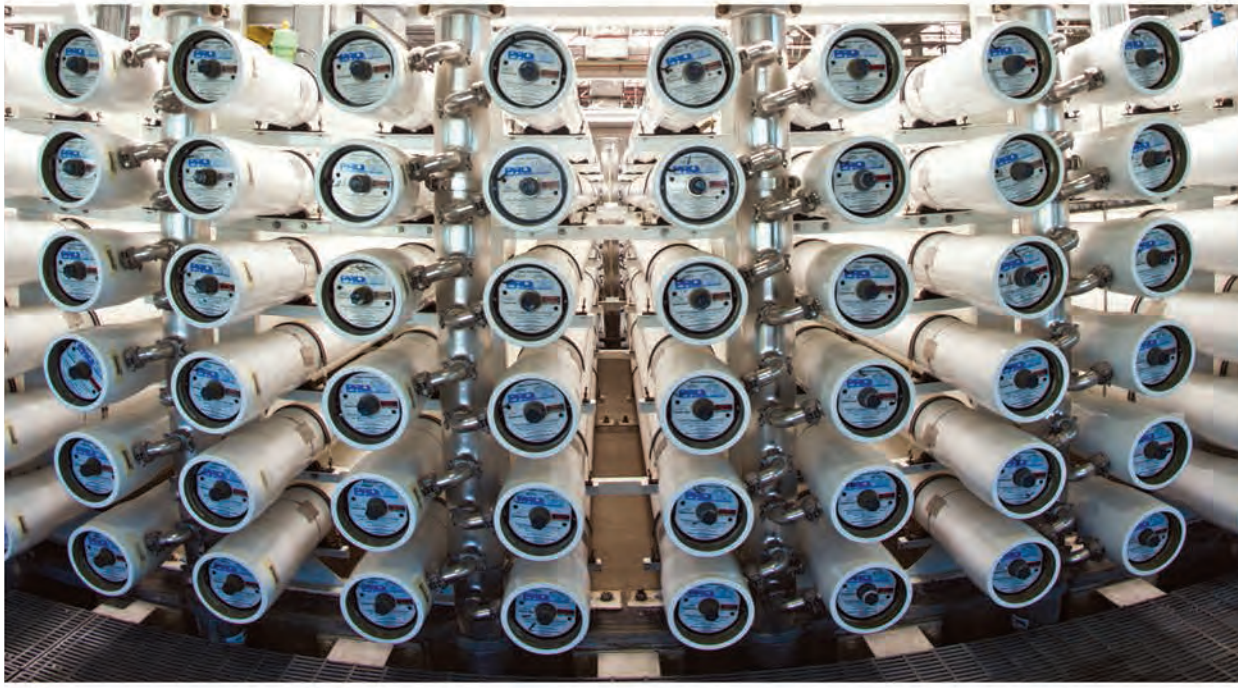
EXPERT PANEL
FINAL REPORT



Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

California State Water Resources Control Board

CALIFORNIA
Water Boards

EXPERT PANEL FINAL REPORT:

Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

Prepared by:

Expert Panel on the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

Adam Olivieri, James Crook, Michael Anderson, Richard Bull, Jörg Drewes, Charles Haas, Walter Jakubowski, Perry McCarty, Kara Nelson, Joan Rose, David Sedlak, and Timothy Wade

Prepared for:

State Water Resources Control Board
Division of Drinking Water
Sacramento, California, USA

Submitted by:

National Water Research Institute
Fountain Valley, California USA
www.nwri-usa.org

August 2016

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A 501c3 nonprofit organization, the National Water Research Institute (NWRI) was founded in 1991 by a group of California water agencies in partnership with the Joan Irvine Smith and Athalie R. Clarke Foundation to promote the protection, maintenance, and restoration of water supplies and to protect public health and improve the environment. NWRI's member agencies include Inland Empire Utilities Agency, Irvine Ranch Water District, Los Angeles Department of Water and Power, Orange County Sanitation District, Orange County Water District, and West Basin Municipal Water District.

For more information, please contact:

National Water Research Institute
18700 Ward Street
Fountain Valley, California 92708 USA
Phone: (714) 378-3278
Fax: (714) 378-3375
www.nwri-usa.org
www.nwri-usa.org/ca-panel.htm

Jeffrey J. Mosher, Executive Director
Gina Melin Vartanian, Publications Manager

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EXPERT PANEL

Expert Panel Co-Chairs:

Adam W. Olivieri, Dr.P.H., P.E., EOA, Inc.

James Crook, Ph.D., P.E., Environmental Engineering Consultant

Expert Panel Members:

Michael A. Anderson, Ph.D., University of California, Riverside

Richard J. Bull, Ph.D., MoBull Consulting

Dr.-Ing. Jörg E. Drewes, Technical University of Munich

Charles N. Haas, Ph.D., Drexel University

Walter Jakubowski, M.S., WaltJay Consulting

Perry L. McCarty, Sc.D., Stanford University

Kara L. Nelson, Ph.D., University of California, Berkeley

Joan B. Rose, Ph.D., Michigan State University

David L. Sedlak, Ph.D., University of California, Berkeley

Timothy J. Wade, Ph.D., United States Environmental Protection Agency

NATIONAL WATER RESEARCH INSTITUTE

Expert Panel Administrator:

Jeffrey J. Mosher, Executive Director

Expert Panel Support Staff:

Brandi Caskey, Administrative Manager

Suzanne Faubl, Project Manager

Gina Melin Vartanian, Publications Manager

DEDICATION

The Expert Panel dedicates this report to the memory of Robert C. Cooper, professor emeritus at the UC Berkeley School of Public Health. He was a teacher, colleague, mentor, and friend to many members of the Expert Panel.

Dr. Cooper taught three generations of environmental engineers and environmental health microbiologists about the relationships between managing water and wastewater and the control of infectious diseases. When he retired from the University of California Berkeley in 1991, he became vice-president of Biovir Laboratories, where he remained active until his death in 2015.

His research ranged from the development of microbiological laboratory methods to microbiological risk assessment. Notably, he was instrumental in the initial investigations on the fate of enteric viruses in the environment and developed methods for virus isolation and detection from environmental media (including reclaimed water). He first applied the concept of microbial health risk assessment in 1971 during work with the United States Army Corp of Engineers to evaluate the management of wastewater disposal in the San Francisco Bay-Delta. These concepts were broadened as part of his work for Governor Brown's Office of Planning and Research on evaluating the public health effects of onsite wastewater disposal systems in California. As principal investigator for the City of San Diego's Total Resource Recovery Health Effects Study (1985 to 1997), he led a team of researchers at six California universities and the California Department of Health Services to study a number of topics related to the indirect potable reuse of recycled water – the findings of which are directly relevant to California's current water challenges.

In addition, Dr. Cooper served on numerous California and national committees, including committees for the National Research Council, California Department of Public Health, and California State Water Resources Control Board. His contributions provided the direction and foundation for the State of California's water reclamation criteria for potable and non-potable reuse. In recent years, his return to the laboratory resulted in a notable contribution to the development of USEPA Method 1682 for the measurement of *Salmonella* in sewage sludge.

Dr. Cooper will long be remembered as a key figure in advancing a clearer understanding of water quality, water recycling, and public health.[1]

[1] Source: sph.berkeley.edu/robert-c-cooper-professor-emeritus-environmental-health-dies-86.

ACKNOWLEDGMENTS

This report is the product of an Expert Panel on the "Development of Water Recycling Criteria for Indirect Potable Reuse through Surface Water Augmentation and the Feasibility of Developing Criteria for Direct Potable Reuse," which was administered by the National Water Research Institute, a 501c3 nonprofit organization based in Southern California, on behalf of the State Water Resources Control Board. The Expert Panel is pleased to acknowledge the organizations and individuals whose support, assistance, and resources made this report possible.

State Water Resources Control Board

The Expert Panel was sponsored by the Division of Drinking Water of the State Water Resources Control Board through Agreement No. 13-21041 with the National Water Research Institute. The Expert Panel is very appreciative of the following staff members at the State Water Resources Control Board for the information, materials, and suggestions received throughout the Expert Panel review process and during the development of this report: Mr. Faraz Asad, Mr. Randy Barnard, Mr. Mark Bartson, Mr. Brian Bernados, Mr. Robert Brownwood, Ms. Jing-Tying Chao (Project Manager), Ms. Cindy Forbes, Mr. Robert Hultquist, Ms. Karen Larson, Mr. Michael McKibben, Ms. Sherly Rosilela, Mr. Kurt Souza, Dr. David Spath, and Ms. Erica Wolski. In particular, the Expert Panel thanks Mr. Mark Bartson and Ms. Jing-Tying Chao for their help in overseeing the panel review process.

National Water Research Institute

The Expert Panel appreciates the outstanding services of staff at the National Water Research Institute, who administered the panel review process, organized and facilitated 12 panel meetings, and helped develop, prepare, and edit this report and other documents related to the panel review process. The Expert Panel thanks Ms. Brandi Caskey (administrative), Ms. Suzanne Faubl (Expert Panel support), Ms. Jaime Lumia (administrative), Mr. Jeff Mosher (Expert Panel oversight, facilitation, and report development), and Ms. Gina Melin Vartanian (report development, editing, and support). In particular, the Expert Panel recognizes the exceptional leadership of Mr. Jeff Mosher for overseeing the panel review process and Ms. Gina Melin Vartanian for editing this report.

Report Contributors

The Expert Panel thanks Dr. Ryan Reinke of the Los Angeles County Sanitation District and Professor Kellogg Schwab of the Johns Hopkins University Bloomberg School of Public Health for their contributions to sections of the report addressing antibiotic resistant bacteria and genes (**Chapter 6**), and Dr. Kevin Crofton of the U.S. Environmental Protection Agency and Professor Michael Denison of the University of California Davis for their contributions on the subject of the use of bioanalytical tools (**Chapter 4**). The Expert Panel also thanks Mr. Jeff Mosher of the National Water Research Institute for his contributions on managerial issues (**Chapter 9**).

Direct Potable Reuse Advisory Group

The Expert Panel recognizes the State Water Resources Control Board's Direct Potable Reuse Advisory Group, which held a number of meetings and prepared specific products to assist the Expert Panel in completing its charge.

The Expert Panel thanks the following individuals who served on the Advisory Group: Mr. Randy Barnard of the California State Water Resources Control Board, Ms. Amy Dorman of the City of San Diego, Mr. Garry Brown of the Orange County Coastkeeper, Mr. Conner Everts of the Environmental Justice Coalition for Water, Mr. Jim Fiedler of Santa Clara Valley Water District, Ms. Julie Labonte of the Water and Energy Committee of the San Diego Regional Chamber of Commerce, Mr. Al Lau of Padre Dam Municipal Water District, Dr. Bruce Macler of the U.S. Environmental Protection Agency, Ms. Traci Minamide of the City of Los Angeles Bureau of Sanitation, Dr. Edward Moreno of the California Conference of Local Health Officers, Monterey County, Mr. Keith Solar of the San Diego County Taxpayers Association, Ms. Frances Spivy-Weber of the California State Water Resources Control Board, Mr. Ray Tremblay of the County Sanitation Districts of Los Angeles County, Ms. Andria Ventura of the Clean Water Action, and Mr. Michael Wehner of the Orange County Water District. Notably, Mr. Garry Brown of Orange County Coastkeeper served as Chair of the Advisory Group.

In addition, the following individuals are recognized for serving on the Advisory Group until changes in employment: Ms. Alisa Reinhardt (previously of the San Diego Regional Chamber of Commerce) and Ms. Marsi Steirer (previously of the City of San Diego).

WateReuse Direct Potable Reuse Research Initiative

The Expert Panel acknowledges the significant time, effort, and investment provided by the Water Environment & Reuse Foundation (WE&RF) (formerly, the WateReuse Research Foundation) and WateReuse California, which together launched the Direct Potable Reuse Initiative in June 2012 to assist the State Water Resources Control Board with its task to determine the feasibility of developing regulatory criteria for direct potable reuse. WE&RF invested in a research portfolio that included 34 projects valued at over $20 million to investigate various aspects of the technical feasibility of implementation direct potable reuse, including the reliability of treatment trains, microbial and chemical water quality, monitoring, and operations. In particular, the Expert Panel would like to thank Ms. Melissa Meeker and Ms. Julie Minton of the WE&RF for leading these efforts. In addition, the following projects contributed significantly to the Expert Panel's review process and the development of this report:

WRRF 14-12 Project Contributors

The Expert Panel appreciated the collaborative support from the team at Trussell Technologies, Inc. (Dr. Brian Pecson and Ms. Sarah Triolo) and Mr. Simon Olivieri of Arcalternatives for their willingness to share and discuss data and concepts as part of conducting the direct potable reuse pilot demonstration project in San Diego, California, for the WRRF 14-12 project on "Demonstrating Redundancy and Monitoring to Achieve Reliable Potable Reuse."

WRRF 14-14 Project Contributors

It is greatly appreciated that WE&RF acted upon a recommendation made by the Expert Panel early on in the panel review process to explore the feasibility of using public health surveillance data to evaluate or inform the implementation of direct potable reuse. The result was the project titled WRRF 14-14 on "White Paper on the Feasibility of Establishing a Framework for Public Health Monitoring for DPR." This project was managed by Ms. Kristan Cwalina of WE&RF and overseen by a Project Advisory Committee made up of experts in the field. The Expert Panel thanks the many contributors to this project, in particular Mr. Jeffrey Soller of Soller Environmental, LLC. (who served as principal investigator), and his team consisting of Dr. Edmund Seto of the University of Washington, Dr. Mary Schoen of Soller Environmental, LLC, and Mr. Andrew Salveson of Carollo Engineers. A complete list of the project participants for WRRF 14-14 is included in the "WRRF Project Contributors" page provided at the end of this report.

WRRF 14-16 Project Contributors

Thanks are extended to the project team for WRRF 14-16, titled "Operational, Monitoring, and Response Data from Unit Processes in Full-Scale Water Treatment, IPR, and DPR," who shared preliminary pathogen data with the Expert Panel, including: Mr. Andrew Salveson, Dr. Eva Steinle-Darling, and Dr. Austa Parker of Carollo Engineers, Inc., as well as Mr. Robert Angelotti of the Upper Occoquan Service Authority and Ms. Marlo Berg of the Texas Commission on Environmental Quality.

WRRF 15-01 Project Contributors

The Expert Panel acknowledges the significant effort undertaken by a number of authors and co-authors to summarize and synthesize the key research results of the 34 projects funded through the WateReuse DPR Initiative. The project, titled "DPR Research Compilation: Synthesis of Findings from DPR Initiative Projects" (WRRF-15-01), was managed by Ms. Julie Minton of WE&RF and overseen by a Project Advisory Committee made up of experts in the industry. The Panel thanks the many contributors to this project, in particular Dr. George Tchobanoglous, Professor Emeritus of the University of California Davis, who served as the technical lead and editor of the final report, and Ms. Gina Melin Vartanian of the National Water Research Institute, who provided editorial assistance. Mr. Jeff Mosher of the National Water Research Institute served as principal investigator of this project. A complete list of authors and other project participants is included in the "WRRF Project Contributors" page provided at the end of this report.

Specialty Seminar on Direct Potable Reuse in California

Finally, the Expert Panel thanks the State Water Resources Control Board, National Water Research Institute, and UC Berkeley School of Public Health for sponsoring the "Specialty Seminar on Direct Potable Reuse in California," held at the David Brower Center in Berkeley, California, in September 2015. The purpose of the seminar was to discuss and review important issues related to direct potable reuse that would be addressed by the Expert Panel throughout the Panel review process and, in particular, focus on the use of bioanalytical tools in water analyses. The Expert Panel appreciated the interest and feedback of over 100 attendees and the presentations provided by a number of speakers, including: Ms.

Frances Spivy-Weber of the California State Water Resources Control Board, Mr. Robert Hultquist of the State Water Resources Control Board, Mr. Jason Dadakis of the Orange County Water District, Dr. George Tchobanoglous of the University of California Davis, Ms. Julie Minton of the WateReuse Research Foundation, Dr. Michael Denison of the University of California Davis, Dr. Kevin Crofton of the U.S. Environmental Protection Agency, Dr. Richard Bull of Washington State University (retired) and Expert Panel member, and Dr. R. Shane Trussell of Trussell Technologies, Inc. The Expert Panel also thanks staff at the National Water Research Institute, led by Mr. Jeff Mosher, for organizing and facilitating the seminar.

Special Appreciation

The Panel appreciates the efforts of Dr. R. Rhodes Trussell of Trussell Technologies, Inc., who served as the original chair of the Expert Panel until June 2014. He continued to attend public meetings of the Expert Panel as an observer and remained an important resource on treatment issues related to direct potable reuse.

Meeting Hosts

Finally, the Panel thanks staff at the following organizations that hosted Expert Panel meetings: City of San Diego Public Utilities Department in San Diego, California; Orange County Water District in Fountain Valley, California; and San Francisco Estuary Institute in Richmond, California. Their support and assistance in holding these meetings was indispensable to the Panel process.

CONTENTS

FIGURES

TABLES

ACRONYMS

AOC	Assimilable organic carbon
AOP	Advanced oxidation process
AOPathway	Adverse outcome pathway
AR	Androgen receptor
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistance genes
ATW	Advanced treated water
AWTF	Advanced water treatment facility
BAC	Biological activated carbon
BAF	Biologically active filtration
BEQ	Bioanalytical equivalent
BNR	Biological nutrient removal
BOD	Biochemical oxygen demand
BPA	Bisphenol A
CAR	Constitutive androstane receptor
CCP	Critical control point
CDC	Centers for Disease Control and Prevention
CDF	Cumulative distribution function
CDPH	California Department of Public Health (now DDW)
CEC	Chemicals of emerging concern (also known as constituents of emerging concern)
CEG	Concentration enabling growth
CFR	Code of Federal Regulations
COC	Constituent of concern (also known as “contaminant of concern”; “constituent” is used in this document)
C-NPR	Centralized non-potable reuse
CT	Residual disinfectant concentration, C (in micrograms per liter), multiplied by the contact time, T (in minutes)
DALY	Disability adjusted life years
DBP	Disinfection byproduct
ddPCR	Digital droplet polymerase chain reaction
DDW	Division of Drinking Water (California State Water Resources Control Board)
DEQ	Oregon Department of Environmental Quality
D-NPR	Decentralized non-potable reuse
DPR	Direct potable reuse
DWR	California Department of Water Resources
DWTF	Drinking water treatment facility
EBT-EC	Effect-based trigger effect concentrations
EC	Effect concentration (**Chapter 5** only)
EC	Electrical conductivity
ED	Electrodialysis

ER	Estrogen receptor
ERα	Estrogen receptor alpha
ESB	Engineered storage buffer
ESWTR	Enhanced Surface Water Treatment Rule
FDA	U.S. Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FRT	Failure and response time
GC	Gas chromatography
GR	Glucocorticoid receptor
GWRS	Groundwater Replenishment System
GWUDI	Groundwater under the direct influence of surface water
HACCP	Hazard analysis and critical control points
HAV	Hepatitis A virus
HI	Hazard index
HPC	Heterotrophic Plate Count
HTP	High-throughput
HTS	High-throughput screening
IPR	Indirect potable reuse
IU	Industrial user
LRV	Log_{10} reduction value
LT2ESWTR	Long-Term 2 Enhanced Surface Water Treatment Rule
LXR	Liver X receptor
MBR	Membrane bioreactor
MCL	Maximum contaminant level
MCLG	Maximum contaminant level goal
MF	Microfiltration
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
MOA	Mode-of-action
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
NCATS	National Center for Advancing Translational Sciences
NCCT	National Center for Computational Toxicology of the USEPA
NCGC	NCATS Chemical Genomics Center
NDMA	N-nitrosodimethylamine
NF	Nanofiltration
NGS	Next generation sequencing
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NPDES	National Pollution Discharge Elimination System

NPR	Non-potable reuse
NRC	National Research Council
NTP	National Toxicology Program
NWRI	National Water Research Institute
0_3	Ozone
O&M	Operation and maintenance
OCSD	Orange County Sanitation District
OCWD	Orange County Water District
OECD	Organization for Economic Cooperation and Development
ORD	Office of Research and Development of the USEPA
PAC	Project Advisory Committee
PCR	Polymerase chain reaction
PDT	Pressure decay test
PFOS	Perfluorosulfonic acid
PHG	Public health goal
POTW	Publicly owned treatment work
PPARα	Peroxisome proliferator activated receptor alpha
PPARγ	Peroxisome proliferation receptor gamma
PR	Progesterone receptor
PXR	Pregnane X receptor
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
QRRA	Quantitative relative risk assessment
RAC	Research Advisory Committee
REF	Relative enrichment factor
RNS	Reactive nitrogen species
RO	Reverse osmosis
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SAR	Structure activity relationship
SAT	Soil aquifer treatment
SCCWRP	Southern California Coastal Water Research Project
SMCL	Secondary maximum contaminant level
SWA	Surface water augmentation
SWP	State Water Project
SWPP	Source Water Protection Program
SWTR	Surface Water Treatment Rule
TCEQ	Texas Commission on Environmental Quality
TDS	Total dissolved solids
TDWD	Tolerable drinking water density
TEQ	Toxic equivalents
TIC	Total ion chromatograph

TMF	Technical, managerial, and financial
TOC	Total organic carbon
TOrC	Trace organic constituent (also known as trace organic contaminant)
TSCA	Toxic Substances Control Act
TSS	Total suspended solids
TTC	Threshold of toxicological concern
USEPA	United States Environmental Protection Agency
UF	Ultrafiltration
UV	Ultraviolet
VTG	Vitellogenin
WE&RF	Water Environment and Reuse Foundation
WEF	Water Environment Federation
WHO	World Health Organization
WRF	Water Research Foundation
WRRF	WateReuse Research Foundation
WWTP	Wastewater treatment plant
ZLD	Zero liquid discharge

ABBREVIATIONS FOR UNITS OF MEASURE

A	Acre; 43,560 ft^2 [(5,280 ft/mi)^2/(640 acre/mi^2)]
AF	Acre-foot (the volume of water that would cover 1 acre of land to a depth of 1 foot); 1 acre-foot (of water) = 325,892 gallons (a unit of water volume used in agricultural irrigation practice)
AF/y	Acre-feet per year
cm	Centimeter
CPU	Colony forming unit
d	Day
D	Dalton
ft	Foot
g/mol	Gram per mole
gal/capita•d	Gallons per capita per day
kg	Kilogram = 1,000 grams
kg $CO_{2e}/10^3$ gal	Kilogram carbon dioxide equivalent per 1,000 gallons
kg CO_{2e}/kWh	Kilogram carbon dioxide equivalent per kilowatt hour
km	Kilometer
kWh	Kilowatt hour
kWh/AF	Kilowatt hour per acre-foot
kWh/m^3	Kilowatt hour per cubic meter
kWh/10^3 gal	Kilowatt hour per 1,000 gallons
L	Liter
L/capita•d	Liter per capita per day
L/d	Liter per day
m	Meter
m^3	Cubic meter
m^3/d	Cubic meters per day
MFL	Million fibers per liter
mg	Milligram
mg/d	Milligram per day
mg/L	Milligram per liter
mgd	Million gallons per day (also Mgal/d)
Mgal	Million gallons
Mgal/d	Million gallons per day (also mgd)
mi	Mile
mJ	Millijoule
mJ/cm^2	Millijoule per square centimeter
mL	Milliliter
ML/d	Million liters per day
mrem	Millirem (a unit of radiation, 1,000 mrem = 1 rem)
ng	Nanogram
ng/L	Nanogram per liter = parts per trillion (ppt)
NTU	Nephelometric turbidity unit
pCi/L	Picocuries per liter (a measure of radioactivity in water)
PFU	Plaque-forming units

pM	Picomolar
ppb	Parts per billion, ~micrograms per liter (µg/L)
ppm	Parts per million, ~milligrams per liter (mg/L)
ppt	Parts per trillion, ~nanograms per liter (ng/L)
ppy	Per person per year
rem	Roentgen equivalent man (a unit of radiation)
tonne	Metric tonne (1,000 kg)
µ	Micron
µg/L	Micrograms per liter = parts per billion (ppb)
µm	Micrometer
µM	Micromolar

PREFACE

P.1 Purpose of the Report

The purpose of this report is to document the efforts and outcomes of an Expert Panel that was mandated by the California Legislature to advise the State Water Resources Control Board on public health issues and scientific and technical matters regarding the feasibility of developing uniform water recycling criteria for direct potable reuse (DPR). Within this report, the Expert Panel provided its opinion and reasoning as to whether it is feasible for the State of California to develop and implement a uniform set of water recycling criteria for DPR that would incorporate a level of public health protection as good as or better than what is provided by current water supplies and by indirect potable reuse (IPR).

P.2 Overview of Direct Potable Reuse

DPR is a strategy being considered today by communities throughout the nation, particularly those in the arid southwest, to help meet future water demands and develop more sustainable water supplies. It involves using treated municipal wastewater effluents (i.e., recycled water) to augment public water supplies. There are two forms of planned DPR:

- **Direct potable reuse producing advanced treated water**. For the first form of DPR, "advanced treated water" produced in an advanced water treatment facility (AWTF) is introduced into the raw water supply immediately upstream of a drinking water treatment facility (DWTF), where it then undergoes surface water treatment before entering the drinking water distribution system. The only two DPR projects in the United States that have been permitted to-date use this form of DPR, both in Texas (i.e., the Big Spring Raw Water Production Facility operated by the Colorado River Municipal Water District and the DPR Project used to produce an emergency water supply for the City of Wichita Falls[2]).

- **Direct potable reuse producing finished drinking water**. For the second form of DPR, "finished water" produced in an AWTF that also is permitted as a DWTF (and meets the requirements of the Surface Water Treatment Rule) is introduced directly into a drinking water distribution system. Finished water is expected to meet all federal, state, and local regulatory requirements for a DWTF.

P.3 Interest in Regulatory Criteria for Direct Potable Reuse in California

As interest in potable reuse has grown, so has the need to provide guidelines for DPR; however, guidance and regulations on DPR do not currently exist for California or nationally. In 2010, the California State Legislature responded to this need through the passage of Senate Bill 918, a law that modified the California Water Code to require the California State Water Resources Control Board (State

[2] The Raw Water Production Facility in Big Spring, Texas, has operated since 2013. The Direct Potable Reuse Project for the City of Wichita Falls, Texas, was designed as a temporary means to assist the city during a time of drought and was decommissioned in 2015 after 1 year of operation and the production of over 2 billion gallons of water.

Water Board)[3] to report to the Legislature by December 31, 2016, on the feasibility of developing uniform water recycling criteria for DPR. Refer to Sections 13560 to 13569 of Chapter 7.3 (entitled "Direct and Indirect Potable Reuse") of the California Water Code (CWC, 2014) for the exact wording of the Legislative mandate (provided herein as **Appendix P1**). Per the California Water Code, the State Water Board was required to examine the following as related to DPR:

- Availability and reliability of recycled water treatment technologies necessary to ensure the protection of public health.
- Multiple barriers and sequential treatment processes that may be appropriate at wastewater and water treatment facilities.
- Available information on health effects.
- Mechanisms that should be employed to protect public health if problems are found in recycled water that is being served to the public as a potable water supply, including (but not limited to) the failure of treatment systems at the recycled water treatment facility.
- Monitoring needed to ensure the protection of public health, including (but not limited to) the identification of appropriate indicator and surrogate constituents.
- Any other scientific or technical issues that may be necessary, including (but not limited to) the need for additional research.

In addition, the State Water Board was required by the California Water Code to convene both (1) an Expert Panel to advise the State Water Board in its efforts and (2) an Advisory Group to advise the State Water Board and Expert Panel on issues related to DPR. Both the Expert Panel and DPR Advisory Group documented their advice in final reports (the Expert Panel in this report and the DPR Advisory Group in Advisory Group [2016]).

P.4 Role of the Expert Panel

The purpose of the Expert Panel is provided in Section 13565 of Chapter 7 of the California Water Code, as follows (emphasis added):

> "13565. (a) (1) On or before February 15, 2014, the department shall convene and administer an expert panel for purposes of advising the department on public health issues and scientific and technical matters regarding development of uniform water recycling criteria for indirect potable reuse through surface water augmentation and ***investigation of the feasibility of developing uniform water recycling criteria for direct potable reuse***. The expert panel shall assess what, if any, additional areas of research are needed to be able to establish uniform regulatory criteria for direct potable reuse. The expert panel shall then

[3] The wording in the California Water Code directs the California Department of Public Health (CDPH) to undertake this legislative mandate. It should be noted that on July 1, 2014, the Drinking Water Program officially transferred from CDPH to the State Water Resources Control Board and was renamed the Division of Drinking Water; thereafter, the State Water Resources Control Board became the entity responsible for investigating and reporting to the Legislature on the feasibility of developing uniform water recycling criteria for direct potable reuse.

> recommend an approach for accomplishing any additional needed research regarding uniform criteria for direct potable reuse in a timely manner."

Because DPR is the subject of this report, emphasis is placed on the Expert Panel's role to advise the State Water Board regarding the development of water recycling criteria for DPR; however, it must be noted that the California Water Code specified that the Expert Panel also provide advice to the State Water Board regarding the State's proposed regulatory criteria for another form of potable reuse, that of IPR using surface water augmentation (SWA). Significantly (and as described in detail in this report), IPR projects involving a surface water reservoir that does not meet the State Water Board's proposed criteria for IPR using SWA could be classified as DPR projects.

With respect to IPR using SWA, the Expert Panel's charge – as stated in Section 13562 of the California Water Code – is as follows:

> "(B) Prior to adopting uniform water recycling criteria for surface water augmentation, the department shall submit the proposed criteria to the expert panel convened pursuant to subdivision (a) of Section 13565. The expert panel shall review the proposed criteria and shall adopt a finding as to whether, in its expert opinion, the proposed criteria would adequately protect public health."

With respect to DPR, the Expert Panel worked with the State Water Board to meet the following State-mandated deadlines, as required in Section 13563 of the California Water Code:

- On or before June 30, 2016, the State Water Board shall prepare a draft report summarizing the research recommendations of the Expert Panel.
- By September 1, 2016, the State Water Board shall complete a public review draft of its report.
- On or before December 31, 2016, the State Water Board is to provide a final report to the Legislature on the feasibility of developing uniform water recycling criteria for DPR.

P.4.1 Establishment of the Panel

In 2013, the State Water Board signed Agreement No. 13-21041 with the National Water Research Institute (NWRI) of Fountain Valley, California, to administer the Expert Panel on the "Development of Water Recycling Criteria for Indirect Potable Reuse through Surface Water Augmentation and the Feasibility of Developing Criteria for Direct Potable Reuse." NWRI is a 501c3 nonprofit research organization with expertise in organizing and facilitating independent, third-party peer review panels for water industry projects and policies. In particular, NWRI has over 15 years of experience in managing panel review processes for potable reuse projects throughout the State of California and elsewhere (see **Appendix P2** for more information about NWRI's Panel Program).[4]

[4] More information about the National Water Research Institute and the Expert Panel can be found online at www.nwri-usa.org/ca-panel.htm, as well as online at http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/RW_SWA_DPRexpertpanel.shtml.

P.4.2 Members of the Expert Panel

With guidance and approval by the State Water Board, NWRI appointed national and international water industry researches, practioners, and consultants to an independent, third-party Expert Panel to provide advice to the State of California on (1) developing uniform water recycling criteria for IPR through surface water augmentation (SWA) and (2) investigating the feasibility of developing uniform water recycling criteria for DPR.

The Expert Panel consisted of 12 individuals who meet the requirement in Section 13565 of the California Water Code that the Expert Panel "shall be comprised, at a minimum, of a toxicologist, an engineer licensed in the state with at least three years' experience in wastewater treatment, an engineer licensed in the state with at least three years' experience in treatment of drinking water supplies and knowledge of drinking water standards, an epidemiologist, a limnologist, a microbiologist, and a chemist." Expert Panel members included:

- *Expert Panel Co-Chair:* Adam W. Olivieri, Dr.P.H., P.E., EOA, Inc. (Oakland, CA)
- *Expert Panel Co-Chair:* James Crook, Ph.D., P.E., Environmental Engineering Consultant (Boston, MA)
- Michael A. Anderson, Ph.D., University of California, Riverside (Riverside, CA)
- Richard J. Bull, Ph.D., MoBull Consulting (Richland, WA)
- Dr.-Ing. Jörg E. Drewes, Technical University of Munich (Munich, Germany)
- Charles N. Haas, Ph.D., Drexel University (Philadelphia, PA)
- Walter Jakubowski, M.S., WaltJay Consulting (Spokane, WA)
- Perry L. McCarty, Sc.D., Stanford University (Stanford, CA)
- Kara L. Nelson, Ph.D., University of California, Berkeley (Berkeley, CA)
- Joan B. Rose, Ph.D., Michigan State University (East Lansing, MI)
- David L. Sedlak, Ph.D., University of California, Berkeley (Berkeley, CA)
- Timothy J. Wade, Ph.D., United States Environmental Protection Agency (Durham, NC)[5]

Brief descriptions of the backgrounds of the Expert Panel members can be found in the Biography section at the end of this report.

P.4.3 Activities of the Expert Panel

The Panel convened 12 times during the period of March 2014 to June 2016 to fulfill its legislative mandate for both IPR using SWA and DPR. Meetings of the Expert Panel were held by NWRI at various locations throughout the State of California, the majority in Orange County and the Berkeley area. Typically, meetings included presentations on relevant subjects and interaction with staff from the State

[5] Dr. Tim Wade's role on the Expert Panel was to provide advice and contributions to the sections in this report on public health surveillance and epidemiology as associated with direct potable reuse.

Water Board.[6] Some portions of these meetings were open to the public. Later meetings involved the development of this report, including outlining, writing, and reviewing drafts of the individual chapters. Staff at NWRI and members of the Expert Panel (often, the Panel Co-Chairs) also interacted with the DPR Advisory Group and Board Members of the State Water Board, as needed.

P.4.4 Specialty Seminar on Direct Potable Reuse in California

To assist the Expert Panel with its mandate regarding DPR, a one-day seminar that was open to the public was held on September 23, 2015,[7] in Berkeley, California. The event, titled the "Specialty Seminar on Direct Potable Reuse in California," was sponsored by the State Water Board, NWRI, and UC Berkeley School of Public Health. The specific purpose of the seminar was to bring together a diverse group of university researchers, the public, regulators, utility representatives, and consultants to review and discuss current issues related to DPR that would be addressed by the Expert Panel and, in particular, focus on the use of bioanalytical tools in water analyses. The agenda featured the following key topics:

- "California Water Supply: Where Does Recycling Fit In?" by Frances Spivy-Weber, California State Water Resources Control Board
- "Regulating Potable Reuse in California" by Robert Hultquist, P.E., State Water Resources Control Board (retired annuitant)
- "Groundwater Replenishment System" by Jason Dadakis, P.G., C.Hg., Orange County Water District
- "The Future of Potable Reuse" by George Tchobanoglous, Ph.D., P.E., University of California, Davis
- "Update on WateReuse DPR Research Initiative" by Julie Minton, WateReuse
- "Design of High-Throughput Screens and Their Application in Biomedical Sciences" by Michael Denison, Ph.D., University of California, Davis
- "Translating High-Throughput Bioassay Results to Risk Estimates" by Kevin Crofton, Ph.D., U.S. Environmental Protection Agency
- "Issues Related to Application of Bioassays to Wastewater and Drinking Water" by Richard Bull, Ph.D., MoBull Consulting and Expert Panel member
- "Demonstrating Redundancy and Monitoring to Achieve Reliable Potable Reuse" by R. Shane Trussell, Ph.D., P.E., Trussell Technologies, Inc.

As a result of this seminar, the Expert Panel was able to better focus its deliberations and identify issues and concerns integral to evaluating the feasibility of developing criteria for DPR in California and identifying potential research needs.

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[6] Downloadable copies of the reports that resulted from meetings of the Expert Panel are available at the State Water Board's website at www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/RW_SWA_DPRexpertpanel.shtml.

[7] Copies of the agenda and slide presentations provided at the "Specialty Seminar on Direct Potable Reuse in California" can be downloaded online from the following links: http://www.nwri-usa.org/dpr-seminar.htm or http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/RW_SWA_DPRexpertpanel.shtml.

P.5 Process to Develop this Report

As discussed in **Chapter 1**, the Expert Panel used a topic-based approach to prepare this report. Key topics were divided into chapters, with these chapters prepared by subgroups of the Expert Panel whose expertise pertained to the subject matter. The entire Expert Panel reviewed each draft of these chapters, as well as the report in its entirety, for technical and editorial accuracy. NWRI staff also provided editorial assistance, as well as formatted the report for organization and consistency. Notably, the Expert Panel used a number of references (e.g., Tchobanoglous et al., 2015; Cotruvo et al., 2012; Bull et al., 2011; Tchobanoglous et al., 2011) in addressing the legislative mandate. Citations and credit to others are shown in the report, as appropriate; however, some text has been reused directly from documents prepared by NWRI that were authored or co-authored by members of the Expert Panel (e.g., Crook, 2010; NWRI, 2013), and citations of that work may not be shown herein on all occasions.

When needed, outside expertise was used to help gather, analyze, and/or document the information contained herein. In addition, the Expert Panel benefited from the support of the WateReuse DPR Research Initiative, which sponsored 34 projects to help investigate the technical feasibility of implementing DPR. Through the DPR Research Initiative, the Expert Panel was privy to a large body of current research data, some in the form of draft or final reports and some of which was in the process of being collected and analyzed. Access to these projects and their principal investigators provided the Expert Panel with useful insight and timely information to help prepare this report.

A full draft of this report was submitted to the State Water Board in July 2016. The State Water Board responded with clarifying questions in mid-August 2016. After considering these questions, the Expert Panel completed and submitted the final report to the State in late August 2016.

P.6 References

Advisory Group on the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse (2016). *Recommendations of the Advisory Group on the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse*, California State Water Resources Control Board, Sacramento, CA.

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EXECUTIVE SUMMARY

Per Section 13565(a)(1) of the California Water Code, the Expert Panel was charged with advising the State Water Resources Control Board (State Water Board) on public health issues and scientific and technical matters regarding the feasibility of developing uniform water recycling criteria for direct potable reuse (DPR).[8] After a yearlong investigation, the Expert Panel finds it is feasible for the State of California to develop and implement a uniform set of water recycling criteria for DPR that would incorporate a level of public health protection as good as or better than what is currently provided in California by conventional drinking water supplies, indirect potable reuse (IPR) systems using groundwater replenishment, and proposed IPR projects using surface water augmentation.

ES.1 Background on Potable Reuse in California

Public water supplies in California come from a variety of sources (i.e., groundwater and surface water), but factors like population growth and extended droughts are stressing these supplies. Consequently, alternative sources of water are needed to help meet current and future water demands and develop more sustainable water supplies. One such alternative is planned potable reuse, in which treated wastewater (or "recycled water") is used to augment public drinking water supplies.

Planned potable reuse has been practiced in the form of IPR for over 50 years in California. With IPR, treated wastewater is introduced into an environmental buffer (e.g., a groundwater basin or an aquifer designated as a source of water supply for a public water system, as defined in Section 13561 of the California Water Code) before being withdrawn and used as a water supply. Longstanding experience in California has demonstrated that IPR can be practiced without having any apparent detrimental effects on public health. In addition, the State Water Board currently is developing a regulation for IPR using surface water augmentation, which is the introduction of highly treated recycled water into a surface water body such as a drinking water reservoir. As part of its charge, the Expert Panel reviewed the State Water Board's proposed regulation for IPR using surface water augmentation and prepared draft findings that were submitted to the State for consideration in developing the proposed regulation (NWRI, 2015c).

A second form of planned potable reuse is DPR, defined in the California Water Code as the "planned introduction of recycled water either directly into a public water system, as defined in Section 116275 of the Health and Safety Code, or into a raw water supply immediately upstream of a water treatment plant." The Expert Panel defines DPR as the delivery of recycled water into a drinking water distribution system or a raw water supply immediately upstream of a drinking water treatment facility (DWTF). Interest exists at both the state and local levels to determine if DPR is protective of public health and feasible to implement in California.

[8] The Expert Panel was formed in 2013 to fulfill two purposes: to advise the State of California on public health issues and scientific and technical matters regarding (1) the development of uniform water recycling criteria for indirect potable reuse through surface water augmentation and (2) investigation of the feasibility of developing uniform water recycling criteria for direct potable reuse.

ES.2 Investigation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

National guidance or regulations currently do not exist for DPR. In 2010, the California State Legislature signed into law SB 918, which requires the State Water Board to report by December 31, 2016, on the feasibility of developing uniform statewide water recycling criteria for DPR. In 2013, the National Water Research Institute (NWRI), with guidance and approval by the State Water Board, appointed international experts to an independent, third-party Expert Panel to provide advice to the State of California, per Section 13565(a)(1) of the California Water Code, on "public health issues and scientific and technical matters regarding the development of uniform water recycling criteria for IPR through surface water augmentation and the investigation of the feasibility of developing uniform water recycling criteria for DPR." Subsequently, the State Water Board is to provide a final report to the Legislature on the feasibility of developing uniform water recycling criteria for DPR.[9]

ES.3 Purpose and Topics of This Report

The specific purpose of this report is to address the legislative mandate that requires the Expert Panel to:

- Advise the State Water Board on public health issues and scientific and technical matters regarding the feasibility of developing uniform statewide water recycling criteria for DPR.
- Assess what, if any, additional research is needed to enable establishing uniform regulatory criteria for DPR, and recommend an approach for accomplishing the additional needed research in a timely manner.

The Expert Panel selected the following main topics to review as part of addressing its legislative mandate:

- Public health surveillance tools and methods to quantify and mitigate risks (**Chapter 3**).
- Analytical approaches for measuring chemical water quality (**Chapter 4**).
- Application of bioanalytical tools (i.e., bioassays) to water analyses (**Chapter 5**).
- Traditional and molecular methods for assessing microbial water quality (**Chapter 6**).
- Antibiotic resistant bacteria and antibiotic resistance genes (**Chapter 7**).
- Performance of DPR systems (**Chapter 8**).
- Potable reuse regulatory feasibility analysis comparing an example DPR system against an existing potable water supply in California that is protective of public health (**Chapter 9**).
- Management controls **(Chapter 10).**

[9] Refer to Sections 13560 to 13569 of Chapter 7.3 (entitled "Direct and Indirect Potable Reuse") of the California Water Code (provided in **Appendix P1**) for a description of required activities of the State Water Resources Control Board and Expert Panel as pertaining to evaluating the feasibility of developing uniform statewide water recycling criteria for direct potable reuse.

In terms of public health protection, microbial contaminants – including bacteria, viruses, and protozoan parasites – are acknowledged as the most critical constituents to regulate in recycled water due to the potential impacts to human health 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 chemicals that can be present in recycled water, some are of concern due to their potential adverse health effects associated with both short-term and long-term exposures. Determining which constituents to regulate can be challenging, but has been done for planned IPR. The possibility of doing the same for DPR is considered in this report.

Notably, although DPR is the subject of this report, many of the key aspects presented and discussed herein can be applied to IPR; accordingly, relevant aspects of IPR also are discussed.

ES.4 Overall Expert Panel Findings Relative to the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

The Expert Panel finds it is feasible to develop uniform water recycling criteria for DPR that would incorporate a level of public health protection as good as or better than what is currently provided in California by conventional drinking water supplies, IPR systems using groundwater replenishment, and proposed IPR projects using surface water augmentation.

For DPR to provide the levels of protection afforded by IPR projects using either groundwater replenishment or surface water augmentation (both of which include the use of an environmental buffer), the functionality provided by the environmental buffer for IPR projects (i.e., storage, attenuation, and response time) must be addressed by other means for DPR projects (e.g., the reliability of mechanical systems and plant performance), thereby ensuring the delivery of a water quality that is protective of human health. To do so, regulations specifying DPR practices need to provide the following features in addition to the requirements already specified in IPR regulations for California:

- The DPR system must be reliable. Reliability is achieved by (1) providing multiple, independent treatment barriers, (2) incorporating the frequent monitoring of surrogate parameters at each step to ensure treatment processes are performing properly, and (3) developing and implementing rigorous response protocols (such as a formal Hazard Analysis Critical Control Point system). See **Chapter 8 (Finding #8-1).** Other key attributes that promote reliability include**:**

    a) Using a treatment train (as described in **Chapter 9**) with multiple, independent treatment barriers (i.e., redundancy) that meet performance criteria greater than the public health threshold $\log_{10}$ reduction value (LRV) goals established for microorganisms.

    b) Ensuring the independent treatment barriers represent a diverse set of processes (i.e., robustness*)* in the treatment train that are capable of removing particular types of contaminants by different mechanisms. This diversity provides better assurance that if a currently unrecognized chemical or microbial contaminant is identified in the future, there is a greater degree of likelihood it will be removed effectively by the treatment train.

    c) Using parallel independent treatment trains (i.e., resilience and redundancy) and providing sufficient replacement parts, along with trained personnel, to rapidly carryout the most frequently needed repairs.

d) Encouraging the use of a probabilistic analysis of treatment train performance at the design stage. The analysis should be based on data from other pilot-scale or full-scale facilities. After the full-scale facility is commissioned, the analysis should be updated periodically using actual performance data and operational experience.

e) Providing the ability to divert advanced treated water that does not meet specifications (i.e., water that is "off-spec").

f) Implementing a rigorous source control program designed to control the discharge of toxic chemicals and other contaminants into the wastewater collection system that serves the DPR project. The source control program must include stringent sewer ordinances and ongoing surveillance.

g) Providing certified operational personnel who are able to conduct rigorous operations and maintenance at advanced water treatment facilities (AWTFs) and DWTFs.

h) Unauthorized short-term peak discharges of chemicals into the wastewater collection system serving a DPR project have the potential to compromise final product water quality; therefore, incorporating a final treatment process (to be specified) after the advanced water treatment train may result in some "averaging" of these potential chemical peaks.

i) Ensuring the operation and performance of each unit treatment process in the DPR treatment train achieves the proposed and/or anticipated $\log_{10}$ reduction values (LRVs) for pathogens.

j) Ensuring the chemical and microbial stability of water in the drinking water distribution system will be maintained after introducing advanced treated water, in particular for a DPR system in which the finished drinking water will enter the distribution system.

- The State Water Board should not codify a specific set of treatment processes as part of developing uniform water recycling criteria for DPR, as it could stifle technological innovation in this growing area of need. The criteria should allow for alternatives to any treatment processes specified in the regulations if it is demonstrated to the State Water Board that the alternatives provide at least an equivalent level of public health protection. See **Chapter 8 (Finding #8-2).**

- The project sponsor needs to show the technical, managerial, and financial (TMF) capacity to reliably implement a DPR project. See **Chapter 10 (Finding #10-1).**

- An approach to stage the introduction of recycled water from a DPR system into a community's drinking water supply should be considered by the State Water Board as part of the review and approval of a project. This approach is consistent with California's regulatory practices for groundwater replenishment using recycled water. See **Chapter 8 (Finding #8-3)**.

- A formal process should be established by the State Water Board that includes an internal process to administer the periodic review of the performance of permitted potable reuse projects by an external expert panel on a 5-year cycle. Based on this review process, the State should incorporate new knowledge into potable reuse regulatory permits. See **Chapter 8 (Recommendation #8-1)**.

ES.5 Expert Panel Research Recommendations Related to the Development of Direct Potable Reuse Criteria in California

As described below, the Expert Panel identified several areas of research that should be conducted to further ensure the protectiveness of DPR. This research, which would best be supported directly by the State of California, could be done either before and/or concurrently with the development of uniform water recycling criteria for DPR, but the absence of better information is not a barrier to the feasibility of establishing this criteria. Additional research needs will emerge in the future.

Research recommendations of the Expert Panel are as follows:

1. To better inform targeted monitoring for source control and final water quality, the State Water Board should be proactive in monitoring the literature on the potential health risks that could present serious harm to health over short durations of exposure to compounds likely to be present in recycled water. Of specific concern are chemicals that adversely affect the development of fetuses and children. Other compounds that produce such effects will undoubtedly be discovered in the future. This activity could be initiated concurrently with the development of DPR regulations and continued as an ongoing effort. A formal process should be established by the State that includes: (1) an internal process to monitor the literature and (2) an external peer review process to address the results of the internal efforts to maintain a high level of awareness of these issues. See **Chapter 4 (Research Recommendation #4-1)**.

2. The State Water Board should adopt the use of probabilistic quantitative microbial risk assessment (QMRA) to confirm the necessary LRVs of viruses, *Cryptosporidium,* and *Giardia* needed to maintain a risk of infection equal to or less than 10^{-4} per person per year. The State should provide oversight, direction, and funding for implementing probabilistic QMRA. The purpose of using probabilistic QMRA is to provide a better assessment of the performance of DPR treatment trains and to provide an opportunity to identify additional effective DPR treatment trains. Input values for pathogen concentrations should be based on descriptive pathogen statistics resulting from additional review of the literature (as well as information collected from **Research Recommendation #3**). Also, as full-scale DPR systems are built, owners and regulators need to take advantage of these systems to sample and assess actual as-built performance and reliability characteristics. See **Chapter 8 (Research Recommendation #8-1)**.

3. To better inform decisions associated with updating LRVs, as well as conducting probabilistic-based QMRA modeling, the State Water Board should include monitoring requirements in a regulatory permit to measure pathogens (i.e., *Giardia* cysts, *Cryptosporidium* oocysts, and several human viruses) in the raw (untreated) wastewater feeding a DPR system to provide more complete information on concentrations and their variability. Improved methods should be used that will allow for the better characterization and improved precision of concentrations of pathogens. See **Chapter 2 (Research Recommendation #2-1)** and **Chapter 8 (Research Recommendation #8-2)**, as well as **Chapter 6** for more information.

4. The State Water Board should investigate the feasibility of collecting pathogen concentration data for raw wastewater associated with community outbreaks of disease and collect such data where possible. See **Chapter 2 (Research Recommendation #2-2)** and **Chapters 6** and **8** for more information.

5. The State Water Board should encourage short-term research be conducted to identify suitable treatment options for final treatment processes that can provide some "averaging" with respect to potential chemical peaks (in particular, for chemicals that have the potential to persist through advanced water treatment). These options might involve: (1) the use of a buffer tank (clear well) of a sufficient size, potentially blended with an alternative water source prior to release into the drinking water distribution system, or using two tanks feeding into the drinking water distribution system; (2) removal of volatile contaminants during a degassing step (decarbonization) similar to the approach that is commonly employed after reverse osmosis treatment in established AWTFs for potable reuse; (3) use of a biologically active filter after reverse osmosis/advanced oxidation processes, to provide an additional opportunity for microorganisms (if microorganisms will be able to survive in that environment) to degrade contaminants that may otherwise pass through the filter; or (4) other options. See **Chapter 8 (Research Recommendation #8-3)**.

6. It is important to focus on non-targeted analysis and, furthermore, low molecular weight compounds. For example, the inability of reverse-phase liquid chromatography/mass spectrometry to detect many uncharged, low molecular weight compounds (e.g., halogenated solvents, formaldehyde, and 1,4-dioxane) problematic for potable reuse projects demonstrates the limitations of current analytical approaches for the detection of unknowns that are likely to pass through reverse osmosis membranes. Research is needed to develop more comprehensive methods to identify low molecular weight unknown compounds. It is possible these compounds may be detected by gas chromatography interfaced with time-of-flight mass spectrometers or hydrophilic interaction liquid chromatography coupled with reversed-phase chromatography prior to triple quadrupole mass spectrometry; however, to date, these methods have not been applied to potable reuse projects to detect these compounds. These methods or others need to be developed to increase the understanding of the make-up of the remaining total organic carbon composed of low molecular weight compounds. In addition, these methods also could address the potential vulnerability of AWTF treatment processes to unintended spills or batch releases of chemicals in the sewershed. See **Chapter 4 (Research Recommendation #4-2)**.

ES.6 Summary of Additional Key Findings and Recommendations Related to Topics Investigated by the Expert Panel

The Expert Panel identified several other key findings to further address possible concerns in the future, some of which would best be directly supported by the State of California and others that would be better led by national and/or international entities.

A brief summary of findings is presented below. More detail on these findings and others are contained at the end of each pertaining chapter in this report, as noted.

- AWTFs sometimes employ an oxidant (e.g., ozone, chlorine, chloramines) prior to or after treatment with reverse osmosis. This practice can result in the formation of toxic byproducts, some of which are low molecular weight compounds that are not removed well during reverse osmosis or might remain after subsequent treatment with advanced oxidation processes. If the water is not subjected to an additional treatment step capable of removing these byproducts, they could be present in the drinking water produced by a DPR system. See **Chapter 4 (Finding #4-5)**.

- Most AWTFs currently under consideration for DPR in California include reverse osmosis as one of the treatment steps. During reverse osmosis, charged compounds and neutral compounds with molecular weights above approximately 200 grams per mole (g/mol) are almost entirely removed. Uncharged, low molecular weight compounds tend to be poorly rejected by reverse osmosis (e.g., N-nitrosodimethylamine [NDMA]), chloroform, and low molecular weight aldehydes). Under normal operating conditions, the concentrations of low molecular weight, neutral compounds in water produced by DPR systems generally are below the low total organic carbon (TOC) method detection limits observed in reverse osmosis permeate (i.e., typically <0.1 milligrams per liter [mg/L]); however, operators of AWTFs have detected short-duration pulses above the TOC method detection limits of acetone and other contaminants in reverse osmosis permeate. These contaminants are believed to originate from discharges to the wastewater collection system by commercial and industrial activities. Chemical monitoring plans for DPR systems need to include high-frequency monitoring of TOC or other surrogate parameters capable of detecting pulses of compounds that are poorly removed in reverse osmosis and subsequent treatment with advanced oxidation. Existing high-frequency TOC analyzers are capable of detecting pulses of elevated concentrations of contaminants in reverse osmosis permeate rapidly enough to allow operators to avoid introducing the final product water into the drinking water supply. In the event a pulse of contaminants arrives at the AWTF that is too low to be detected by a high-frequency TOC analyzer, the Expert Panel believes that subsequent removal in later treatment processes (e.g., during advanced oxidation) would result in concentrations of contaminants that may not pose unacceptable risks to public health. See **Chapter 4 (Finding #4-6** and **Recommendation #4-3)**.

- Bioassays have a potential role in the identification of yet-to-be-discovered contaminants, but the Expert Panel does not recommend the routine use of bioassays in monitoring programs for DPR projects at this time. Bioassay-directed fractionation is a useful research tool for identifying compounds in recycled water that merit further evaluation. For this reason, research efforts that employ bioassays and non-target screening analysis simultaneously are encouraged to be used to discover new contaminants of concern in municipal wastewater and water produced by DPR projects. See **Chapter 5 (Finding #5-1** and **#5-2).**

- Antibiotic resistance is a valid and serious worldwide public health concern that goes well beyond DPR projects. While risk levels associated with antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water have not been determined, concentrations of ARB and ARG in waters subjected to DPR treatment processes would likely be lower than that from current water sources entering DWTFs, suggesting that risk levels would be comparable to, or less than, those associated with current source waters. Further, considering all the available information, a combination of secondary wastewater treatment and advanced water treatment processes (i.e., a sequence of treatment train processes such as microfiltration/ultrafiltration, reverse osmosis, and ultraviolet disinfection/advanced oxidation processes) leading to a finished potable water is likely to reduce ARB and ARG concentrations in recycled water to levels well below those found in conventional treated drinking water. See **Chapter 7 (Findings #7-1, 7-2,** and **7-9)**.

- The role of public health surveillance is to: (1) establish partnerships, engagement, and communication between water utilities and public health partners; (2) identify sources of data to characterize baseline public health conditions and track trends over time; and (3) help determine if transient treatment failures and contamination events lead to adverse health

outcomes. Within the context of potable reuse, local public health partners should be informed when a DPR project is being considered. Points of contact should be identified and available surveillance data sources should be reviewed. In addition, processes for regular engagement, information sharing, and notification should be established with an emphasis on tracking, reporting, and communicating notifiable acute (primarily) waterborne diseases. The State Water Board also should work with DPR project sponsors and local health agencies to consider the feasibility of enhanced public health surveillance for communities with DPR systems. Such efforts may include syndromic surveillance, sentinel surveillance, or serological surveys for waterborne infections. See **Chapter 3 (Recommendations #3-1** and **3-2).**

- All current and proposed IPR regulations in the State of California include the use of a regulatory-defined environmental buffer; however, there are likely to be potential potable reuse projects where an environmental buffer is available, but does not meet the proposed operational and performance criteria for an IPR project using surface water augmentation. Notably, the proposed criteria for IPR projects using surface water augmentation do not include an alternatives clause (NWRI, 2015b) like that in the regulations for IPR using groundwater replenishment (CCR, 2015), where a project may be allowed to use an alternative to ***any*** requirement if it "assures at least the same level of protection to public health." Consequently, an IPR project for SWA using an environmental buffer that does not meet regulatory criteria would be defined as DPR. This situation creates a regulatory "Gap" between IPR projects with smaller environmental buffers and DPR projects with no environmental buffers. Based on a previous analysis of the environmental buffer conducted by the Expert Panel during the review of proposed criteria for IPR using SWA (NWRI, 2015a,b), the Expert Panel considers IPR projects with a theoretical hydraulic retention time of <2 months in the reservoir to be a DPR project (i.e., the Gap covers IPR-SWA projects with hydraulic retention times of ≥2 months and <4 months). See **Chapter 9 (Sections 9.1.2** and **9.3.2).** In effect, the Gap represents a transition between the currently proposed criteria for IPR using SWA and DPR. Given the above considerations, the Expert Panel supports the following approach by the State Water Board:

  a) Incorporate an alternatives clause that covers Gap projects into the proposed criteria for IPR using surface water augmentation.

  b) Require that agencies proposing potable reuse projects failing to meet the criteria for IPR using surface water augmentation demonstrate – through hydrodynamic and public health risk modeling – public health protection equivalent to that achieved by full compliance with criteria.

  c) Establish a consistent framework as part of the established regulatory process for preparing project-related engineering reports and subsequently reviewing and permitting Gap projects.

  d) Conduct a peer review of several Gap project proposals and engineering reports to assist in the establishment of a consistent technical basis for Gap projects.

  e) Encourage the State Water Board to consider the potential benefits of environmental buffers, irrespective of size, as a means of taking advantage of temperature equalization, storage, and peak attenuation.

ES.7 References

CCR (2015). *Water Recycling Criteria*, Title 22, Division 4, Chapter 3, California Code of Regulations: Sacramento, CA. https://govt.westlaw.com/calregs/Browse/Home/California/CaliforniaCodeofRegulations?guid=IE8ADB4F0D4B911DE8879F88E8B0DAAAE&originationContext=documenttoc&transitionType=Default&contextData=(sc.Default) (accessed 9/3/2015).

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PART I: BACKGROUND

CHAPTER 1: INTRODUCTION

- Brief history of water reuse in California.
- Difference between planned and unplanned potable reuse.
- Difference between indirect potable reuse and direct potable reuse (including the environmental buffer).
- Critical public health considerations for direct potable reuse.
- Purpose and organization of this report.

Potable water supplies are derived from a variety of sources, including local and imported surface water, groundwater, desalinated brackish water and seawater, and recycled water. As a result of population growth, urbanization (especially in coastal areas), droughts, and climate change, public water supplies in some parts of the United States are becoming stressed, and the opportunity to develop new sources of water supply from groundwater or surface water is becoming more difficult, if not impossible. Although conservation can reduce per capita water demand, the remaining supplies most likely will be insufficient to meet overall water needs. As a consequence, alternative strategies are needed to help meet future water demands and develop more sustainable water supplies (Tchobanoglous et al., 2015). One such strategy is planned potable reuse, in which highly treated municipal wastewater (i.e., recycled water) is used to augment public water supplies.

The practice of indirect potable reuse (IPR) involves using recycled water to (1) recharge groundwater aquifers via surface spreading or direct injection (i.e., groundwater replenishment), or (2) augment a stream or reservoir that serves as a source of drinking water (i.e., surface water augmentation [SWA]). For perspective, in 2010, approximately 1.35-million cubic meters per day (m^3/d) [or 355-million gallons per day (mgd)] of recycled water was used for IPR nationwide, which represents less than 1 percent of all municipal wastewater effluents generated in the United States; however, for communities practicing IPR, the average contribution of recycled water to their drinking water supplies can be as high as 30 percent, with some consumers receiving drinking water in which more than 50 percent originated from recycled water (Drewes and Khan, 2011).

An alternative option to IPR is direct potable reuse (DPR), in which recycled water is added directly into a drinking water distribution system or into a raw water supply immediately upstream of a drinking water treatment facility (DWTF). The feasibility of developing uniform water recycling criteria for DPR in the State of California is the subject of this report.

1.1 Overview of Water Reuse in California

Water supplies in the State of California tend to rely on runoff associated with melting snowpack. Over the next few decades, supplies are likely to diminish because climate change is predicted to cause more precipitation to fall as rain rather than as snow, with runoff occurring earlier in the season (Harris-Lovett and Sedlak, 2015). In response to the challenges of climate change and population growth, the California State Water Resources Control Board (State Water Board) has adopted a water recycling policy that declared independence from relying on the vagaries of annual precipitation and has moved

towards the sustainable management of surface waters and groundwater, together with enhanced water conservation, water reuse, and the use of stormwater (SWRCB, 2013). The policy of the State Water Board includes the following goals related to water recycling:

- Substitute recycled water for potable water as much as possible by 2030.
- Increase the use of recycled water over 2002 levels by at least 1-million acre-feet per year (AFY) (3.3-million m^3/d) by 2020 and by at least 2-million AFY by 2030 (6.6-million m^3/d).

The main drivers for water recycling include the following:

- Manage or alleviate water stress (i.e., the need for water).
- Replace the use of existing supplies of potable water (i.e., reduce the use of freshwater).
- Abate pollution.
- Address the need for reliable supplies of water.
- Address the need for cost-effective alternative supplies of water.
- Use wastewater as a source of new water.
- Respond to or comply with regulatory policies and regulations.

A brief overview is provided in **Sections 1.1.1** to **1.1.3** of the following water reuse practices as pertaining to California: non-potable reuse, planned potable reuse (including the potential role of DPR in a community's water supply), and unplanned (*de facto*) potable reuse. A more detailed summary of the history of water reuse in California is available in Harris-Lovett and Sedlak (2015).

1.1.1 Non-Potable Reuse

The planned use of recycled water for non-potable reuse applications[10] has been practiced for many years in the United States and other countries. The reuse of municipal wastewater was first practiced on a large scale shortly after cities began using flush toilets and sewers. In coastal areas, pipes transported sewage to the sea, where it was discharged far enough offshore to prevent aesthetic problems; however, surface water discharges presented problems for many inland communities. An alternative to dilution was needed for managing sewage. One such alternative was planned non-potable reuse of municipal wastewater, first implemented in the late nineteenth century with the development of sewer farms in England, Australia, Germany, France, and Italy. By 1900, sewer farms were numerous in these countries; about a dozen also existed in the United States (Fuller, 1912), including 10 in California (Ongerth and Ongerth, 1982). For example, one of the first sewer farms in California was established when the City of Pasadena purchased a 120-hectare (300-acre) plot of land outside the city, named it the Pasadena Sewer Farm, and piped in raw sewage to irrigate crops. This sewer farm produced walnuts, pumpkins, hay, and corn, and became a profitable business for the City (Holder, 1904). Other Southern California cities also turned to sewer farms as a means to profit from human waste while sending it away from homes. For example, in 1909, residents of the coastal city of Redondo Beach voted down a proposed sewer outflow to the ocean and instead insisted the City adopt the sewer

[10] 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).

farm model for reuse (Barkley, 1909). To the City, sewage was a source of water and nutrients that could make the dry landscape of Southern California produce useful crops.

By 1910, as many as 35 communities in California were using sewage for irrigation: 11 without any treatment and 24 after septic tank treatment (Ongerth and Ongerth, 1982). The sewage farms gave way to wastewater treatment plants (WWTPs) when the land area required for the treatment of wastes grew too large to be feasible, urban areas began to encroach on sewer farms, and concerns grew about odors and health risks associated with putting raw sewage on farm fields (Harris-Lovett and Sedlak, 2015). Biological waste treatment – developed in the early twentieth century – required much less land and permitted the discharge of wastewater effluents to bays, rivers, and streams. Until the early twentieth century, there were no significant regulations or restrictions on the use of wastewater for agricultural irrigation. As the scientific basis of disease became more widely understood, concerns grew among public health officials about the possible health risks associated with irrigation using wastewater and other non-potable uses of recycled water. This concern led to the establishment of guidelines and regulations to control the use of wastewater for agricultural irrigation, which was the first application of reclaimed water to be regulated.

Water reuse began to increase in both the number of projects and types of reclaimed water applications as wastewater treatment, disinfection processes, and microbiological analytical techniques became more sophisticated during the first half of the twentieth century. Similarly, water reuse standards evolved to regulate the use of reclaimed water for irrigation. During this time, water resources generally were adequate to meet all potable and non-potable needs, and the use of reclaimed water often was based on opportunity, convenience, and economics. In general, projects were implemented when water reuse constituted the most economical method of sewage disposal (Crook et al., 1994); however, burgeoning population growth in the second half of the twentieth century began to strain available freshwater resources and increased water demands in certain areas in California to the point where natural freshwater was no longer readily available and the development of additional supplies became necessary. It was in these water-stressed areas that reclaimed water first came to be viewed as a beneficial resource. The development of non-potable water recycling practices in California includes the milestones listed in **Table 1-1**.

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. 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 dramatic 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 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 ***chemicals*** 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. A summary is provided in **Table 1-2** of the progression of water recycling policies and regulations in California.

Table 1-1: Milestones for the Development of Non-Potable Water Recycling Practices in California

Year	Description of Milestone
1890	Sewer farms in use in several California communities.
1909	The City of Redondo Beach votes down a proposed sewer outflow to the ocean and instead insists that the City adopt the sewer farm model for reuse.
1929	The City of Pomona begins using recycled water for the irrigation of lawns and gardens in a suburban, semirural home development area.
1932	Golden Gate Park initiates recycling for filling ornamental lakes and landscape irrigation from a specially constructed water reclamation plant (which is terminated in 1981).
1943	Recycled water is first used at military installations to irrigate landscape in recreational areas.
1961	The City of Santee uses recycled water to develop recreational lakes for fishing and boating, and studies an experimental swimming operation.
1965	The City of Burbank begins using recycled water for power plant cooling.
1977	The Irvine Ranch Water District initiates the first major residential landscape irrigation project with a dual water system delivering recycled water.
1998	Monterey Regional Water Pollution Control Agency initiates the first project in California using tertiary-treated recycled water to irrigate food crops eaten raw.

Table 1-2: Summary of Water Recycling Policies and Regulations in California

Year	Recycling Policies and Regulations in California
1906	The California State Board of Health endorses septic tank effluent for crop irrigation.
1907	The California State Board of Health recommends against the use of septic tank effluent for irrigation of food crops eaten raw.
1918	First regulations (for crop irrigation) go into effect, prohibiting the use of raw sewage and septic and Imhoff tank effluents for the irrigation of food crops eaten raw.
1933	Requirements are added for cross-connection control and disinfection reliability.
1967	The Legislature sets policy (included in the California Water Code) for water recycling, and regulations are developed for the quality of recycled water for non-potable applications.
1968	More restrictive criteria are developed for crop irrigation. Requirements are added for landscape irrigation and impoundments.
1975	Requirements are added for treatment reliability.
1978	More restrictive criteria are developed for open access landscape irrigation. General groundwater replenishment requirements are added.
2000	Changes are made to criteria for treatment and quality, addressing additional types of uses and adding use area requirements (which were previously used as guidelines).
2014	Requirements are added for indirect potable reuse via groundwater replenishment.

1.1.2 Planned Potable Reuse

Planned potable reuse involves the use of recycled water to augment drinking water supplies. Two forms of planned potable reuse exist:

- **Indirect potable reuse (IPR)**: Treated wastewater is introduced into an **ENVIRONMENTAL BUFFER** (i.e., a groundwater system or surface water system) before the blended water is introduced into a water supply system. The **CALIFORNIA WATER CODE** provides regulatory defined definitions for the environmental buffer.
- **Direct potable reuse (DPR)**: Highly treated wastewater is introduced either directly into a public water system or into the raw water supply immediately upstream of a DWTF.

Environmental Buffer

A surface water system (e.g., reservoir, lake, or river) or groundwater system (i.e., aquifer) that receives treated recycled water and serves as a source of potable raw water.

In California, the practice of planned potable reuse has occurred in the form of IPR for over 50 years (Crook, 2010; Drewes and Khan, 2011; Drewes and Horstmeyer, 2016). Longstanding experience in California (and worldwide) has demonstrated that planned potable reuse using IPR can be practiced without having any apparent detrimental effects on public health (NRC, 1998; USEPA, 2012; NRC, 2012; Khan, 2013). A key element of an IPR system is its reliance on an environmental buffer. While some environmental buffers might offer opportunities for further treatment, ***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 indirect potable reuse in California (as defined by the California Water Code) are shown in **Figure 1-1**, which depicts advanced treated water being introduced into an environmental buffer as part of the raw water supply upstream of a DWTF. In **Figure 1-1 (a,b)**, the environmental

State of California Terminology for Potable Reuse

Per Chapter 7, Section 13561(b-d), of the California Water Code:

INDIRECT POTABLE REUSE FOR GROUNDWATER REPLENISHMENT 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.

SURFACE WATER AUGMENTATION means the planned placement of recycled water into a surface water reservoir used as a source of domestic drinking water supply.

DIRECT POTABLE REUSE means the planned introduction of recycled water either directly into a public water system, as defined in Section 116275 of the Health and Safety Code, or into a raw water supply immediately upstream upstream of a water treatment plant.

California Regulations Regarding the Environmental Buffer

SURFACE WATER SYSTEM: The Expert Panel's review of the State Water Board's proposed criteria for indirect potable reuse using surface water augmentation (NWRI, 2015a,b) considered the reservoir dilution criteria (100:1 or 10:1 + treatment) as a "performance" criterion for the reservoir and the theoretical hydraulic residence time, tr, as the reservoir "operational" criterion. The reservoir has a unique and central role to play in surface water augmentation, and these two criteria define how the reservoir is to be operated for surface water augmentation

GROUNDWATER SYSTEM: California regulations (CCR, 2015) addressing groundwater replenishment projects define the function of soil aquifer treatment in the vadose zone and groundwater system.

buffer is a groundwater aquifer; therefore, the project must meet regulations for groundwater replenishment (CCR, 2015). For such a project, advanced treated water can be applied by surface application (spreading) or subsurface application (direct injection), whereas tertiary effluent is applied by spreading to take advantage of soil aquifer treatment. In **Figure 1-1 (c)**, the environmental buffer is a surface water reservoir, so the project must meet the draft criteria for IPR using SWA (i.e., the reservoir has a theoretical hydraulic retention time of ≥4 to 6 months)[11] (NWRI, 2015a,b,c).

Because a key element of an IPR system is its reliance on a regulatory defined environmental buffer, by default, all potable reuse projects that do not meet California regulations for groundwater replenishment or the draft criteria for IPR using SWA are considered DPR. In this report, the term ***"the Gap"*** refers to the continuum between in the proposed regulations for an IPR project using SWA and a DPR project (as defined in the California Water Code). In particular, the Gap covers IPR projects using SWA with hydraulic retention times of ≥2 months and <4 months. A schematic of a potable reuse project falling under the Gap is illustrated in **Figure 1-2 (a)**.[12]

With DPR, the environmental buffer is reduced (i.e., the Gap) or eliminated and recycled water is piped directly into the raw water supply near the inlet of a DWTF or into the drinking water distribution system; therefore, the core functions of the environmental buffer used for IPR would need to be provided and maintained in some other way for DPR to ensure public health protection. The schematics of DPR are illustrated in **Figure 1-2 (a, b,** and **c)**. In **Figure 1-2 (a)**, advanced treated water is introduced with a smaller SWA environmental buffer or **(b)** without the use of an environmental buffer into the raw water supply immediately upstream of a DWTF. To date, permitted operational DPR projects in the United States involve this form of DPR (i.e., the Big Spring Raw Water Production Facility operated by the Colorado River Municipal Water District and the DPR Project used to produce an emergency water supply for the City of Wichita Falls. The Wichita Falls project was used on an emergency basis and has been discontinued.). In **Figure 1-2(c)**, finished product water is introduced directly into a drinking water distribution system.

[11] 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 reviewed the proposed regulations and provided recommendations to the State Water Board in 2015 (NWRI, 2015a,b,c). More information is available at http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/RecycledWater.shtml.

[12] More specific details and some guidance with respect to the Gap are provided in **Chapters 8** and **9** that can be used by the State Water Resources Control Board to evaluate the feasibility of all proposed potable reuse projects consistent with the overall objective to protect public health.

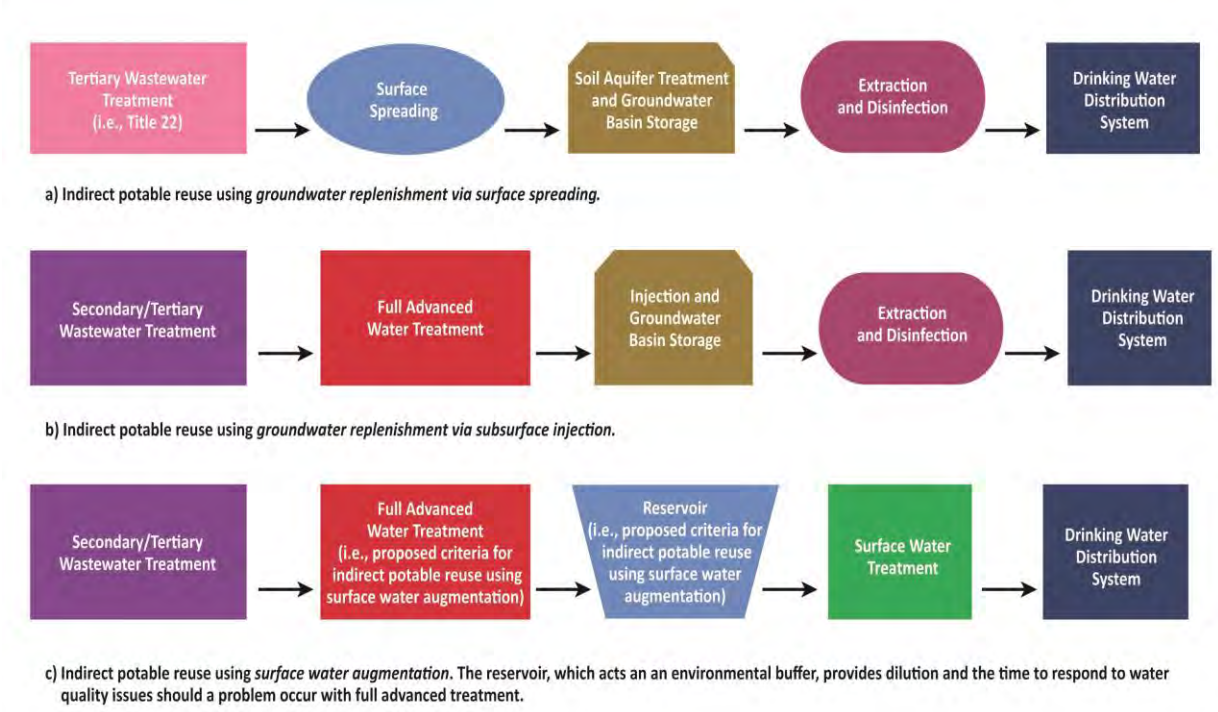

Figure 1-1: Schematics of indirect potable reuse in California using groundwater replenishment (a,b) and surface water augmentation (c). The environmental buffer is represented by a groundwater aquifer in (a) and (b), and by a reservoir in (c). Wastewater treatment could include either secondary or tertiary 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 ultraviolet disinfection combined with advanced oxidation. Drinking water treatment for surface water meets California drinking water standards.

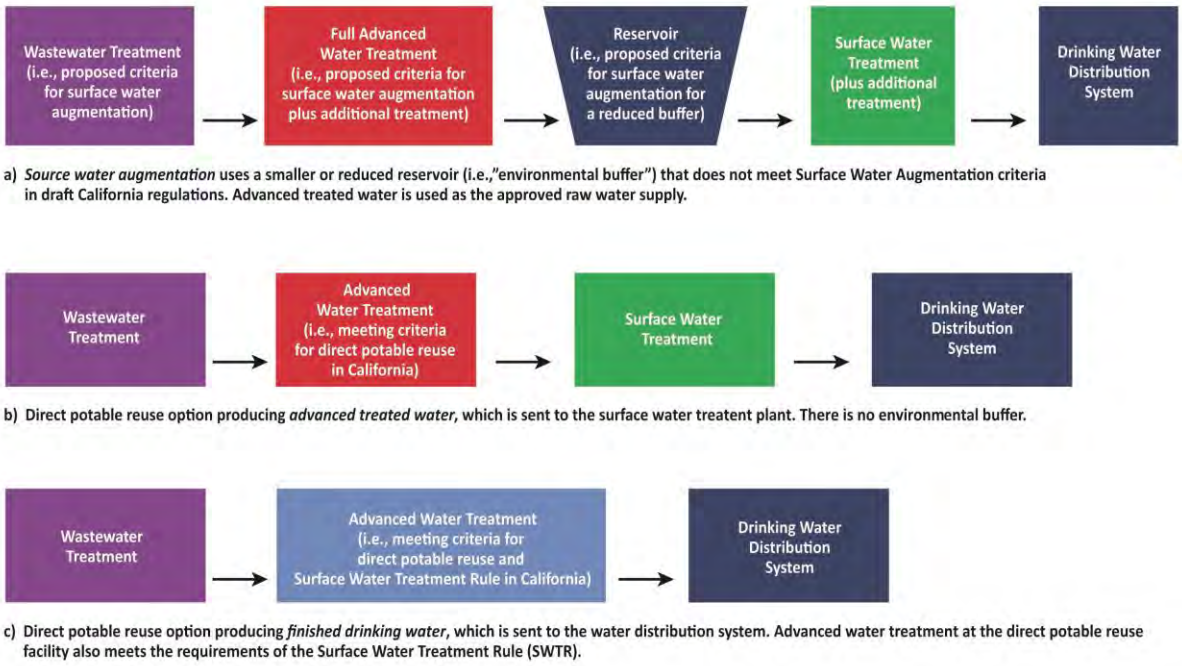

Figure 1-2: Potable reuse, showing the transition of (a) indirect potable reuse using an environmental buffer that does not meet California's proposed criteria for indirect potable reuse using surface water augmentation (the "Gap") to (b,c) direct potable reuse.

1.1.3 Unplanned (*De Facto*) Potable Reuse

Unplanned (d*e facto)* potable reuse was defined in NRC (2012) as the unplanned or incidental presence of treated wastewater in a downstream water supply source. A schematic of unplanned potable reuse is provided in **Figure 1-3**. Unplanned potable reuse is a common occurrence in a number of drinking water supplies derived from surface water sources (SWPCA and CDWR, 2011), principally rivers (NRC, 2012), and has been understood for at least 100 years, including how to address its challenges (Hazen, 1914); however, the practice is not recognized officially (USEPA, 2012). A recent analysis by Rice and Westerhoff (2015) of 2,056 surface water intakes serving 1,210 DWTFs covering 82 percent of the population of the United States indicates that 50 percent of the DWTFs are potentially impacted – under average stream flow conditions – by upstream discharges, but typically at relatively low percentages (i.e., <1 percent). The Rice and Westerhoff analysis also indicates that under low-stream flow conditions (e.g., drought), some DWTFs receive up to 50 percent of their water from wastewater effluent discharges.[13]

Figure 1-3: Schematic of unplanned (*de facto*) potable reuse, which involves the discharge of treated wastewater effluent from one community into a surface water body that is used as a source of drinking water supply for another community. Depending upon the location and local requirements, wastewater treatment could vary, but for the most part freshwater discharges in California include secondary treatment followed by filtration and disinfection. Surface water treatment includes filtration and disinfection.

As an example, unplanned potable reuse occurring in the Sacramento-San Joaquin River Delta, or "Delta," is addressed in this report from a comparative perspective relative to planned potable reuse. The State Water Project provides drinking water to approximately two-thirds of California's population and is the nation's largest state-built water development project. The watershed of the State Water Project is composed mainly of the 27,000-square-mile Sacramento River and the 13,000-square-mile San Joaquin River watersheds. Twelve WWTPs discharge directly into the Delta, and a number of others discharge to tributaries of the Delta. Currently, the average dry weather wastewater discharge is roughly 350 mgd (1.32-million m^3/d), based on average dry weather flow. The design capacity of all WWTPs is estimated to be 560 mgd (2.12-million m^3/d), indicating a future increase in volumes of wastewater to the receiving waters (SWPCA and CDWR, 2011).

The California Department of Water Resources (DWR) modeled wastewater discharges from three of the largest WWTPs that discharge approximately 82 percent of the volume of wastewater into the Delta; results indicate that the volume of wastewater in the Delta's surface water ranges from zero to about 3 percent (SWPCA and CDWR, 2011). In addition, based on State requirements, most WWTPs have been upgraded to include filtration, with several other facilities required to upgrade within the next 10 years (SWPCA and CDWR, 2011).

[13] The low-flow analysis was limited to 80 of the 2,056 locations that had stream gauges. Of the 80 sites, 32 had an estimated increase to roughly 50-percent wastewater in the raw water supply.

1.1.4 Amount of Water Used for Planned Potable Reuse

As a result of indoor and outdoor water uses and other nonresidential municipal consumptive uses, neither DPR nor IPR can replace all current potable water demands, nor can all collected wastewater be used as part of a potable reuse project. Based on a recent estimate, roughly 30 percent of all wastewater collected in California – or about 50 percent of the water now discharged to the ocean – could be used by 2020 for either DPR or IPR projects (Raucher and Tchobanoglous, 2014). The actual amount of water available will vary by region, depending on site-specific factors, such as discharge locations for wastewater effluents (Tchobanoglous et al., 2015).

1.2 Public Health Considerations as a Condition of Potable Reuse

Potable reuse provides a number of benefits, including: (1) the use of alternative freshwater supplies; (2) energy savings; (3) conservation of conventional freshwater resources; and (4) reduced amount of wastewater discharged into the environment (Asano et al., 2007). The use of DPR rather than IPR, however, has the potential to modify conventional public health practices by removing the physical separation (i.e., environmental buffer) between wastewater disposal and water supply. Consequently, it is imperative to develop and implement basic principles for the safe design and operation of DPR systems that provide continuous protection against short-term and long-term exposures to contaminants (Haas and Trussell, 1998; NRC, 2012).

Public health protection requires that microbiological pathogens and chemicals in wastewater be removed to the extent practical 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 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 waters 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-term and long-term exposures (NRC, 2012). Microbial and chemical contaminants in water produced for potable reuse can have adverse effects on human health. In addition, wastewater used as a direct 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). What is important is regulating important constituents that may result in adverse human health impacts. Determining which constituents to regulate can be challenging, but has been done for both unplanned potable reuse and planned IPR. The possibility of doing the same for DPR is considered in this report.

1.2.1 Overview of Health Risk Assessments

To understand the development of existing drinking water regulations and the application of these regulations to potable reuse, it is useful to:

- Consider how health effects are assessed.
- Review health effects considered in potable reuse studies conducted by the National Research Council.
- Review epidemiological, risk assessment, and toxicological health effects studies conducted for potable reuse.

1.2.1.1 Studies Used to Assess Human Health Effects

Human health effects assessments[14] can be based on studies using (1) test animals, (2) biochemical or cellular systems, and (3) humans. Examples include epidemiological, microbiological, and toxicological studies. Brief descriptions of these studies are provided in **Table 1-3**.

1.2.1.2 Limitations of Epidemiological, Microbiological, and Toxicological Studies

Neither epidemiological nor toxicological studies are sensitive to the low levels of exposure usually found in drinking water. Microbiological risks have been determined based on disease outbreaks attributable to a specific organism in public water supplies. In contrast, the contribution of a chemical to a specific adverse health outcome (e.g., bladder cancer) must be differentiated from other causes of that outcome (e.g., smoking), which is difficult to do.

With a single epidemiological study, care should be exercised in accepting either positive or negative results. The results must be confirmed independently with replication on other study populations. Multiple studies frequently are required before an association or lack thereof can be accepted as fact. When evaluating individual chemicals, these studies are conducted at high doses with the assumption that the effects observed can be extrapolated to environmental exposures at doses that are orders of magnitude lower.

Some animal studies have been conducted using concentrated samples of organic chemicals in water to accomplish the same goal; however, these studies have the additional goal of detecting the effects of unidentified chemicals that might be in water. As with epidemiological studies, these animal studies addressed a narrow range of potential adverse health effects (largely cancer, limited neurotoxicity screening, and reproductive outcomes), but have not focused on other chronic diseases and subtle effects on development. Some recycled water studies have been conducted with this goal in mind and are summarized in Anderson et al. (2010).

[14] Key information can be found on the risk assessment webpage of the U.S. Environmental Protection Agency (USEPA) (https://www.epa.gov/risk/risk-assessment-guidelines) and the risk assessment webpage of the State of California (http://oehha.ca.gov/risk-assessment), as well as other sources such as Asano et al. (2007), Cotruvo (1987), Haas et al. (2014), ILSI (1996, 2000), and NRC (2012).

Table 1-3: Brief Description of Epidemiological, Microbiological, and Toxicological Studies Used to Assess Human Health Effects

Type of Study	Description
Epidemiological studies	• **Purpose:** Identify and quantify changes in the incidence or processes of disease in human populations observed in an exposed population as compared to control groups (i.e., unexposed populations or those experiencing less exposure). • **Examples**: Ecological epidemiology studies (which compare aggregated data from different populations) and analytical epidemiology studies (which require more detailed controls or information from individuals within the exposed and control populations). • **Note:** In general, it is difficult to detect low incremental risks or differentiate these risks from the occurrence of background disease. • **Consideration:** Because exposure to chemicals from food, water, and the environment is difficult to quantify, care must be taken to identify and quantify the exposure as accurately as possible and to control for variables (e.g., ethnic distribution, genetics, and social factors) that may confound the outcome or result in exposure misclassifications.
Microbiological studies	• **Purpose**: Used to estimate the risks of infection by pathogens that cause human disease at various exposure levels encountered from water. • **How It Works**: Controlled dose-response infectivity studies are conducted with a known exposure to measure indications of harmful health effects through time following exposure. • **More Information**: ILSI (1996, 2000) and Haas et al. (2014).
Toxicological studies	• **Purpose:** Conducted in humans and on experimental animals for varying lengths of time and with multiple dose levels to identify no-effect levels and to obtain a dose-response relationship. • **How It Works:** The process of using animal data for human safety assessments goes through two stages: first, adverse health outcomes are identified and dose-response relationships are established that can be extrapolated to humans. • **Note:** Descriptive toxicological studies in animals tend routinely employ doses much greater than human exposures from drinking water (usually to maximally tolerated dose). This practice is done to increase the sensitivity of the animal studies, which (for practical reasons) can employ only small numbers of animals relative to the human populations exposed to drinking water. Consequently, the dose-response relationship must be extrapolated to low doses (see the U.S. Environmental Protection Agency and California risk assessment websites noted in **Section 1.2.1.1** of this report). • **Consideration:** "Safe" does not indicate zero risk, but rather that acceptable risks are likely to occur at doses represented by maximum contaminant levels.

Source: Adapted from Tchobanoglous et al. (2015).

1.2.2 National Research Council Studies on Potable Reuse

Within the past 20 years, two assessments have been conducted by the National Research Council (NRC, 1998, 2012) in which potential challenges were identified and appropriate solutions were suggested to ensure potable reuse is a safe practice from the perspective of public health. Notably, the 1998 study focused solely on IPR, while the 2012 study addressed both IPR and DPR. In the intervening years between the two studies, significant advances were made in treatment technologies and monitoring capabilities, along with increased research, interest, and need to consider potable reuse as a source of drinking water supply. The findings from NRC (2012) with respect to chemical and microbial constituents are summarized in **Table 1-4**.

Table 1-4: Findings from NRC (2012) as Related to Risks from Chemical and Microbial Constituents

Type of Risk	Findings
Risk from chemical constituents	Water quality is ensured through source control programs, treatment technologies that meet drinking water maximum contaminant levels and other limits, and monitoring for constituents that present a public health risk. For advanced water treatment trains, most chemicals are not detected; those that are detected are found at levels lower than those found in conventionally treated drinking water supplies (NRC, 2012).
Risk from microbial constituents (i.e., pathogens)	The risk from pathogens in potable reuse "does not appear to be any higher, and may be orders of magnitude lower, than currently experienced in at least some current (and approved) drinking water treatment systems (i.e., *de facto* reuse)" (NRC, 2012).

Sources: NRC (2012) and Tchobanoglous et al. (2015).

1.2.3 Epidemiological, Risk Assessment, and Toxicological Health Effects Studies on Potable Reuse

Several epidemiological and toxicological health effects studies have been conducted in the last 30 years to evaluate the public health implications of potable reuse. These studies are summarized in NRC (1998). Health effects data from some existing and demonstration potable reuse facilities, including the first DPR project in the world (located in Windhoek, Namibia), are summarized in Tchobanoglous et al. (2015). Results have shown no health impacts, based on both epidemiological studies of groundwater replenishment (i.e., the Montebello Forebay groundwater replenishment project) and whole animal studies of recycled water intended for potable reuse in several locations (e.g., Denver, Tampa, and Singapore); however, the limited sensitivity and scope of these toxicological and epidemiological studies (as described in **Table 1-3**) prevent the use of these results to support the contention that potable reuse projects have been shown to be safe. Despite these complications, the results provide some assurance that risks to public health are low.

In addition, a Science Advisory Panel formed by the State Water Board reviewed the results of many key studies conducted over the past 40 years on chemicals of emerging concern (CECs) in recycled water and their toxicological relevance to humans (Anderson et al., 2010). On the basis of this review, the Science Advisory Panel noted "…that appropriately treated recycled water represents a safe source of water to

Cryptosporidium and the Long-Term 2 Enhanced Surface Water Treatment Rule

Cryptosporidium oocysts are among the most difficult microorganisms to treat in water because of their small size (~3 to 6 micrometers) and resistance to chlorination.

The LT2ESWTR assigns log_{10} reduction credits to a variety of technologies, and individual states can assign credits for other technologies based upon performance data (e.g., membrane credits are based upon challenge testing). Log credits for disinfectants are based upon CT values (i.e., residual disinfectant concentration, C, in mg/L, multiplied by the contact time, T, in minutes).

Water supplies that can demonstrate low risk of *Cryptosporidium* contamination and wish to avoid filtration can meet requirements by using two disinfectants that can control *Cryptosporidium*, such as ozone, ultraviolet disinfection, or chlorine dioxide.

supplement potable drinking water supplies. The predominantly negative findings described above do not preclude the need to monitor recycled water to assure its continued safety."

Finally, several narrowly focused risk-based studies have been conducted to evaluate the risks to human health associated with the use of recycled water for groundwater replenishment and other types of potable reuse (NRC, 2012, Anderson et al., 2010).

1.2.4 Defining a Tolerable Level of Public Health Risk

To quantify the potential for human health effects resulting from exposure to microbial and chemical constituents, regulatory agencies have adopted the concept of a "tolerable level of risk" to assist in setting water quality guidelines or standards.

In the regulatory realm, a "de minimis risk" is a risk that is too small to be concerned with (i.e., a "virtually safe" level) or is "below regulatory concern." Traditionally, for drinking water supplies, de minimis risk levels are related to public health criteria (i.e., the toxicity of the constituent, characteristics of the population, and exposure). For microbial constituents of concern, the original Surface Water Treatment Rule (40 CFR 141.70-141.75) required, in part, that DWTFs using surface water and groundwater under the direct influence of surface water (GWUDI) must filter and disinfect the water and must achieve 4-log_{10} reduction of virus and 3-log_{10} reduction of *Giardia* spp.

More recently, the **LONG TERM 2 ENHANCED SURFACE WATER TREATMENT RULE** (LT2ESWTR) (71 FR 654, Vol. 71, no. 3, Jan. 5, 2006) dealt primarily with ensuring the control of *Cryptosporidium,* as well as other microbial constituents. While the LT2ESWTR did not change the long-standing informal public health risk goal of one in 10,000 infections per year, it was the first drinking water standard to establish a minimum required treatment level at individual DWTFs. It should be noted that the one in 10,000 goal is similar to the drinking water guideline recommended by the World Health Organization (WHO) of one in 1,000,000 disability adjusted life years (DALYs) for microbial disease risk. Specifically, for surface waters and GWUDI, public health protection is to be achieved through installing sufficient treatment technologies to achieve log_{10} reductions of *Cryptosporidium* ranging from 2 log_{10} (multiple disinfection types with source water concentrations of <0.01 oocyst per liter) to 3 log_{10} (conventional surface drinking water filtration and disinfection treatment) to 5.5 log_{10}, depending upon the concentrations of *Cryptosporidium* measured in 24-monthly source water samplings.

Note that different risk levels are commonly used, depending on the specific situation and type of contaminant. The Office of Drinking Water of the USEPA uses a "regulatory window" for chemical

carcinogens of 10^{-6} to 10^{-4} risk per person per lifetime. For pathogens, the treatment requirements as defined in Surface Water Treatment Rule and its amendments were derived using a value of 10^{-4} infections per person per year as the tolerable risk goal.

Performance goals for potable reuse projects in California have been proposed that are based on a low tolerable risk level of 10^{-4} annual risk of infection (NWRI, 2013). These tolerable risk levels refer to final drinking water quality and apply independent of whether potable reuse is practiced as IPR or DPR.

1.3 Recycled Water as a Potable Water Source

The framework for the Safe Drinking Water Act was established between the 1970s and 1990s, when the focus of regulatory efforts was limited to sources of water from streams, rivers, lakes, and groundwater aquifers. Due to competing demands for these natural water sources (e.g., in-stream flow, agricultural use, and concentrated population growth in arid portions of the United States), consideration is now being given to recycled water as a source of drinking water supply. In addition, advances have been made in research and practical experience has been gained regarding the removal of pollutants and naturally occurring constituents. The efficacy and cost-efficiency of wastewater and drinking water treatment technologies considered routine today have changed substantially from those used when the Safe Drinking Water Act was first drafted (Cotruvo, 2014). Also, advanced water treatment technologies like advanced oxidation processes were, at best, research concepts when the Safe Drinking Water Act was reauthorized for the second and third times.

At present, a sound technical basis exists for developing water recycling programs incorporating IPR and/or DPR that are protective of public health. By building on key elements of the existing framework of the Safe Drinking Water Act, the water industry can move forward to incorporate properly treated recycled water as a source of raw drinking water supply.

1.4 Purpose of This Report

The purpose of this report is to address the legislative mandate contained in Section 13565(a)(1) of the California Water Code **(see Appendix P1)** that, in summary, requires the Expert Panel to:

- Advise the State Water Board on public health issues and scientific and technical matters regarding the feasibility of developing uniform water recycling criteria for DPR.
- Assess what, if any, additional areas of research are needed to establish uniform regulatory criteria for DPR, and recommend an approach for accomplishing the additional needed research in a timely manner.

The Expert Panel selected the main topics listed in **Table 1-5** for investigation as part of addressing the legislative mandate. In the following chapters, more details are provided on each of the main topics listed in **Table 1-5**, including the Expert Panel's assumptions, conclusions, and recommendations to address these topics, as well as the feasibility of developing uniform water recycling criteria for DPR. Although DPR is the subject of this report, many of the key aspects presented and discussed herein also can be applied to IPR; accordingly, relevant aspects of IPR are discussed.

Table 1-5: Main Topics Addressed by the Expert Panel in This Report

Topic Selected by Expert Panel	Description of Topic	Location in Report
Public health surveillance	Example programs, ongoing national and state programs, health endpoints, sensitivity and interpretation of data, non-health based data, and the feasibility of a surveillance program for direct potable reuse.	Chapter 3
Chemical analytical methods and tools	Approaches for assessing the chemical water quality of advanced treated water and drinking water.	Chapter 4
Bioanalytical tools	Issues related to the use of *in vitro* bioassays for advanced treated water and drinking water.	Chapter 5
Traditional and molecular pathogen monitoring methods	Monitoring indicators, surrogates, and pathogens in advanced treated water and drinking water.	Chapter 6
Antibiotic resistant bacteria and antibiotic resistance genes	State-of-the-science, relative sources, potential exposure pathways, and relative significance of concern.	Chapter 7
Direct potable reuse system performance	Multiple barriers (e.g., redundancy, inherent performance, and mechanical reliability); online monitoring tools (e.g., sensors, surrogates and indicators); and performance objectives (e.g., process and overall facility compliance).	Chapter 8
Potable reuse regulatory feasibility analysis	Relative comparison of an example direct potable reuse system against an existing California potable water supply and an indirect potable reuse project with a reduced environmental buffer.	Chapter 9

1.5 Organization of This Report

This report is organized into the following chapters:

In **Chapter 1**, the concept of potable reuse is introduced to the reader, including the history of reuse in California, differences between IPR and DPR, and public health considerations for DPR, among other topics. An overview also is provided of the purpose and organization of this report.

In **Chapter 2**, the potential hazards of potable reuse are discussed, including microbial and chemical constituents of concern.

In **Chapter 3**, information is provided about efforts to manage public health risks, such as the Safe Drinking Water Act and other regulations, and surveillance tools and methods to quantify and mitigate these risks.

The focus of the next four chapters is on monitoring potential hazards. In **Chapter 4**, the Expert Panel addresses analytical methods and tools to measure chemical water quality, describing the elements of a potential chemical monitoring program for DPR. In **Chapter 5**, bioanalytical tools (specifically, *in vitro* bioassays) are evaluated as a potential tool to analyze water produced by DPR projects, while a discussion is included in **Chapter 6** on traditional and new molecular methods to detect, characterize, and quantify pathogenic microorganisms in wastewater and advanced treated water. The topic of antibiotic resistance is addressed in **Chapter 7**, with emphasis on the sources of antibiotic resistant bacteria and antibiotic resistance genes, methods to detect them in water, and their occurrence and removal during wastewater treatment and advanced water treatment.

For **Chapter 8**, an in-depth discussion is provided on the performance of DPR systems, including technical issues associated with the implementation of DPR: (1) source control to reduce constituents in wastewater; (2) the types and combinations of unit processes (i.e., treatment trains) used for DPR; (3) reliability of treatment process (i.e., how the DPR system performs mechanically and how it performs in meeting water quality objectives); and (4) facility operations and maintenance.

In **Chapter 9**, the Expert Panel describes the approach used to evaluate the feasibility of developing uniform water recycling criteria for DPR. This feasibility analysis was performed for microbial pathogens using *Cryptosporidium* as the reference pathogen and involved the following three key steps: (1) define alternative drinking water supply options; (2) define the analysis approach and assumptions; and (3) conduct a feasibility analysis for a reference pathogen.

In **Chapter 10**, a summary is provided of topics related to managing DPR systems, such as: (1) operator training and certification; (2) the technical, managerial, and financial capabilities of the utilities building and operating DPR projects; and (3) other issues instrumental to the successful operation of DPR projects.

Finally, in **Chapter 11**, the Expert Panel lists its key findings and recommendations to the State Water Board regarding the feasibility of developing uniform water recycling criteria for DPR. In particular, research needs are identified to address information gaps related to public health.

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CHAPTER 2: POTENTIAL HAZARDS OF POTABLE REUSE

- Microbial pathogens of concern, and chemical constituents of concern.
- Regulatory mechanisms to manage potential risks.
- Public health surveillance tools and methods to quantify and mitigate risk.

Recycled water derived from municipal wastewater (historically known as sewage) comes from a variety of sources, including homes, schools, hospitals, and commercial and industrial facilities. Depending on the makeup of a community, the quantity and quality of untreated wastewater will vary. Untreated wastewater is expected to contain a variety of chemicals and microbial constituents that may be of concern to public health. In general, exposure to untreated and/or poorly treated wastewater has not been a concern in the United States because all communities are required to comply with the Clean Water Act, a federal law that regulates the discharge of pollutants into the nation's waters. Disinfected secondary wastewater treatment – and, often, filtered disinfected secondary wastewater treatment – now is used in in many locations throughout California.

Discharges to surface waters from industries or publicly owned treatment works (POTWs) for industrial sectors are controlled by National Pollutant Discharge Elimination System (NPDES) permits and effluent guidelines. The National Pretreatment Program was created as part of the Clean Water Act to address the discharge of toxics from non-domestic sources to POTWs (USEPA, 2014). Pretreatment requirements (e.g., source control) have been established for chemical discharges to municipal wastewater systems. Ambient water quality criteria have been established to classify water-quality specifications according to designated use (which can include municipal drinking water supplies). Together with the California Water Code, the implementation of this combined legislation has resulted in higher-quality treated wastewater and a better understanding of what microbial and chemical constituents remain in treated wastewater effluents throughout California. Public health concerns associated with recycled water (particularly, potable reuse) are related directly to the degree and effectiveness of the following:

- Source control programs and wastewater treatment regulated under the Clean Water Act.
- Effectiveness and reliability of advanced water treatment.
- Quality of the raw or finished product water (i.e., concentrations of microbial and chemical constituents of concern).

As described in **Chapter 1**, public health concerns related to non-potable reuse and indirect potable reuse (IPR) using groundwater replenishment have been addressed successfully in California through state regulations (CCR, 2015), practical experience with numerous projects, and research. For this chapter, the Expert Panel reviewed potential public health concerns associated with direct potable reuse (DPR) as part of evaluating the feasibility of developing uniform water recycling criteria for DPR that are protective of public health. Notably, this review was limited to potential microbial and chemical constituents of concern in water produced for potable reuse; therefore, other sources of exposure (e.g., food, household products, and personal contact) – whose impact to public health could be more significant – are not addressed in this report.

2.1 Overview of Microbial Pathogens of Concern

Infection is the invasion of an organism's body tissues by disease-causing agents, the multiplication of these agents, and the reaction of host tissues to these agents and the toxins they produce. Disease occurs when the impairment of normal functions ensues. Infectious disease (also known as "transmissible disease" or "communicable disease") is illness resulting from an infection. When considering the implications of infectious disease due to human exposure to raw and treated wastewater, the following factors need to be considered: (1) for waterborne illness or disease to occur, an agent of disease (e.g., a pathogen) must be present; (2) the agent must be present in sufficient concentration to produce a probability of infection or disease in an unacceptable fraction of the population; and (3) a susceptible host must come into contact with the dose in a manner that results in infection or disease (adapted from Cooper et al., 1986; Cooper, 1991).

Although a wide range of pathogens has been identified in raw wastewater, relatively few have been documented as responsible for the majority of the waterborne illnesses caused by pathogens of wastewater origin (Mead et al., 1999; Scallan et al., 2011). Based on foodborne disease in the United States, pathogens of public health concern have been identified by the Centers for Disease Control and Prevention (CDC) (Mead et al., 1999, Scallan et al., 2011). In characterizing food-related illness and death in the United States, Mead et al. (1999) estimated the annual total number of illnesses caused by known pathogens (adjusted for the fact that many illnesses are not reported) to be 38.6 million cases, with 5.2-million cases (13.5 percent) resulting from bacterial pathogens, 2.5-million cases (6.5 percent) resulting from parasitic pathogens, and 30.9-million cases (80 percent) resulting from viral pathogens. Noroviruses have been reported to account for 23,000,000 illnesses each year, of which 60 percent are estimated to be non-foodborne. Rotavirus accounts for 3,900,000 illnesses each year, of which 99 percent are non-foodborne (Mead et al., 1999). With this background, it follows that many illnesses can be caused by pathogens found in domestic wastewater, thereby emphasizing the need to reduce microbial pathogens to acceptable levels in drinking water. A review of CDC research data indicates that 85 to 90 percent of all non-foodborne cases (i.e., cases related to other routes of transmission, such as waterborne) in the United States are caused by viral pathogens (i.e., enteric viruses). The relative importance of viral pathogens in the transmission of waterborne disease is supported by data from WHO (1999) and by research conducted over the last 20 years on exposure to waterborne pathogens through recreational activities, such as swimming (Cabelli, 1983; Wade et al., 2003; Soller et al., 2010).

2.1.1 Review of Performance Criteria for Microbial Pathogens

To address the legislative mandate, the first action taken by the Expert Panel was to document and review pathogen performance criteria (defined as $\log_{10}$ reduction values, or "LRVs") applied to IPR projects. This documentation and review process are presented in **Sections 2.1.1.1** to **2.1.1.3** as three distinct steps: (1) a review of the derivation of LRVs; (2) application of the LRVs to groundwater replenishment; and (3) a probabilistic review to establish a baseline for the DPR feasibility analysis.

2.1.1.1 Review of the Derivation of California Log_{10} Reduction Values

For IPR using groundwater replenishment, the State Water Board developed minimum LRV requirements for target pathogen groups (i.e., enteric viruses and parasites) (CCR, 2015). An analogous approach has been used in developing proposed criteria for IPR using surface water augmentation (SWA). The LRV requirements were determined using the following assumptions:

Assumption 1: The tolerable annual risk of infection is 10^{-4} per person per year (based upon guidance from the U.S. Environmental Protection Agency [USEPA] in developing the Surface Water Treatment Rule (Regli et al., 1991). This acceptable risk level is applied independently to each organism group (i.e., enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts).

Assumption 2: The tolerable concentrations of enteric viruses and *Giardia* cysts in finished drinking water that correspond to an annual risk of infection of 10^{-4} per person per year were determined using the dose-response models from Regli et al. (1991). For *Cryptosporidium* oocysts, it was determined by the dose-response model from the Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA, 2006). The dose-response models are based on infection (rather than illness) as an endpoint, providing a margin of safety particularly for vulnerable populations because, for most pathogens, only a fraction of those infected exhibit symptoms of a range of severity, depending on factors such as the host's immune status.

Assumption 3: The intent was to evaluate the necessary treatment for the worst-case exposure scenario for microbial infection; therefore, the "maximum organism density reported for raw wastewater in general (rounded up to one significant figure)" (see **Table 2-1**) was specified and assumed to be the concentration in raw wastewater used as a source water for IPR.

The analysis by the State Water Board that resulted from these assumptions is summarized in **Table 2-1**. Based upon the maximum concentration assumed to be in raw wastewater and the tolerable drinking water density determined for finished drinking water, the required LRVs for IPR-based potable reuse schemes were calculated to be 12-$\log_{10}$ reduction for enteric virus, 10-$\log_{10}$ reduction for *Giardia* cysts, and 10-$\log_{10}$ reduction for *Cryptosporidium* oocysts (referred to as "12/10/10").

Point estimate-based quantitative microbial risk assessment (QMRA) was the approach used by the State Water Board to determine the LRVs in **Table 2-1**. Estimating risk using such assessments is necessary in situations where the fraction of infection that could be attributed to water (e.g., potable drinking water, recreational exposure, or exposure through non-potable reuse) is low or extremely difficult to measure through health surveillance programs or epidemiological studies. The point estimate-based assessment approach used by the State Water Board relies on a single concentration value for the target organism, an assumed single volume rate of water consumption, and single values of the parameters defining the dose-response relationship. This approach is consistent with guidelines from the World Health Organization (WHO) for regulating microbial risks from drinking water and recycled water (WHO, 2011; WHO, 2006) and also is the basis for the USEPA's Safe Drinking Water LRVs for enteric virus and *Giardia* cysts.

The development of the LRV criteria is reviewed in detail in NWRI (2013), which concluded that the LRV criteria of 12/10/10 are sufficiently conservative to maintain risk below the ***acceptable risk*** level of 10^{-4} per person per year for virus, *Giardia* cysts, and *Cryptosporidium* oocysts, respectively. The analysis and findings of NWRI (2013) provide a strong base of evidence supporting these criteria and should be consulted for more in-depth discussion. Furthermore, as suggested in NWRI (2013), any treatment train that achieves 10-$\log_{10}$ reduction of *Cryptosporidium* oocysts will ensure a 10-$\log_{10}$ reduction of *Giardia* cysts (i.e., *Giardia* cysts are more easily disinfected than *Cryptosporidium* oocysts and are larger in size than *Cryptosporidium* oocysts; therefore, *Giardia* cysts are removed more readily by membranes than *Cryptosporidium* oocysts).

Table 2-1: Assumed Concentration Values that Result in the Required Log_{10} Reduction Values as Determined by the State of California (CCR, 2015)

	Enteric Virus	*Giardia*	*Cryptosporidium*[15]
Raw wastewater maximum density	10^5 virus per liter[a]	10^5 cysts per liter[b]	10^4 oocysts per liter[b]
Tolerable drinking water density (TDWD)	2.2×10^{-7} virus per liter[c]	6.8×10^{-6} cysts per liter[c]	1.7×10^{-6} oocysts per liter[d]
Ratio of TDWD to wastewater density	2.2×10^{-12}	6.8×10^{-11}	1.7×10^{-10}
Required log_{10} reduction values (LRVs)	12	10	10

[a] These high enteric virus and cyst concentrations are reported in Table 3-7 of Asano et al. (2007).

[b] An oocyst concentration of 10^4, rounded up, based on data from Norway (Robertson et al., 2006) and Melbourne (Tetra Tech, 2011).

[c] Regli et al. (1991).

[d] Used the high infectivity rate from the Long Term 2 Surface Water Treatment Rule [Federal Register: January 5, 2006 (Vol. 71, No. 3)] [Rules and Regulations] [Pages 653-702].

The Expert Panel reviewed the LRV assumptions in the context of DPR projects (see **Appendix 2A**) and determined that the LRVs are acceptable as a basis from which to construct an approach to evaluate the feasibility of DPR criteria. Specifically, probabilistic QMRA is now a well-developed approach in which input parameters are represented with descriptive statistics that capture uncertainty and variability (Haas et al., 2014). As discussed in **Section 2.1.1.3**, the Expert Panel constructed an example probabilistic review of *Cryptosporidium* for comparisons against the point estimate to provide a more rigorous approach from which to evaluate the feasibility of DPR criteria and to provide an approach that the State Water Board can use to evaluate future applications of DPR.

2.1.1.2 Application of Log_{10} Reduction Values to Groundwater Replenishment

IPR projects using groundwater replenishment (and the currently proposed criteria for IPR using SWA) must meet the overall minimum LRVs of 12/10/10 (CCR, 2015). A summary of LRV credits approved by the State Water Board for treatment unit processes at select existing groundwater replenishment projects is provided in **Table 2-2**. Also included in this table are the maximum LRV credits currently established by the State Water Board for each unit process.

[15] Per a discussion with the State Water Resources Control Board, the original estimated log_{10} reduction (LRV) calculations were based on an assumed infective dose of one organism per infection and an annual consumption of 1,000 liters of water per year. All the current LRV estimates contained in the regulations for IPR using groundwater replenishment and the proposed criteria for indirect potable reuse (IPR) using surface water augmentation (SWA) are based on organism-specific dose-response functions and an annual consumption of 2 liters of water per day for 365 days (or 730 liters per year). While this information does not explain the actual difference between the WateReuse 11-02 report (Trussell et al., 2013) and the CDPH (2014) reference in use today, it provides some background on the fundamental modifications made between the original draft LRV estimates and those used to define the current regulations for IPR using groundwater replenishment and the proposed criteria for IPR using SWA.

Table 2-2: Approved Log_{10} Reduction Values (LRVs) for Selected Groundwater Replenishment Projects in California[a]

Process	Current Maximum Allowable LRVs	WRD Alamitos Gap Barrier Project[a]	Cambria CSD[a]	LABOS Dominguez Gap Barrier Project[a]	Upper San Gabriel Valley MWD[a]	OCWD GWRS Mid-Basin Project[a]	Comments on Potential LRV Credit (Conservative Regulatory Assumptions)
Secondary activated sludge	1.9 V 1.2 C 0.8 G[b]	2.0 V 1.0 C 2.0 G	2.0 V 1.0 C 2.0 G	1.9 V 1.2 C 0.8 G	None claimed	None claimed	WRD claimed credit via a dataset (Rose et al., 2004) using a long solid retention time (SRT). Note that Appendix A of Soller et al. (2007) contains a reanalysis of the dataset and expanded the data to produce descriptive statistics for LRVs. In addition, Cooper et al. (2012) included a literature review for the dataset and raw wastewater pathogen distributions (see Tables 2.3, 2.4, 2.5, and 3.2 in the Cooper report). Later assessments of the dataset recommend the lower 10th percentile of the entire dataset (see Table 5-2 of Larry Walker Associates, Inc. et al., 2015) and are consistent with the previous reanalysis.
Microfiltration or ultrafiltration	0 V 4.0 C 4.0 G	0 V 4.0 C 4.0 G	0 V 4.0 C 4.0 G	0 V 4.0 C 4.0 G	None	0 V 4.0 C 4.0 G	No virus credit was given due to the inability to monitor sensitivity at a resolution of 0.01 micrometers with a pressure decay membrane integrity test. A 4 log_{10} credit for protozoa was granted through a demonstration study conducted by the State of California (CDPH, 2011) using the *Membrane Filtration Guidance Manual* (USEPA, 2005), provided a daily pressure decay membrane integrity test is conducted and any needed repairs are made.
Special Study	--	--	--	--	--	1.0 V	Based on a site-specific study.
Filtered and disinfected tertiary	5.0 V 0 C 0 G	None claimed	None claimed	None claimed	5.0 V 0 C 0 G	--	Combination of secondary wastewater treatment, filtration, and disinfection.
Reverse osmosis	2.0 V 2.0 C 2.0 G	1.5 V 1.5 C 1.5 G via TOC (online)	None claimed	1.0 V 1.0 C 1.0 G via EC	None	2.0 V 2.0 C 2.0 G (assumed via TOC control of 0.1 mg/L)	Limited credit is given due to the inability to accurately monitor membrane integrity beyond an acceptable level of certainty. Traditionally, total dissolved solid (TDS) removal has been used as a surrogate. Because the TDS of wastewater is not as high as seawater, the log_{10} reduction is <1.5; if the influent is 2,500 mg/L and the effluent is 250 mg/L, the log_{10} reduction is 1. Sensitive online total organic carbon (TOC) monitors can demonstrate a log_{10} reduction of <2 (if 10 mg/L in and 0.1 mg/L out, the log_{10} reduction is 2); however, the average removal rate of TOC at the indirect potable reuse project at the Orange County Water District in 2014 was 98 percent, which is a log_{10} reduction of 1.7 (under review by the State Water Board). Newer monitoring tools have been proposed, but have yet to be approved by the State Water Board, such as online TRASAR dye (which claims a demonstrated log_{10} reduction of >3).

Process	Current Maximum Allowable LRVs	WRD Alamitos Gap Barrier Project[a]	Cambria CSD[a]	LABOS Dominguez Gap Barrier Project[a]	Upper San Gabriel Valley MWD[a]	OCWD GWRS Mid-Basin Project[a]	Comments on Potential LRV Credit (Conservative Regulatory Assumptions)
Free chlorine post-reverse osmosis	4.0 V 0 C 3.0 G via free Cl	None claimed	2.0 V 0 C 0 G via free Cl	4.0 V 0 C 3.0 G via free Cl	--	--	Chlorination in a pipeline using free chlorine CT.[c]
Ultraviolet/ hydrogen peroxide	6.0 V 6.0 C 6.0 G	6.0 V 6.0 C 6.0 G	6.0 V 6.0 C 6.0 G	6.0 V 6.0 C 6.0 G	None	6.0 V 6.0 C 6.0 G	6-$\log_{10}$ reduction of virus (including adenoviruses) and 6-$\log_{10}$ reduction of protozoa, assuming the ultraviolet dose is >300 millijoules per square centimeter (mJ/cm^2) (based on advanced oxidation, typically >900 mJ/cm^2).
Subsurface application retention time	6.0 V 0 C 0 G	6.0 V 0 C 0 G	2.0 V 0 C 0 G	6.0 V 0 C 0 G	--	--	CCR 60320.208 (b) - For each month water is retained underground, the project is credited with 1-$\log_{10}$ reduction of virus. Time must be verified by a tracer study.
Surface application retention time	6.0 V 10.0 C 10.0 G (for 6-month retention time)	--	--	--	7.0 V 10.0 C 10.0 G (7-month retention time)	3.0 V 0 C 0 G (3-month retention time)	CCR 60320.108(c) - A groundwater replenishment reuse project using surface application that demonstrates at least 6-months retention underground will be credited with 10-$\log_{10}$ reduction of *Giardia* cysts and 10-$\log_{10}$ reduction of *Cryptosporidium* oocysts.

[a] The full names of the projects are as follows: WRD Alamitos Gap Barrier Project = Water Replenishment District Alamitos Gap Barrier Project; Cambria CSD = Cambria Community Services District; LABOS Dominguez Gap Barrier Project = Los Angeles Bureau of Sanitation Dominguez Gap Barrier Project; Upper San Gabriel Valley MWD = Upper San Gabriel Valley Municipal Water District; OCWD GRWS Mid-Basin Project = Orange County Water District Groundwater Replenishment System Mid-Basin Project.

[b] Waiting for the results of WRRF 14-02 regarding potential additional information that may support additional $\log_{10}$ reduction credits for wastewater treatment plants.

[c] CT = Residual disinfectant concentration, C, in milligrams per liter, multiplied by the contact time, T, in minutes.

General Notes:

- Developed based in part on information provided by Brian Bernados, State Water Resources Control Board.
- For ozone and ozone/hydrogen peroxide treatment processes, the potential maximum allowable $\log_{10}$ removal values (LRVs) are based on USEPA CT tables (i.e., 6-$\log_{10}$ reduction of virus, 3- to 6-$\log_{10}$ reduction of *Giardia*, and 1- to 2-$\log_{10}$ reduction of *Cryptosporidium*).
- Useful background information regarding LRVs is provided in WRRF 11-02 (Trussell et al., 2013).
- The State Water Resources Control Board's expectation is that LRVs will be met at or above the 95th percentile LRV (i.e., 95 percent of the time equal to or better than the 95th percentile LRV).
- LRV = Log_{10} reduction value. V = Virus. C = *Cryptosporidium*. G = *Giardia*. TOC = Total organic carbon. EC = Electrical conductivity. mg/L = Milligram per liter. Cl = Chlorine.

The Expert Panel evaluated the current approach used to give credit for existing IPR projects to determine if it is sufficient for DPR and whether any changes would be needed in the process of establishing regulations for DPR. The following is a summary of the Expert Panel review:

Primary and Secondary Wastewater Treatment LRV Credit: Currently, the maximum credits assigned for primary and secondary wastewater treatment (combined) are 2/1/1. These values are believed to be conservative for the reasons described in **Table 2-2**; however, there is widespread agreement that a deeper understanding is needed as to what factors influence removal. Future research may lead to a reassessment of the LRV credits for primary and secondary wastewater treatment.

Microfiltration LRV Credits: The maximum credits assigned for microfiltration are 0/4/4. The process for assigning these credits is the same as that used in the report on *Surface Water Treatment Rule Alternative Filtration Technology* (CDPH, 2011). This approach also is adequate for DPR; however, the Expert Panel recommends that large volume sampling and molecular methods should be used to assess the removal of pathogens by membrane technologies installed at full-scale DPR facilities (see **Chapter 6**).

Chlorination LRV Credits: The approach for assigning credits for chlorination depends on the point in the treatment train at which chlorination occurs. If chlorination occurs after tertiary wastewater treatment, the maximum credits assigned for chlorination are 5/0/0. This value is derived from the California non-potable water recycling regulations, which require a CT value of 450 milligram-minutes per liter (mg-min/L) (CCR, 2015); however, these regulations do not distinguish between free and combined chlorine. A previous evaluation of this subject is contained in Cooper et al. (2012), which includes the recommendation that "Because the use of free chlorine can offer significant advantages over the use of combined chlorine, especially when coupled with the use of membrane bioreactors in satellite applications, it is recommended that the California Department of Public Health undertake a comprehensive study of the required CT values based on free chlorine for wastewater treatment processes that nitrify completely. Ultimately, it is envisioned that the required CT values would be based on the wastewater treatment technology, process control, and process monitoring instrumentation." The Expert Panel suggests that this recommendation also should apply to DPR projects, and that virus removal credit be assessed during the startup of DPR facilities.

If chlorination occurs after reverse osmosis, an additional maximum credit of 3-$\log_{10}$ LRV can be assigned for *Giardia*, based on the residual free chlorine concentration, and using the CT tables of the USEPA's Surface Water Treatment Rule. This approach is reasonable and consistent with existing regulations for drinking water.

Ultraviolet-Based Advanced Oxidation Process LRV Credits: To date, only advanced oxidation processes (AOPs) based on ultraviolet (UV) disinfection have been permitted in California. The maximum LRVs are 6/6/6, based on the application of a UV dose of >300 millijoules per square centimeter (mJ/cm^2). To date, adenovirus is the most UV-resistant waterborne pathogen described in the literature. These maximum LRVs are believed to be adequate, based on the following:

- Laboratory studies (e.g., Meng et al., 1996, in which a dose of 30 mJ/cm^2 is reported for 1-$\log_{10}$ inactivation of adenovirus).
- An assessment by the USEPA for the *UV Guidance Manual*, which was designed to provide adequate inactivation of adenoviruses (e.g., 4-$\log_{10}$ reduction credit is given for a UV dose of 186 mJ/cm^2) (USEPA, 2006).

An important concern is that the UV reactor system must be designed to prevent short-circuiting, as an extremely small amount of short-circuiting could result in some pockets of water receiving a lower dose and result in less than 6-$\log_{10}$ inactivation. Multiple reactors in series likely are needed (e.g., see Lawryshyn and Hofmann, 2015). Consequently, it is important that rigorous evidence of reactor hydraulics is provided as part of the DPR project's engineering report.

Ozonation LRV Credits: Ozonation is a unit process that has not been proposed yet and, therefore, has not been permitted for an IPR project using groundwater replenishment. LRVs for viruses and *Giardia* cysts have been established, but currently there is a knowledge gap regarding the inactivation of *Cryptosporidium* oocysts. Projects like WRRF-15-10 on "Optimization of Ozone-BAC Treatment Processes for Potable Reuse Applications" and others may address this gap. It is expected that the CT concept can be used to assign LRV credits for viruses, *Giardia*, and *Cryptosporidium,* although additional verification should be undertaken in real wastewater matrices.

Finally, the expectation is the LRVs will be met at or above the 95th percentile LRV (i.e., 95 percent of the time equal to or better than the 95th percentile LRV). Hamilton et al. (2006) focused on the 95th percentile to be conservative with respect to public health protection. In Tanaka et al. (1998), both the 90th and 95th percentiles were considered, and focus was placed on the 95th percentile based on criterion in the Surface Water Treatment Rule that turbidity in finished water remain below the maximum level at least 95 percent of the time.

Overall, the Expert Panel concludes a similar process for assigning LRV credits for individual unit treatment processes is feasible for DPR; however, additional process monitoring is recommended to ensure reliable treatment. A probabilistic approach could be used to establish the basis from which to measure overall DPR plant performance. In addition, future research may be necessary to provide evidence for assigning higher credits in some cases (e.g., reverse osmosis, chlorination).

2.1.1.3 Probabilistic Approach for Developing Health-Based Pathogen Log_{10} Reduction Values

A more accurate estimate for required LRVs can be obtained using a probabilistic approach, in which (1) pathogen concentrations are represented with probability distributions and (2) a Monte Carlo simulation is used to estimate the LRVs. A probabilistic approach could be used to estimate health-protective pathogen LRVs for each pathogen individually, corresponding with the USEPA's drinking water health-based goal of an annual rate of infection risk of 10^{-4} per person per year. Notably, the State Water Board considers a one in 10,000 (i.e., 1×10^{-4}) mean risk of infection to be an acceptable risk from exposure to treated wastewater effluent (CDPH, 2010). As an example, *Cryptosporidium* spp. was used to illustrate the concordance between both the simpler maximum value type estimate and the probabilistic-based approach. The probabilistic approach that was used accounted for the following:

- Variation in pathogen density in raw wastewater.
- Daily exposures (assuming that 1 liter per day [L/d] was consumed for each day of the year).
- Dose-response model with uncertainty.

To find the estimated pathogen LRVs from **Section 2.1.1.1**, the annual probability of infection from *Cryptosporidium* for a specific exposure (i.e., consumption) was solved to determine the Tolerable Infection Risk (**Equation 2-1**).

$$\text{Tolerable Infection Risk} = 1 - D_r(V \times 10^{\log(C) - LRV})^n \quad \text{(Equation 2-1)}$$

Where:

- The Tolerable Infection Risk is set at an annual infection risk of 10^{-4} per person per year.
- D_r is a dose response function (for *Cryptosporidium* spp, Exponential [infection]).
- $r_{lower} = 0.04$, $r_{upper} = 0.16$ (USEPA, 2006).
- V is the volume of water ingested per use event (set at 1 L/d, which is the mean unboiled tap water consumption in the United States).
- n is the number of exposure events per year (set at 365 days per year).
- C is the pathogen concentration distribution in raw wastewater of *Cryptosporidium* (oocysts per liter [L]) with a lognormal distribution mean of $\log_{10}$ 2.85 +/- SD (standard deviation) of $\log_{10}$ 1.75 (based on data from Rose et al., 2004).
- LRV is the estimated $\log_{10}$ reduction value (the equation is solved for LRV).

A Monte Carlo analysis approach was used to capture both (1) the natural variability in the input parameters (for this example, just the pathogen concentration) and (2) uncertainty in the dose-response parameterization. Ten thousand simulations were run using **Equation 2-1** and plotted as a cumulative distribution function (CDF), as shown in **Figure 2-1.**

The results of the probabilistic example with the data used indicates that an LRV of 9.1 (for the dose-response function in USEPA, 2006) will achieve a reduction of health risk to the acceptable annual level of infection (i.e., 10^{-4} per person per year) 95 percent of the time. In this case, the simpler maximum value estimation approach (see **Section 2.1.1.1**) provides a reasonable upper-bound LRV; however, a more thorough analysis should be done based on a more comprehensive review of *Cryptosporidium* oocyst concentrations in raw wastewater and other dose-response relationships, such as those recently published by Messner and Berger (2016). A preliminary evaluation of one of the new suggested dose-response relationships (i.e., Beta-Poisson) was conducted using the above raw wastewater concentrations for *Cryptosporidium* and other noted exposure assumptions. A comparison of the estimated $\log_{10}$ reduction results between the USEPA exponential model and new Beta-Poisson model indicates that the 95-percent estimated LRV decrease by roughly 0.3 $\log_{10}$, which is within the upper-bound LRV estimated by the simpler maximum value approach discussed above. A similar probabilistic analysis should be conducted for enteric virus and *Giardia* cysts. Because new data and research results are published regularly, an assessment of the assumptions should be conducted periodically to confirm the LRV criteria for all three pathogen groups.

2.1.2 Outbreak Considerations

An important consideration, which was not addressed in **Sections 2.1.1.1** to **2.1.1.3**, is that none of the studies of raw wastewater pathogen concentrations reported that samples were collected during an outbreak of illness. It is possible that pathogen concentrations in raw wastewater when an outbreak occurs could be higher than the measured values found in literature.

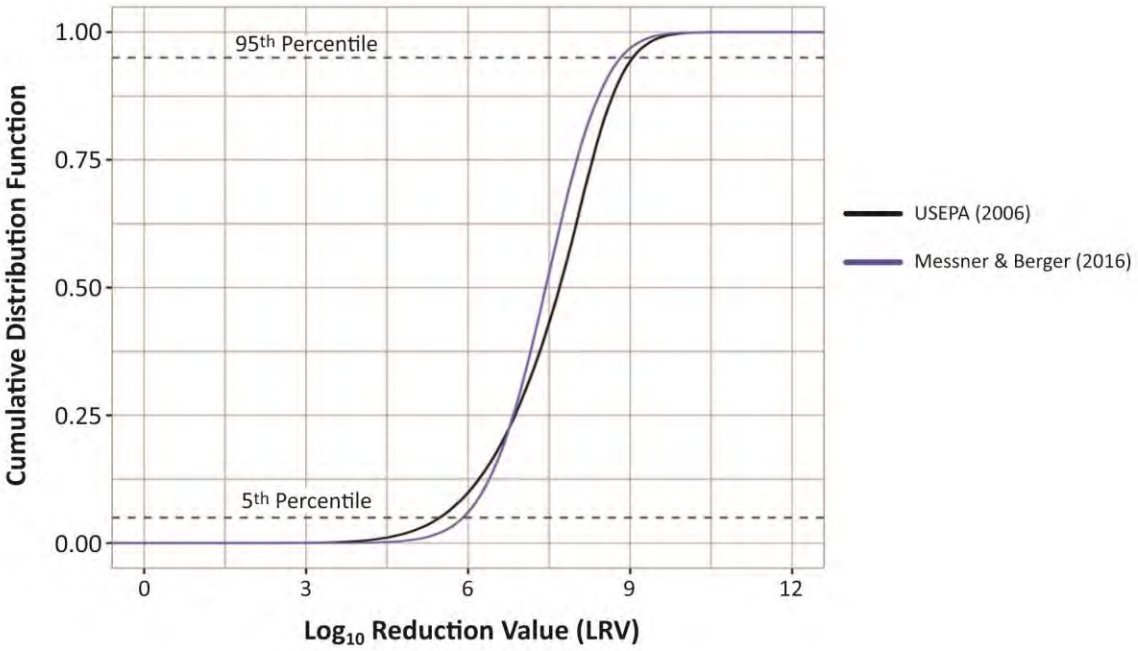

Figure 2-1: Cumulative distribution function (CDF) plot of estimated log_{10} reduction values for *Cryptosporidium* spp. to meet an annual tolerable risk of infection of 10^{-4} per person per year (USEPA [2006] is shown as the black line, and Messner and Berger [2016] is shown as the blue line).

One recent article (Hellmér et al., 2014) provides useful information on this question. In this study, the presence of seven pathogenic viruses (i.e., norovirus [NoV], astrivirus, adenovirus, Aichi virus, parechovirus, hepatitis A virus [HAV], and hepatitis E virus) was investigated in raw wastewater to explore whether their identification could be used as an early warning for outbreaks (Hellmér et al., 2014). Daily raw wastewater samples were collected every second week over a period of 15 weeks and were pooled to represent a weekly composite sample during January through April 2013 from a treatment plant in Gothenburg, Sweden. In the study, the amount of NoV genogroup II (GII) in wastewater peaked 2 to 3 weeks before the infection spread in hospital wards and nursing homes (Hellmér et al., 2014). The concentration data of NoV GII in raw wastewater collected over the entire study period (i.e., before, during, and after the outbreak) ranged from 10^4 to 10^5 genome copies (GC)/L (unadjusted for recovery) and 10^5 to 10^6 GC/L (adjusted for recovery). The results from Hellmér et al. (2014) generally correspond with other investigations reporting concentrations ranging from 10^1 to 10^9 GC/L for NoV genogroup I (GI) and from 10^4 to 10^7 GC/L for NoV GII (da Silva et al., 2007; Hellmér et al., 2014; Flannery et al., 2012; Victoria et al., 2010; Seto et al., accepted). It is possible that the other references also captured outbreak conditions given the range of concentrations. Notably, no attempt was made to normalize the detection methods used in these different studies. Furthermore, the general observation that the concentrations of NoV GII are greater than NoV GI appears to be consistent with the results of other noted investigations.

Barker et al. (2013) conducted a QMRA using NoV, *Giardia,* and *Campylobacter* as reference pathogens to determine the level of treatment required to meet the tolerable annual disease burden of 10^{-6} DALYs per person per year (similar to 10^{-4} annual infection per person year) for a small remote community in Antarctica. The researchers evaluated and compared two scenarios, one relying on published municipal

wastewater pathogen loads and the second based on estimating pathogen loads that might occur during a gastroenteritis outbreak. For the municipal wastewater scenario, estimated required LRVs would be for 6.9 for norovirus, 8.0 for *Giardia,* and 7.4 for *Campylobacter*. For the outbreak scenario, the LRVs would be 12.1 for norovirus, 10.4 for *Giardia*, and 12.3 for *Campylobacter* (95th percentiles) (Barker et.al, 2013). Under outbreak conditions, pathogen concentrations were noted to be higher as a function of the relatively greater degree of contact between community members in a small population. The researchers concluded that while the estimates of outbreak conditions may overestimate wastewater concentrations of pathogens, the results suggest that for small communities, additional treatment barriers might be required for outbreak conditions as compared to non-outbreak conditions, as expected. The LRVs determined to be necessary for an outbreak (12.1 for virus and 10.4 for *Giardia*), however, are still about the same as those used currently by the State of California for IPR.

Finally, based on this one key paper (Hellmér et al., 2014), it appears that the range of norovirus concentrations during an outbreak fall within the overall range of concentrations reported in the literature for raw wastewater. In larger community wastewater systems, it is likely that larger flow volumes will dampen pathogen loads from localized outbreaks; however, pathogen concentrations in raw wastewater may increase as wastewater volumes decrease due to water conservation measures.

As discussed later in **Section 2-3**, a future research project should be routine pathogen monitoring in raw wastewater to capture and record concentrations during outbreaks. For example, the City of Milwaukee (in collaboration with the local health department and water and sewerage agencies) conducted monthly monitoring of total culturable viruses in raw and treated wastewater, as well as two drinking water sources, for a 9-year period (Sedmak et al., 2005).

2.2 Overview of Chemicals of Concern

Numerous potential chemical constituents in DPR sources (Anderson et al., 2010; NWRI, 2013) may have adverse health effects if they survive typical treatment processes and are found in finished drinking water at sufficient concentrations. Notably, advanced water treatment technologies capable of producing advanced treated water that meets all drinking water standards have been demonstrated in numerous investigations and full-scale AWTFs. In general, the advanced treated water is of higher quality than most conventional treated drinking waters with respect to total organic carbon (TOC) and total dissolved solids (TDS), as well as trace constituents.

The microbial and chemical treatment standards for providing public drinking water and assessing raw water supplies developed under the Safe Drinking Water Act provide a comprehensive basis for evaluating both the (1) acceptability of finished drinking water and (2) protection of sources of water supply. Typical drinking water treatment processes (e.g., chemical coagulation, sedimentation, filtration, and disinfection) have been effective at limiting the levels of microbial and chemical constituents in finished drinking water. The best management approach would be to ensure that appropriate multiple barrier technologies – conceptually similar to the multiple-barrier risk reduction approach of the Safe Drinking Water Act – are installed and operated to specification (i.e., an optimized and reliable treatment system) and key constituents are identified to verify the performance of these technologies. Federal maximum contaminant levels (MCLs) have been developed taking into account the opportunity for meaningful national public health risk reduction. In the case of DPR systems, the potential risk would be to smaller populations; therefore, a focused effort is warranted to identify additional compounds of concern.

Numerous sources of health-based benchmarks exist for chemicals, in addition to the drinking water standards that can be used if unregulated constituents are detected. Some of these sources include the WHO *Guidelines for Drinking Water Quality* (WHO, 2011), U.S. EPA's Drinking Water Health Advisories (U.S. EPA, 2012, 2015b), and U.S. EPA's "human health benchmarks for pesticides" in drinking water (U.S. EPA, 2015c). Detailed risk assessments, including the use of "Margins of Exposure" (MOEs) applied to scientifically defensible points of departure, can be applied to chemicals to arrive at figures that are essentially as protective to health as MCLs, PHGs, or HAs. These methodologies can be used to arrive at health-based benchmark values.

"Thresholds of Toxicological Concern," (TTC) and the related Threshold of Regulation (TOR) are approaches that can be used as means of assessing whether small concentrations can be neglected, but this approach is applied only to chemicals for which there are no data and excludes chemicals in classes with particularly high potency (e.g., nitrosamines, dibenzofurans/dioxins and polychlorinated biphenyls, azoxy compounds, aromatic amines and nitrates, azo compounds, highly chlorinated compounds, hydrazines, α-nitrofuryl compounds, steroids, strained ring structures, and vinyl compounds [Cheeseman et al., 1999]). Some improvements in this methodology that go beyond the Cramer classification scheme address various toxicological endpoints with more specificity, which might be useful for identifying health-based benchmark levels (see the various procedures introduced into Toxtree at EURL ECVAM [2016]).[16] These predictions are based on formal rulebases, but generally are restricted to well-studied classes of chemicals (see the Benigni/Bossa rulebase of mutagenicity and carcinogenicity, a module of Toxtree[17] [Benigni et al., 2008]).

2.3 Managing Potential Public Health Risks

DPR as a source of drinking water supply would be subject to existing drinking water quality requirements in California derived from the Safe Drinking Water Act, as well as California-specific potable reuse regulations. Federal regulations like the Safe Drinking Water Act are considered in **Sections 2.3.1** and **2.3.2**, while a brief discussion of Source Water Protection Programs is included in **Section 2.3.3.**

2.3.1 Safe Drinking Water Act

The Safe Drinking Water Act is the federal law that establishes the minimum quality standards for drinking water in the United States. Passed by Congress in 1974, the Safe Drinking Water Act replaced existing Public Health Service standards to provide a new overarching regulatory framework and to vest the USEPA with oversight of the law's implementation. Amended in 1986 and 1996, the law also requires a number of actions to protect drinking water and its sources: rivers, lakes, reservoirs, springs, and groundwater. The 1996 amendments enhanced the existing law by recognizing source water protection, operator training, funding for water system improvements, and public information as important components of safe drinking water. This approach was designed to ensure the quality of drinking water by protecting it from ***source to tap***. Importantly, the decision to regulate under the Safe Drinking Water Act involves careful analysis to weigh the public health benefits of requiring the control of contaminants with the costs associated with imposing such controls.

[16] https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxtree (last accessed July 7, 2016).

[17] https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/doc/EUR_23241_EN.pdf (last accessed July 7, 2016).

Under the Safe Drinking Water Act, the USEPA sets national health-based standards for drinking water to protect against both naturally occurring and man-made constituents that may be found in drinking water. Subsequent implementation is through California state primacy, with oversight by the public water systems that implement these standards. State drinking water regulations must be at least as stringent as national drinking water regulations.

2.3.2 National Drinking Water Regulations

Regulations of the Safe Drinking Water Act include primary and secondary drinking water standards (USEPA, 2015a). Primary standards, known as Primary MCLs, are established for contaminants that may pose a health risk when present in drinking water supplies and are known or anticipated to occur in public water systems. As enforceable maximum permissible levels of regulated constituents in drinking water, MCLs are set at concentrations that are as close as possible to levels that are not anticipated to have public health consequences with a margin of safety (known as Maximum Contaminant Level Goals).

In setting MCLs, the USEPA takes into account the availability of treatment options, suitable analytical methods, and the costs of control measures. "Treatment technique" requirements are established for constituents in which control is important to public health, but monitoring is not feasible technically and economically to set MCLs. Requirements for both MCLs and treatment techniques include minimum monitoring reflective of the nature of the health risk and effectiveness of the control measures.

Secondary standards, known as Secondary Maximum Contaminant Levels (SMCLs), are established for constituents that have aesthetic effects. SMCLs are not enforceable under federal law, but some states (e.g., California have incorporated them into their enforceable standards (USEPA, 2015b).

2.3.3 Managing Risk from Source to Tap

The concept of multiple barriers has been a design consideration in drinking water treatment facilities (DWTFs) for more than half a century. It now formally includes the protection of water supply sources. In the 1996 amendments to the Safe Drinking Water Act, provisions were included to require a deliberate assessment of water supply sources for hazards so that additional risk mitigation measures could be taken. Currently, Source Water Protection Programs include the following tasks:

- Risk identification (i.e., delineation and source inventories).
- Risk ranking and screening (i.e., susceptibility analyses).
- Risk management measures (prevention programs).
- Preparation for unexpected drinking water supply replacement emergencies (i.e., contingency planning).

Four fundamental elements of Source Water Protection Programs (USEPA, 2015c) are listed in **Table 2-3**.

Table 2-3: Fundamental Elements of Source Water Protection Programs

Barrier	Description
Risk prevention	The best approach to protect drinking water is to keep constituents from entering source water.
Risk management	The public water system is the first line of defense to reduce or eliminate constituents in source water. The Safe Drinking Water Act, which regulates these systems, develops standards and guidance to help public water systems reach the goal of providing safe and reliable drinking water. Public water systems must collect and treat water, hire trained and qualified operators, and have an emergency response plan in case of a natural disaster or terrorist attack.
Risk monitoring and compliance	Dealing effectively with risks to drinking water requires the constant evaluation of water quality. Water is monitored in one or more locations: at the (1) source; (2) treatment plant, after it has been treated and disinfected; (3) drinking water supply distribution system, which delivers water through pumps and pipes to homes; and (4) (in some cases) the consumer's tap (though it is not regulated at all of these locations).
Individual action	What occurs in the watershed can directly impact the quality of water that arrives at the treatment plant. The more the public knows about their drinking water, the better equipped they are to protect it.

Source: USEPA (2015c).

The success of the Source Water Protection Program is assessed by conducting sanitary surveys on a routine basis to prevent the contamination of drinking water supplies (i.e., both source water and finished drinking water). Furthermore, sanitary surveys provide an opportunity to work and communicate with water system personnel in a preventative mode. The USEPA has defined a sanitary survey as:

> *"…an onsite review of the water source (identifying sources of contamination using results of source water assessments where available), facilities, equipment, operation, maintenance and monitoring compliance of a public water system to evaluate the adequacy of the system, its sources and operations and the distribution of safe drinking water."* (40 CFR 141.2)

The seamless integration of Source Water Protection Program principles and the elements of a POTW source control program, as discussed in **Chapter 8**, are necessary for a successful DPR program.

2.4 Findings of the Expert Panel

In regards to the potential hazards of potable reuse, the Expert Panel concludes the following:

- **Finding #2-1:** Overall, the current approach by the State Water Board for assigning LRV credits for individual unit treatment processes as part of IPR projects is feasible for DPR projects; however, additional process monitoring is recommended to ensure reliable treatment. Also, a probabilistic approach could be used to establish the basis from which to measure overall DPR plant performance with DPR criteria. In addition, future research may be necessary to provide evidence for assigning higher credits in some cases (e.g., reverse osmosis, chlorination).

- **Finding #2-2:** Based on the review of available limited information, it appears that the range of norovirus concentrations during an outbreak fall within the overall range of concentrations reported in literature for raw wastewater. In larger community wastewater systems, it is likely that larger flow volumes will dampen pathogen loads from localized outbreaks; however, pathogen concentrations in raw wastewater may increase as wastewater volumes decrease due to water conservation measures.

2.5 Recommendations of the Expert Panel

The Expert Panel has the following general recommendations, which are not listed in preferential order, for the State Water Board:

- **Recommendation #2-1:** Given the large LRVs likely to be assigned to UV/AOP reactors (i.e., up to 6-$\log_{10}$ removal for all three pathogens), it is important to provide evidence of excellent reactor hydraulics to ensure that short-circuiting does not compromise the efficiency of disinfection. This information should be included as part of the DPR project's engineering report.

- **Recommendation #2-2:** The data for assigning LRVs to each unit process and the total LRV credits for each AWTF should be presented in the DPR project's engineering report.

2.6 Research Recommendations of the Expert Panel

The Expert Panel recommends the following research activities be pursued by the State Water Board. These recommendations are not listed in preferential order.

- **Research Recommendation #2-1:** To better inform decisions associated with updating LRVs, as well as conducting probabilistic-based QMRA modeling, the State Water Board should include monitoring requirements in regulatory permits to measure pathogens (i.e., *Giardia* cysts, *Cryptosporidium* oocysts, and several human viruses) in the raw (untreated) wastewater feeding a DPR system to provide more complete information on concentrations and variabilities. Improved methods should be used that will allow for the better characterization and improved precision of measuring concentrations of pathogens. Note this recommendation also is listed in **Chapter 8** (see **Research Recommendation #8-2**). In addition, see **Chapters 6** and **8** for more information**.**

- **Research Recommendation #2-2**: The State Water Board should investigate the feasibility of collecting pathogen concentration data for raw wastewater associated with community outbreaks of disease and collect such data where possible. See **Chapters 6** and **8** for more information.

2.7 References

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PART II: MONITORING POTENTIAL HAZARDS

CHAPTER 3: Public Health Surveillance Tools and Methods

- Public health surveillance tools and methods.
- Elements of a public health surveillance system.
- Waterborne disease surveillance.
- Potential applications in potable reuse systems.

For this chapter, the Expert Panel examined: (1) the role of public health surveillance; (2) how it is distinguished from epidemiology studies; and (3) the potential role for both in waterborne disease risk characterization for potable reuse applications. Acceptable or tolerable risks of 10^{-4} to 10^{-6} per year are beyond the statistical power of even the largest epidemiology studies to measure and quantify with accuracy, and risk is appropriately quantified and managed by risk assessment and the associated $\log_{10}$ reduction credits provided by various treatment processes; however, as emphasized in NRC (1998), public health surveillance will play an important role in communities receiving drinking water treated by direct potable reuse (DPR) or indirect potable reuse (IPR).

As noted in NRC (1998):

> "Every community using reclaimed waters as drinking water should implement well-coordinated public health surveillance systems to document and possibly provide early warning of any adverse health events associated with the ingestion of reclaimed water…."

An additional recommendation was:

> "…epidemiologic studies should be conducted at the national level using alternative study designs and more sophisticated methods of exposure assessment and outcome measurement to evaluate the potential health risks associated with reclaimed water…" (NRC, 1998).

More recently, Rodriguez et al. (2009) included similar recommendations in regard to IPR with some modification (emphasis added):

> "Regulators approving IPR projects need to implement a well-coordinated public health surveillance system to document possible warning signs of any adverse health events associated with the ingestion of recycled water. ***Existing surveillance systems, such as those for notifiable communicable diseases, should be used and/or enhanced to meet these needs. Surveillance systems must be jointly planned and operated by health departments, water utilities, and other relevant agencies. Key individuals in each agency need to be appointed to coordinate planning and rehearse emergency procedures. The surveillance plan, its purpose, the monitoring results, and the system process performance should be available to the community and interested stakeholders.*** Surveillance systems may indicate whether an epidemiological study is required. However, epidemiological surveillance is considered relatively slow and is reactive as it is based on disease outcomes."

Due to the increasing availability and accessibility of large health datasets, it is expected that well planned or *ad hoc* epidemiology studies will be conducted in these communities. With this expectation in mind, the goals of this chapter include:

- Describe the different types of public health surveillance.
- Identify sources of available public health surveillance data.
- Describe waterborne disease surveillance and discuss data sources and limitations.
- Provide guidance for interpreting the results of epidemiological studies.
- Discuss potential applications for public health surveillance in the context of DPR.
- Describe epidemiological study designs used to study waterborne disease and their potential applications to DPR.

3.1 Public Health Surveillance

Public health surveillance is defined as the "ongoing systematic collection, analysis, and interpretation of outcome-specific health data for use in the planning, implementation, and evaluation of public health practice" (Thacker and Berkelman, 1988) or, alternatively, the "ongoing systematic collection, analysis, and interpretation of outcome-specific health data for the purpose of preventing and controlling disease, injury, and other health problems" (Stroup et al., 2003). It is distinct from the "surveillance" of individuals with serious communicable diseases (such as smallpox or Ebola) to implement isolation measures, but rather focuses on trends in populations (Thacker, 2010). Recently, public health surveillance has expanded to focus not only on health outcomes, but also on other determinants of health (e.g., health behaviors, health care, and physical environment). As a result, more recent definitions replace "outcome-specific" health data with "health-related data" and emphasize that public health surveillance must be "closely integrated with the timely dissemination of these data to those who need to know" (Thacker, 2010). Public health surveillance has the following purposes, as described by the World Bank (Garcia-Abreau and Halperin, 2002):

- Recognize cases or clusters of cases to trigger interventions to prevent transmission or reduce morbidity and mortality.
- Assess the public health impact of health events or determine and measure trends.
- Identify the need for public health intervention programs and resources, and allocate resources during public health planning.
- Monitor the effectiveness of prevention and control measures and intervention strategies.
- Identify high-risk population groups or geographic areas to target interventions and guide analytical studies.
- Develop hypotheses that lead to analytical studies about risk factors for disease causation, propagation, or progression.

Although there are many variations of public health surveillance, there are two general types: one type tracks long-term trends in population health, and the other provides a warning system of potential adverse events. A public health surveillance system could serve both purposes, but an early warning

system will require different data and expertise than a program designed to track long-term trends. Information obtained from public health surveillance is used to guide public health departments in providing services to people, including education and planning, and to inform and evaluate public health programs. On its own, surveillance data usually lack sufficient detail and resolution for in-depth epidemiologic evaluations, but can be used to identify trends that require formal investigation and follow up (Buehler, 2008). By providing information on the natural history of a disease and its occurrence among different age groups, geographical areas, and demographic groups, public health surveillance can inform the descriptive epidemiology of a disease or condition.

Public health surveillance is distinct from formal analytical epidemiologic research, although surveillance data can be used to inform and develop epidemiological studies. Whereas surveillance information can be used to identify research, service, and training needs, surveillance does not encompass epidemiologic research studies that are "related but independent public health activities that might not be based on surveillance" (Thacker, 2010). Public health surveillance is descriptive in nature and describes the occurrence of disease and its determinants in the population. Analytical epidemiology studies use a formal comparison group to test hypotheses regarding potential causes and risk factors associated with disease risk and transmission (Choi, 2012). Surveillance data usually are limited in detail and relatively inexpensive to obtain, whereas research data often are detailed and expensive to obtain. Additional distinctions between public health surveillance and epidemiologic research are shown in **Table 3-1** (Thacker and Gregg, 1996).

Table 3-1: Differences Between Public Health Surveillance and Epidemiology Research[a]

	Surveillance	**Epidemiology Research**
Purpose	Detection and description Suggest hypotheses Monitor trends Early warning	Hypothesis testing
Frequency	Ongoing	Time limited
Methods	Routine	Specifically tailored for study
Data analysis	Simple and descriptive	Often complex
Dissemination of information	Timely, regular, and targeted to public health agencies	Not timely, infrequent, and targeted to academics and a clinical audience
Case definition	May be broad	Typically, should be specific

[a] Source: Thacker and Gregg (1996).

Despite the limitations of public health surveillance data, it can be used in epidemiological analyses. When other appropriate data sources are considered in the context of a well-planned epidemiology study, public health surveillance data can provide important insights regarding the determinants of disease at the population level. For example, in a widely cited and influential study, Pope et al. (2004) used mortality surveillance data to demonstrate an association between cardiovascular mortality and fine particulate matter. The success of studies relying on surveillance data depend on integrating other

appropriate data sources, conducting appropriate and well-designed analyses, recognizing the limitations of available data, and developing hypotheses that are grounded in and supported by plausible biological and physiological mechanisms.

The earliest types of public health surveillance focused on vital records, such as "death reports," beginning in the late 1600s (Buehler, 2008). In the mid-1900s, public health surveillance focused on local reports of communicable diseases to enable prevention and control activities and then transitioned into broader national systems for tracking infectious disease. More recently, public health surveillance has extended beyond infectious diseases to include health-risk factors, chronic diseases (e.g., cancer and cardiovascular disease), birth defects, and occupational illnesses.

In the United States, the Centers for Disease Control and Prevention (CDC) is the lead national agency for public health surveillance and disseminates regular summaries for a wide range of surveillance activities in the Morbidity and Mortality Weekly Report (MMWR).

3.1.1 Types and Examples

Some examples of public health surveillance systems are shown in **Table 3-2**. More information on the various types of surveillance systems is included in **Appendix 3A**, and more information on epidemiological studies is included in **Appendices 3B** and **3C**.

3.1.2 Passive Versus Active Surveillance

Passive and active surveillance systems are distinguished by the way the surveillance is conducted. In a passive surveillance system, the organization conducting the surveillance relies on health care providers and others in the health industry to report the occurrence of a disease. An example of a primarily passive surveillance system is the CDC's National Notifiable Diseases Surveillance System (NNDSS), which relies on local health authorities to report notifiable diseases. Passive surveillance systems are relatively inexpensive and can cover large areas, but because passive surveillance depends on people in different institutions to provide data, the quality and timeliness of data are difficult to control. Active surveillance systems involve regular outreach, and the organization conducting the surveillance initiates procedures to obtain reports and data, such as regular phone calls or contact with physicians and hospitals (Choi, 2012). The CDC's Emerging Infections Program (EIP) is an example of an active surveillance program that includes the Foodborne Diseases Active Surveillance Network and Active Bacterial Core Surveillance. Active surveillance provides the most accurate and timely information, but is expensive.

3.1.3 Elements of a Public Health Surveillance System

In planning and implementing a public health surveillance system, the following should be considered:

- Purpose and/or statement of the problem.
- Case definition.
- Population under surveillance.
- Established reporting procedures.

Table 3-2: Examples of Public Health Surveillance Systems

System	Agency	Outcome/Endpoint	Notes
Surveillance, Epidemiology and End Results (SEER)	National Cancer Institute/Centers for Disease Control and Prevention (CDC)	Cancer	http://seer.cancer.gov/
National Vital Statistics System	National Center for Health Statistics (of the CDC)	Birth, death, fetal death	http://www.cdc.gov/nchs/nvss/
National Health and Nutrition Examination Survey (NHANES)	CDC	General health status	http://www.cdc.gov/nchs/nhanes/
Behavioral Risk Factor Surveillance System (BRFSS)	CDC	Health related risk behaviors, chronic health conditions	http://www.cdc.gov/brfss/
Emerging Infections Program (EIP)	CDC	FoodNet, Influenza, Active Bacterial Core Surveillance	http://www.cdc.gov/ncezid/dpei/eip/
National Notifiable Disease Surveillance System (NNDSS)	CDC/Council of State and Territorial Epidemiologists	Range of health outcomes	https://wwwn.cdc.gov/nndss/
California Birth Defects Monitoring Program	California Department of Public Health	Population based registry on birth defects, stillbirths, miscarriages	http://www.cdph.ca.gov/programs/CBDMP/Pages/default.aspx
National Outbreak Reporting System (NORS)	CDC	Outbreaks of acute gastrointestinal infections, as well as waterborne outbreaks of non-enteric disease	http://www.cdc.gov/nors/about.html
Waterborne disease outbreak reporting system (WBDOSS)	CDC	Outbreaks a reported to NORS determined to be waterborne	http://www.cdc.gov/healthywater/statistics/wbdoss/nors/
National Syndromic Surveillance Program	CDC	Bioterrorism related events	http://www.cdc.gov/nssp/
National Hospital Care Survey	CDC	Tracking of trends in hospital care	http://www.cdc.gov/nchs/nhcs/index.htm
California Occupational Health and Surveillance Program	California Department of Public Health	Track work related injuries	http://www.cdph.ca.gov/programs/ohsep/Pages/default.aspx
Los Angeles County Automated Disease Surveillance Section	Los Angeles County	Syndromic surveillance	http://www.publichealth.lacounty.gov/acd/ADSS.htm

Elements of a public health surveillance system (Garcia-Abreau and Halperin, 2002) include:

- Detection and notification of a health event.
- Investigation and confirmation.
- Collection of data.
- Analysis and interpretation of data.
- Feedback and dissemination of results.
- Response (e.g., a link to public health programs, specific actions for prevention and control).

Data sources for public health surveillance (Choi, 2012) include:

- Health surveys (e.g., NHANES; see **Table 3-2**).
- Administrative data (vital records, hospitalization).
- Mandatory reports (e.g., NNDSS; see **Table 3-2**).
- Voluntary reports (e.g., adverse outcomes due to drugs).
- Special or targeted groups (e.g., people with HIV/AIDS).

3.2 Waterborne Disease Surveillance

Routinely collected public health surveillance data have played a long, if sometimes controversial, role in the management of drinking water and assessment of waterborne risks. In 1854, a sharp increase of cholera cases in London led John Snow to conduct an epidemiologic investigation implicating sewage contamination of drinking water sources (Johnson, 2006). Elevated cancer mortality in and around New Orleans in communities receiving water from the Mississippi River as compared to communities receiving groundwater were important in the development of the original Safe Drinking Water Act, which was enacted soon after these reports were first published in 1974 (Page et al., 1976). Occasionally, public health surveillance and/or reports from clinicians serve as actionable warnings of acute failures in water treatment. In Milwaukee, one of the first indications that drinking water was contaminated by *Cryptosporidium* was an increase in school absences and shortages of anti-diarrheal medications (Hrudey and Hrudey, 1995; MacKenzie et al., 1995; Morris et al., 1998). Arguably, these situations are rare examples and are not likely to be representative of a well-run DPR system, where the water will be of extremely high quality; nevertheless, for a DPR system in which a failure could result in serious and wide-ranging health consequences, these examples highlight the (1) need for interaction between water utilities and health officials and (2) awareness of the potential uses and limitations of routinely collected health surveillance data.

Surveillance for waterborne disease presents some challenges. One major concern is the lack of specificity in the health endpoints associated with waterborne disease. For example, most of the pathogens responsible for acute infectious disease also are transmitted by food, person-to-person contact, or contact with contaminated objects through the fecal-oral route of transmission. Indeed, at the population level, water may represent only a small, infrequent pathway for some potential waterborne pathogens, which are predominantly transmitted by food or person-to-person contact (e.g., *Campylobacter, Salmonella*, and norovirus). Surveillance programs that target these infections may not

provide much value in terms of "waterborne" disease surveillance. Similarly, there are challenges in the interpretation of syndromic surveillance programs that focus on indicators or targets for non-specific health endpoints commonly associated with waterborne disease, like acute gastrointestinal infections. In the United States, acute gastrointestinal infections resulting from all sources (e.g., water, food, and person-to-person contact) is thought to range from 0.1 to 3.5 episodes per person per year, depending on the definition used (Roy et al., 2006). Notably, because many enteric infections often are mild and do not result in a visit to a health care provider, surveillance systems focused on laboratory testing data for a specific infection will underestimate the total burden of disease in the population.

The "disease pyramid" (Frost et al., 1996) describes how only a fraction of total cases of waterborne disease are ever detected. In the case of waterborne infection, among those infected, only a fraction are symptomatic, a fraction of those report to a doctor's office, a fraction of those are actually tested for evidence of infection, and fewer still are hospitalized or die. Because many waterborne infections are often mild and asymptomatic, those that actually are observed in a surveillance program represent only a small fraction of all cases.

Serological surveys measure antibodies-based tests in serum or saliva as a biomarker of waterborne and other infections and can be used to monitor the incidence or prevalence of infection in a population. These types of surveys have been used to evaluate the impacts of water treatment and to compare prevalence rates in different communities. Serological surveys target specific waterborne pathogens and potentially could use samples collected for other purposes to monitor trends in the population over time. One potential source of data could be blood banks. Frost et al. (2002, 2003) applied this approach using available samples from blood banks and other routinely collected blood samples to compare serological responses to *Cryptosporidium* in several communities with different sources of drinking water.

3.2.1 Notifiable Waterborne Diseases

As discussed in **Section 3.1.2**, the National Notifiable Disease Surveillance System (NNDSS) compiles reportable disease information reported by states, territories, and other localities. Each state has laws requiring certain diseases be reported at the state level, but it is voluntary for states to provide information or notifications to the CDC at the federal level. Reporting to the CDC is voluntary, while reporting diseases required by individual state law is mandatory. Diseases that are reportable to the State of California include several infections that are primarily waterborne, such as Cryptosporidiosis, Giardiasis, and Legionellosis, and other infections that are partially or potentially waterborne, such as Leptospirosis, Hepatitis A, Campylobacteriosis, Salmonellosis, and typhoid fever (CCR, 2011).

3.2.2 Waterborne Disease Outbreaks

Since 1920, waterborne disease outbreaks have been tracked in some form in the United States. Since 1971, the U.S. Environmental Protection Agency (USEPA), CDC, and Council of State and Territorial Epidemiologists (CSTE) have maintained the Waterborne Disease and Outbreak Surveillance System[18] (Craun et al., 2010). In 2009, the CDC launched an electronic National Outbreak Reporting System[19] to collect reports of enteric diseases outbreaks, as well as waterborne outbreaks of non-enteric disease.

[18] http://www.cdc.gov/healthywater/surveillance/drinking-surveillance-reports.html (last accessed July 7, 2016)

[19] http://www.cdc.gov/nors/about.html (last accessed July 7, 2016).

To be defined as a waterborne disease outbreak, two or more persons must be epidemiologically linked by time, location of water exposure, and case illness characteristics, and the epidemiological evidence must implicate water as the probable source (Beer et al., 2015). Outbreaks are classified according to the strength of evidence in implicating water as the vehicle of transmission based on the strength of the available clinical, epidemiological, and environmental data (Brunkard et al., 2011).

Regular summaries of waterborne disease outbreaks have helped identify trends in waterborne disease transmission patterns and emerging pathogens, as well as provided some measure of the magnitude of the overall impact on health. For example, in a review of data covering 35 years of outbreaks associated with drinking water, Craun et al. (2010) identified several trends, including: (1) the emerging importance of *Legionella* outbreaks and outbreaks associated with premise plumbing; (2) decreased outbreaks in public water utilities; (3) reduction in outbreaks associated with surface water systems following the implementation of the Surface Water Treatment Rule in 1989; and (4) a relative increase in outbreaks associated with untreated groundwater systems (Craun et al., 2010). The increase in outbreaks in groundwater systems helped provide justification for the Groundwater Treatment Rule in 2006.

Information obtained from waterborne disease outbreaks has several limitations and must be interpreted with caution. The reporting of outbreaks varies across states and localities, and is dependent on public health agencies to recognize, report, and investigate outbreaks, as well as the ability of diagnosticians to recognize and confirm particular infectious agents. As a result, the sensitivity of the current surveillance system to detect outbreaks is unknown. In addition, only a small fraction of the total cases of waterborne disease outbreaks are detected, which is when cases exceed a threshold (i.e., epidemic cases) and result in alerts to public health authorities. The persistent low level of disease that does not exceed a reporting threshold is called ***endemic*** disease and, for acute gastrointestinal infection in the United States, is thought to range from 0.1 to 3.5 episodes per person per year (Roy et al., 2006). The total burden of acute gastrointestinal infection attributable to drinking water (including endemic, epidemic, and sporadic cases) has been estimated to be in the range of 4.3-million to 11.7-million cases annually (Colford et al., 2006).

3.3 Potential Applications in Potable Reuse Systems

3.3.1 Public Health Surveillance

In the context of potable reuse applications, the potential roles of public health surveillance include:

- Establish partnerships, engagement, and communication between water utilities and public health partners.
- Identify sources of data to characterize baseline conditions and track trends over time.
- Serve as a warning system of treatment failures and contamination events.

As noted in **Section 3.2**, many waterborne outbreaks have been detected by alert clinicians or public health practitioners noting an increase in cases of waterborne infections like *Giardia* or *Cryptosporidium*. Other outbreaks have been identified through consumer complaints regarding taste, odor, or turbid drinking water (Hrudey and Hrudey, 2004). In the context of DPR, the response time to correct failures and out-of-compliance water will be reduced greatly because the environmental buffer is eliminated. A quick and coordinated response to complaints and any increases in clinical reports is needed to rule out

a water treatment failure or to take corrective action; however, due to the insensitivity of public health surveillance systems to rapidly detect increases in endemic disease, by the time confirmed cases are identified in the population, the optimistic assumption would be that any failure already will have been detected and corrected. If it has not, immediate action will be necessary to prevent additional and widespread waterborne disease. On the other hand, repeated over-reaction to "false-positives" will result in the unnecessary loss of time and resources and a loss of confidence in the surveillance system.

Preliminary statistical power calculations conducted as part of a White Paper titled the "Feasibility of Establishing a Framework for Public Health Monitoring for DPR" (WRRF 14-14; Soller et al., in press) has provided some additional insight regarding the uses and limitations of public health surveillance as an early warning system. Analyses were configured to determine the number of people required to participate in a surveillance program to provide 90-percent certainty in detecting a specified increase in a health outcome (e.g., acute gastrointestinal infection) as significant at the 5-percent level. Power calculations were conducted to determine the required number of people assuming either: (1) surveillance cannot discriminate the DPR-related health outcomes from background health outcomes, or (2) surveillance can discriminate DPR-related health outcomes. Calculations were conducted assuming both normal operations of the DPR system (producing water with a level of microbiological risk that did not exceed the one infection per 10,000 persons per year) and conditions in which treatment processes were not operating in a manner consistent with these criteria ("off-spec" water is being produced). The results of these analyses indicate that the number of people required to participate in a surveillance program to provide 90-percent certainty in the detection of a relatively small change in gastrointestinal illness as significant at the 5-percent level is extremely (and impractically) high (i.e., greater than 3×10^8 population) if the DPR system is operating in a manner that produces water with a risk of microbial infection not greater than one in 10,000 per year. If the DPR system were to exhibit a gross treatment failure, causing treatment effectiveness to be reduced by a factor of ~10,000 (e.g., a catastrophic failure), the number of people required is on the order of $\sim 3 \times 10^3$. These results confirm it is unlikely that public health surveillance would be able to detect a change in annual infection risks of acute gastrointestinal infection of 10^{-4} under normal operating conditions; however, a surveillance program could serve to identify acute failures in treatment, and procedures should be established for the joint utility and public health investigation of surveillance alerts.

The USEPA has developed "A Water Quality Surveillance and Response System," which provides a systematic framework to detect and respond to emerging water quality issues before they become problems. Four aspects are covered, including: (1) Online Water Quality Monitoring; (2) Enhanced Security Monitoring; (3) Customer Complaint Surveillance; and (4) Public Health Surveillance (USEPA, 2015a). The latter two reports, Customer Complaint Surveillance (USEPA, 2015b) and Public Health Surveillance (USEPA, 2015c), are relevant to this chapter and are included in **Appendices 3C** and **3D**.

The USEPA report on Public Health Surveillance (USEPA, 2015c) states "communication between water utilities and public health partners has often been insufficient to provide timely detection and response to waterborne disease outbreaks. Incorporating public health surveillance into a Surveillance and Response System (SRS) helps ensure that data acquisition, analysis, and information sharing is coordinated between the drinking water utility and public health partners, resulting in earlier detection of possible contamination events." This report identifies potential data sources and describes potential partners (as well as exemplary design goals) for a public health surveillance system. The major design elements include:

- Integration of Public Health System Capabilities (e.g., emergency medical services, hospitals, local public health, and poison control).
- Communication and Coordination (i.e., strengthen partnerships, regular engagement, and information sharing).
- Alert Investigation Procedures (i.e., notification and documentation).

The report recommends the following approaches for public health surveillance (USEPA, 2015c):

- Meet with local public health partners to establish relationships, exchange contact information, and learn how these partners could support the detection of and response to contaminated drinking water.
- Evaluate public health surveillance data streams currently monitored by public health partners to determine if the data have the potential to provide the timely detection of contaminated drinking water.
- Establish procedures for the joint utility and public health investigation of public health surveillance alerts that might be indicative of contaminated drinking water.

In addition to these recommendations, a Public Health Assessment Interview Form has been developed that can be used to engage local public health partners (USEPA, 2015d). The responses to these interview questions can be used as a starting point for discussions between the utility and public health partners.

Consumer complaints are another data stream distinct from public health surveillance that also should be monitored to provide alerts to changes in drinking water quality that may be indicative of water treatment failures or problems. The USEPA provides recommendations on tracking these complaints and applying algorithms to identify unusually high call volumes or spatially clustered complaints. In addition, the USEPA provides both a "Threshold Analysis Tool" and an "Alarm Estimation Tool," which can be used to develop thresholds and alerts based on customer service complaints.[20]

Ultimately, for DPR systems, the nature and elements of public health surveillance should be specific to the characteristics of the local public health system, characteristics of the source water and drinking water distribution system, and responsive to local health concerns. At minimum, partnerships should be made with local public health officials, and existing sources of surveillance data should be evaluated using the USEPA reports provided in **Appendices 3C** and **3D** or similar approaches for guidance. Of particular focus for a public health surveillance system should be acute cases of notifiable primarily waterborne diseases, such as Cryptosporidiosis, Giardiasis, and possibly Legionellosis. Other potential waterborne health endpoints (e.g., birth outcomes, cancer, and blood lead surveillance) could be monitored to track trends over time and provide some assurance regarding the safety and quality of the drinking water provided. Tracking trends of waterborne disease over time also may help demonstrate the health benefits of improved water sources and provide public acceptance of DPR and other potable reuse applications. Alternate and novel data sources also should be considered. For example, the use of serology to track trends in *Cryptosporidium* infection or other waterborne infections over time may provide valuable insights into the safety or risks associated with water reuse and provide information for more targeted studies.

[20] See https://www.epa.gov/waterqualitysurveillance/customer-complaint-surveillance-resources (last accessed July 7, 2016).

3.3.2 Epidemiological Studies

Few well-designed epidemiological studies have been conducted for potable reuse systems. Recently, available epidemiological evidence was reviewed in NRC (2012). A range of health endpoints have been studied in a variety of reuse applications, including a DPR system in Namibia, groundwater replenishment in Los Angeles, and a dual-reticulation system in Australia (NRC, 2012). The health endpoints studied include cancer, overall mortality, infectious disease, and respiratory complaints. Overall, no consistently plausible associations were identified, though these studies were mostly *ad hoc* in nature, relied on existing surveillance data, had limited exposure characterization, and were likely underpowered to detect any association.

Epidemiology studies are unable to accurately quantify annual infection risks in the 10^{-4} range, which is the "acceptable risk" targeted by drinking water treatment (see **Chapter 2**). As a result, these studies are unlikely to provide evidence that potable reuse systems are meeting their risk targets. Most epidemiology studies can identify risks only magnitude of orders higher. With a sample size of approximately 600 individuals, Payment et al. (1991) found that 35 percent (or 3,500 cases per 10,000 per person-year) of acute gastrointestinal infections were attributable to drinking water using a randomized intervention design or approximately (though it may be an overestimate due to the lack of blinding of study participants). Colford et al. (2005) also conducted a double-blinded randomized intervention study (N = 456) where no difference in acute gastrointestinal infections was observed between the controls and a group receiving additional water treated with 1-micron filtration and ultraviolet light. A subsequent risk assessment based on raw water concentrations of pathogens in the source water (i.e., water from the Mississippi River) estimated the annual risk of acute gastrointestinal infection to be 13.25 cases per 10,000 persons, whereas the Colford study was powered to detect 1,100 cases of acute gastrointestinal infection per 10,000 persons (Eisenberg et al., 2006). This paper further estimated that to detect an annual risk of acute gastrointestinal infection of 100 cases per 10,000 persons per year, a sample size of 416,000 persons would be necessary.

Because of these limitations, care should be taken to avoid the misuse of poorly designed and under-powered epidemiology studies that provide little evidence for the safety of potable reuse in terms of attaining acceptable risk levels; however, as communities implement DPR systems (especially those serving large populations), there may be a role for well-designed epidemiological research, covering multiple communities, to provide assurance of the safety of potable reuse or to evaluate risk, so long as the limitations and study power are communicated clearly. Theoretically, large well-designed epidemiology studies also could demonstrate the health benefits of DPR in terms of the reduction of acute gastrointestinal infections or other illnesses resulting from the improved quality of drinking water. *Ad hoc* studies that use existing surveillance data and are retrospective, cross-sectional, or ecological in design likely will be conducted by health researchers because they are relatively inexpensive and health and geographic data are becoming widely accessible. These studies should be interpreted cautiously, but some well-designed studies (e.g., before-after natural experiments in large communities) may be used for hypothesis generation and further investigation, although they probably cannot reliably inform risk.

Ideally, any epidemiological research will be conducted in collaboration with water utilities, have a clearly defined causal association and hypotheses, avoid "data dredging" (i.e., conducting a large number of poorly justified analyses and only presenting statistically significant associations), and address issues like misclassification and study power. Epidemiology studies that target infection rather than illness may be less prone to bias and random error, especially if primarily waterborne infections are

targeted. Ongoing public health surveillance activities implemented by communities may provide information for the design of epidemiology studies targeted on a specific health endpoint, which can be used to either demonstrate the safety of the DPR system or to identify it as a potential source of risk.

3.4 Findings of the Expert Panel

The Expert Panel has the concluded the following regarding public health surveillance and epidemiology studies.

3.4.1 Public Health Surveillance

- **Public Health Surveillance Finding #3-1:** Public health surveillance is a valuable tool to track trends in disease over time and to inform public health policy. Public health surveillance can be used to identify trends for further investigation and formal study.

- **Public Health Surveillance Finding #3-2:** Public health surveillance is distinct from analytical epidemiology. Public health surveillance is general and descriptive in nature, whereas epidemiology studies collect more detailed data to test research hypotheses.

- **Public Health Surveillance Finding #3-3:** Existing data sources for waterborne disease surveillance include vital records, nationally notifiable diseases, syndromic surveillance, and waterborne disease outbreaks. Consumer confidence reports may be another data source that can provide insights into changes in drinking water quality.

  Public Health Surveillance Finding #3-4: In the context of potable reuse systems, the local or state public health agency should be encouraged to consider the use of public health surveillance to establish baseline conditions and potentially identify anomalous events that require further action. The agency should recognize that significant resources may be required to investigate false positives.

  Public Health Surveillance Finding #3-5: The USEPA has developed guidance for Public Health Surveillance and Customer Complaint Surveillance for water utilities.

- **Public Health Surveillance Finding #3-6**: The results of the preliminary statistical power calculations conducted as part of a White Paper titled the "Feasibility of Establishing a Framework for Public Health Monitoring for DPR" (WRRF 14-14) indicate that the number of people required to participate in a surveillance program to provide 90-percent certainty in the detection of a relatively small change in acute gastrointestinal illness as significant at the 5-percent level is extremely (and impractically) high (i.e., greater than 3×10^8 population) if the DPR system is operating in a manner that produces water with a risk of microbial infection not greater than one in 10,000 per year. If the DPR system was to exhibit a gross treatment failure, causing treatment effectiveness to be reduced by a factor of ~10,000 (e.g., a catastrophic failure), the number of people required is on the order of ~3×10^3.

3.4.2 Epidemiology

Epidemiology Finding #3-1: Epidemiology studies lack the accuracy and statistical power to measure risk associated with tolerable waterborne disease risks of 10^{-4} to 10^{-6}; however, well-designed epidemiology studies in large populations may be able to bound risk estimates.

Epidemiology Finding #3-2: Epidemiology studies should be designed with a causal model, clearly defined objectives, and consideration toward an appropriate sample size. Systematic or random bias, generalizability, and multiple testing should be considered in interpreting the results of epidemiological studies.

3.5 Recommendations of the Expert Panel on Public Health Surveillance

The role of public health surveillance is to: (1) establish partnerships, engagement, and communication between water utilities and public health partners; (2) identify sources of data to characterize baseline public health conditions and track trends over time; and (3) help determine if transient treatment failures and contamination events lead to adverse health outcomes. As such, the Expert Panel recommends the following for consideration by the State Water Board Resources Control Board (State Water Board). These recommendations are not listed in preferential order.

- **Recommendation #3-1:** Within the context of potable reuse, local public health partners should be informed when a DPR project is being considered. Points of contact should be identified, and available surveillance data sources should be reviewed. In addition, processes for regular engagement, information sharing, and notification should be established, with an emphasis on tracking, reporting, and communicating notifiable acute, primarily waterborne diseases. Refer to the USEPA Public Health Surveillance for Water Quality Surveillance and Response Systems (USEPA, 2015b) for additional information (see **Appendix 3D**).

- **Recommendation #3-2:** The State Water Board should work with DPR project sponsors and local health agencies to consider the feasibility of enhanced public health surveillance for communities with DPR systems. Such efforts may include syndromic surveillance, sentinel surveillance, or serological surveys for waterborne infections.

- **Recommendation #3-3:** Power calculations to detect changes in waterborne diseases under a range of assumptions should be done to help put the findings of epidemiological analyses of public health surveillance in the proper context. In addition, when epidemiological studies are under consideration, power calculations also should be done to help guide the feasibility and design of epidemiology studies.

- **Recommendation #3-4:** Communities that rely on multiple sources for their drinking water supplies and that deliver these types of water into different pressure zones have an opportunity to develop and conduct a pilot public health surveillance effort for a DPR-augmented service area in comparison to a service area receiving conventional supplies.

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CHAPTER 4: ANALYTICAL APPROACHES FOR MEASURING CHEMICAL WATER QUALITY

- Periodic monitoring of chemical contaminants.
- Short-duration releases of chemical contaminants.
- Chemical contaminants that compromise aesthetics.
- Chemical contaminants of commercial or industrial origin.
- Indicator chemicals and surrogate parameters.
- Frequency and location of monitoring.
- Responding to off-specification water.

4.1 Introduction

Concentrations of chemical contaminants are monitored in drinking water to validate that water treatment processes are adequately protective of public health. Chemical monitoring protocols (including the selection of target analytes, frequency of analysis, and necessary sensitivity of analytical methods) are well established for drinking water obtained from sources of surface water and groundwater. Other chemical monitoring approaches have been developed for situations in which municipal wastewater effluent (i.e., the use of impaired water sources and indirect potable reuse [IPR]) accounts for a significant fraction of the source water. The purpose of this chapter is to assess chemical monitoring approaches appropriate for direct potable reuse (DPR) projects.

4.1.1 Interest in Chemical Monitoring

As the State Water Board develops an approach for permitting DPR projects, it will become necessary to determine if proposed DPR chemical monitoring programs will provide the information needed to protect the public from the potential adverse effects of recognized chemical contaminants. Although it is unrealistic to review all possible approaches that could achieve this goal, it was important to the Expert Panel's evaluation of the feasibility of developing criteria for DPR to assess (1) available tools, (2) approaches used in other types of monitoring programs, and (3) issues requiring attention prior to the establishment of regulations. A viable DPR project must have a chemical monitoring program to ensure the treatment process is providing a level of public health protection comparable to or more stringent than monitoring programs currently used in other drinking water applications.

4.1.2 Scope of the Review on Chemical Monitoring

In developing this chapter, the Expert Panel considered existing regulatory requirements, peer-reviewed scientific publications, and information provided by utilities and consultants in the water industry. For those chemicals already included in established regulatory requirements (e.g., chemicals monitored as part of the Safe Drinking Water Act), it was assumed that monitoring requirements would be as strict as or stricter than existing requirements. For other chemicals, the Expert Panel considered information on known occurrence and toxicity, but did not employ risk assessment to prioritize the analysis or establish

threshold values for specific contaminants in drinking water. The Expert Panel also considered the use of indicator compounds and surrogate parameters to monitor system performance, but did not make recommendations about which specific contaminant(s) or parameters (e.g., surrogates and/or indicators) should be monitored.

4.2 Background

Water from DPR projects could contain chemical contaminants that pose human health risks, compromise the aesthetic properties of water (e.g., impact taste and odor), or have other undesirable consequences (e.g., interfere with the maintenance of residual disinfectant in the drinking water distribution system). The ability to detect – in a timely manner – chemical contaminants at concentrations that compromise water quality is critical to the assessment of treatment performance and water quality assurance, as well as to ensure these chemicals do not present a health hazard. Irrespective of the source of water, certain chemical contaminants are monitored routinely in drinking water; therefore, DPR projects will need to meet these requirements, plus additional monitoring requirements specific to the characteristics of the DPR process.

In general, the operators of conventional drinking water treatment facilities (DWTFs) monitor a suite of regulated chemical contaminants and water quality parameters at prescribed intervals (e.g., quarterly sampling) and locations (e.g., after the final stage of treatment, in the drinking water distribution system), as specified by the Safe Drinking Water Act and more stringent conditions established by the State of California (CDPH, 2010). IPR projects in California (CDPH, 2014) often are required to monitor the same set of regulated chemical contaminants, plus an additional set of chemicals that are known or suspected to be present in recycled water (e.g., NDMA and 1,4-dioxane).

The operators of advanced water treatment facilities (AWTFs) and conventional DWTFs engaged in potable reuse typically employ sensors to continuously monitor water quality parameters (e.g., residual chlorine, turbidity, conductivity). These data are used to alert operators of process upsets, fluctuations in the composition of incoming water, or changes in the performance of a treatment process. Although these data are not always considered part of chemical contaminant monitoring plans, they would be important to the oversight of DPR systems. The use of this information also could ensure performance reliability, as discussed in **Chapter 8**.

In addition to monitoring a set of chemical contaminants already included in monitoring plans for drinking water and IPR, it is appropriate to include additional chemical contaminants and monitoring approaches for DPR projects. One important difference between DPR and other approaches to providing drinking water is the absence of an environmental buffer that meets IPR requirements established by the State of California. The absence of this buffer could expose consumers of water from DPR systems to chemicals originating from industrial or commercial operations for short periods if relatively large quantities of these chemicals are discharged into wastewater collection systems over short periods. Given the concerns expressed by members of the public about the potential presence of yet-to-be-discovered chemical contaminants in municipal wastewater, it also may be appropriate to use non-targeted monitoring techniques to screen for yet-to-be-discovered contaminants when municipal wastewater is used as a source of water for potable reuse projects. The inclusion of methods to detect such chemicals could enhance public confidence in the DPR treatment process and ensure that water quality would not be compromised if a change occurs with the types and amounts of chemicals entering the wastewater collection system.

Decisions about the safety of water produced by AWTFs will require an appropriate set of chemical monitoring tools that provide accurate and precise data in a timely manner. For chemical monitoring plans for AWTFs, factors such as the following need to be considered: (1) the practicality of the methods used (e.g., whether it will be conducted by utility or commercial laboratories using available equipment); (2) time required to analyze samples; (3) reliability of the methods; and (4) overall costs of implementing the chemical monitoring program. Decisions to include expensive or complex analyses will need to be balanced against costs and the value of the data produced.

4.3 Elements of Chemical Monitoring Programs

Chemical monitoring programs for DPR projects should consist of three main elements:

- A list of chemical contaminants and performance surrogate parameters to be monitored.
- Specifications about the frequency, locations, and methods used for monitoring.
- A plan for how treatment plant operators will respond when monitoring data indicate that one or more chemical contaminants or surrogate parameters exceed a predetermined value (e.g., initiating additional monitoring efforts or shutdown procedures when high concentrations of a contaminant are detected).

All three aspects of chemical monitoring plans are discussed in the following sections, with an emphasis on monitoring needs specific to DPR projects.

4.3.1 Chemical Contaminants

Chemicals contaminants are included in water quality monitoring programs for a variety of reasons. The primary objective of a chemical monitoring program is to ensure the public is not exposed to concentrations of chemical contaminants that pose an unacceptable health risk; therefore, contaminants of human health concern known to be present in wastewater or water produced by AWTFs must be included in chemical monitoring plans. Other contaminants that could compromise water quality (e.g., by affecting the appearance, smell, or taste of water) also need to be monitored to ensure consumer confidence in the water supply. To assess the performance of the treatment system, the monitoring plan also may include indicator compounds or water quality surrogates. A complimentary strategy for assessing contaminants that could pose potential human health risks involves the use of screening techniques designed to detect chemicals not already included in routine chemical monitoring programs. Each of these strategies is summarized in **Sections 4.3.1.1** to **4.3.1.5**.

4.3.1.1 Periodic Monitoring of Chemical Contaminants

At minimum, chemical monitoring programs for DPR systems need to include regulated drinking water contaminants, which normally are monitored through the collection of composite or grab samples at predetermined time intervals (e.g., quarterly, annually) and analysis with standardized procedures. These chemicals fall into two categories: primary and secondary drinking water contaminants.

- **Primary drinking water standards** (see **Appendix 4A**) are included in drinking water monitoring plans because regulators have determined these chemicals are likely to occur in drinking water at concentrations posing unacceptable human health risks at frequencies high enough to merit routine monitoring, even if there is no specific evidence that the water is contaminated with

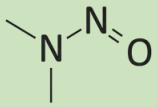
these compounds. Based on similar logic, chemical contaminants likely to be present at concentrations of concern in municipal wastewater effluent should be monitored in potable reuse projects.

- **Secondary drinking water standards** (see **Appendix 4A**) include chemicals that could compromise the use of the water. For example, in drinking water, elevated concentrations of iron – resulting either from its presence in source water or the corrosion of water distribution pipes – can stain household water fixtures and clothing. Similarly, elevated concentrations of dissolved ions (i.e., concentrations of total dissolved solids exceeding the secondary standard of 500 mg/L) tend to elicit negative responses from the public due to aesthetic issues (i.e., taste, stains on water fixtures). The State Water Board is strongly encouraged to include the monitoring of secondary drinking water standards in DPR projects, as well as the development of a program to explain the potential implications of any excursions above secondary drinking water standards to consumers who receive water from DPR projects.

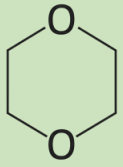
Examples of Compounds of Interest for Direct Potable Reuse

Among the over 100 compounds listed in **Tables 4A-1** and **4A-23** (primary and secondary drinking water standards, see **Appendix 4A**), several are worth noting with respect to chemical monitoring plans for direct potable reuse systems due to their frequency of occurrence in municipal wastewater effluent or water produced by advanced water treatment facilities. These compounds include:

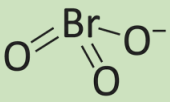
N-NITROSODIMETHYLAMINE (NDMA) often is present at elevated concentrations in wastewater from certain industries (e.g., chemicals used for the treatment of metal plating baths; Sedlak et al., 2005). It also is produced during the chloramination of municipal wastewater effluent (Mitch and Sedlak, 2004) and ozonation of surface water contaminated with pesticides (Schmidt and Brauch, 2008; Chen and Young, 2008) or industrial chemicals (Kosaka et al., 2009).



1,4-DIOXANE is a compound used as a stabilizer for halogenated solvents (Mohr, 2010). It also is used to manufacture inks and adhesives.



BROMATE is formed when ozone is used to disinfect water, especially if the water contains elevated concentrations of bromide (von Gunten, 2003). In many locations in California, drinking water and wastewater effluent contain relatively high concentrations of bromide, which results in the formation of high concentrations of bromate when ozone is used. The presence of ammonia (e.g., in wastewater that has not been nitrified) tends to decrease the formation of bromate.

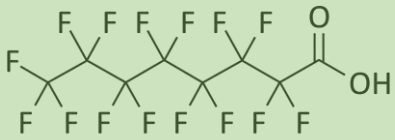


The State Water Board has identified a group of chemical contaminants of concern for which the federal government has not established maximum contaminant levels (MCLs). These contaminants are monitored because California's regulators have encountered situations under which these contaminants are present in source waters or finished drinking water at concentrations that pose unacceptable health risks (CDPH, 2010). Monitoring programs for drinking water and IPR in California frequently include notification levels for these unregulated contaminants **(Appendix 4A**). When concentrations of contaminants in finished drinking water exceed the specified notification levels, a process is initiated in which the State Water Board recommends the drinking water purveyor notify the community. At higher concentrations (e.g., typically at concentrations 10 times higher than the notification levels), the State Water Board requests that the purveyor stop delivering water to consumers and initiate a series of actions to address the issue. The Expert Panel believes this approach is reasonable and balances the need to protect public health with the resources required for compliance monitoring. The State Water Board should allocate resources to continue its practice of conducting research and performing occurrence surveys for the purpose of identifying new chemical contaminants to include in water produced by potable reuse projects.

Additional Compounds of Interest

There also are some compounds of concern for DPR projects that are not included in **Tables 4A-1** or **4A-2** in **Appendix 4A**, for example:

PERFLUOROOCTANOIC ACID (PFOA) is a compound that is present or formed from the breakdown of chemicals used in the production of stain-resistant coatings, fluorinated surfactants, and other specialty chemicals. As a result of its use in consumer products and industrial operations, PFOA and other poly- and per-fluoalky substances often are present in municipal wastewater effluent (Schultz et al., 2006). These compounds are removed by reverse osmosis (Tang et al., 2007), but are nearly impossible to remove by advanced oxidation and other chemical treatment methods (Vecitis et al., 2009).



With respect to the challenge of identifying new chemical contaminants to include in monitoring programs for potable reuse projects, California continues to be the worldwide leader. The Expert Panel is unaware of any efforts by other state regulators or federal agencies that have resulted in the identification of chemical contaminants to monitor that have not already been considered by the State Water Board. Internationally, regulators look to California for guidance on the issue of identifying chemical contaminants to monitor. One possible exception is the approach taken in Australia.

In 2008, Australia's regulatory authority developed national guidelines for IPR that included a list of chemicals to monitor to ensure the protection of public health (NRMMC et al., 2008). For those chemicals not already regulated, guidelines were developed from available health, toxicological, or structural information based upon the "threshold of toxicity" approach, which employs numerous safety factors that yield values considerably lower than drinking water guidelines when applied to chemicals for which MCLs or notification levels already exist. The guidelines developed through this approach were intended as screening values that could be used to eliminate some compounds from consideration and prioritize the assessment of other compounds. They were not intended for use in the manner that California uses MCLs and notification levels. The Expert Panel does not recommend using the threshold of toxicity approach for routine chemical monitoring at DPR facilities; rather, the use of this approach should be limited to prioritizing research efforts to assess the need to monitor additional contaminants in water produced by DPR projects.

4.3.1.2 Monitoring Short-Duration Releases of Chemical Contaminants

For DPR projects, it may be appropriate to monitor for other chemicals in addition to the chemicals routinely monitored in drinking water and IPR facilities. These other chemicals fall into two categories:

- Chemicals that are difficult to remove by the application of reverse osmosis, advanced oxidation processes, or activated carbon adsorption (e.g., 1,4-dioxane).

- Chemicals that are removed more readily by certain DPR treatment processes, but may be present at elevated concentrations in wastewater entering water recycling systems.

In situations in which difficult-to-remove chemicals have been detected in wastewater at elevated concentrations, the presence of these chemicals often has been traced back to commercial or industrial activities that discharge wastes to municipal wastewater collection systems. Frequently, these excursions in contaminant concentrations are associated with discharges from batch processes or the intermittent use of a chemical in a process; therefore, infrequent monitoring (e.g., the monthly collection of 24-hour composite samples) is unlikely to detect the presence of these contaminants. Identifying sources of these contaminants and controlling their releases are discussed in **Chapter 8**.

Most treatment trains currently under consideration for DPR projects in California include reverse osmosis as one of the treatment steps. During reverse osmosis, charged compounds and neutral compounds with molecular weights above approximately 200 grams per mole (g/mol) are removed almost entirely, with rejections frequently exceeding 99 percent (Bellona et al., 2004). Uncharged, low molecular weight compounds (e.g., NDMA, chloroform, low molecular weight aldehydes) tend to be poorly rejected by reverse osmosis systems. For example, under conditions encountered at full-scale AWTFs, concentrations of NDMA in recycled water typically decrease by 25 to 75 percent during reverse osmosis treatment, depending on feed water temperature conditions (Fujioka et al., 2012).

Because of the lack of an adequate environmental buffer or substantial opportunities for dilution through blending, short-duration releases of chemical contaminants could be problematic for DPR projects that rely upon reverse osmosis to remove chemical contaminants. Contaminants that are difficult to remove during reverse osmosis (see **Chapter 8**), such as acetone, methyl ethyl ketone, and methanol, are widely used in commercial, industrial, and research applications. Although the release of these chemicals to municipal wastewater collection systems is regulated under the Clean Water Act through its industrial source control provisions, pulse releases of these contaminants can lead to periods of several hours in which elevated concentrations of chemicals enter WWTPs. In some cases, concentrations of solvents in untreated wastewater can exceed several parts per million (i.e., mg/L).

Concentrations of solvents in wastewater will decrease substantially as the pulse passes through the treatment train of conventional and advanced treatment processes, through removal (i.e., most solvents are removed partially during biological wastewater treatment), and the attenuation of the peak through mixing during treatment. In AWTFs used for DPR, solvent concentrations will decrease further during treatment with reverse osmosis and advanced oxidation processes. Nonetheless, elevated concentrations of solvents may be present in finished drinking water when the treatment processes do not fully attenuate the peak as it passes through the treatment train (see the example of an acetone excursion at the Groundwater Replenishment System, an IPR project that uses full advanced treatment, in **Figure 4-1**).

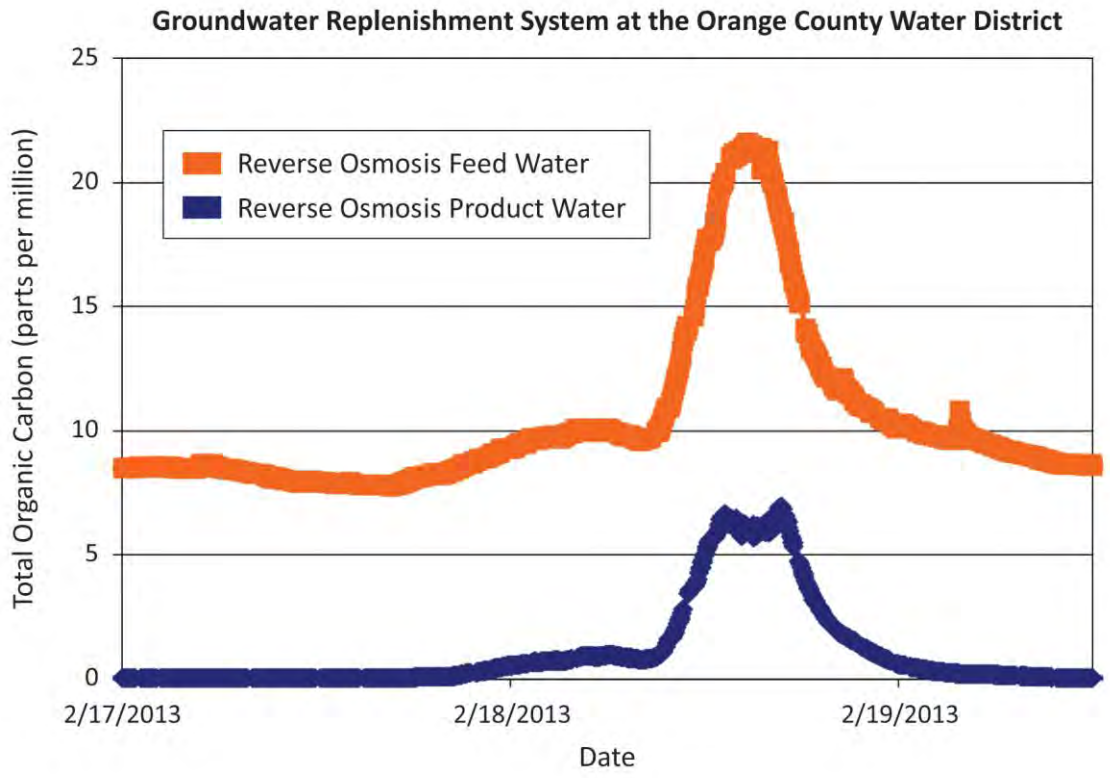


Figure 4-1: Results from online monitoring of total organic carbon before and after reverse osmosis (RO) at the Orange County Water District's Groundwater Replenishment System in February 2013 (Dadakis and Dunivin, 2013).

The detection of short-duration pulses of solvents or other chemicals through routine sampling methods is challenging because 24-hour composite samples (i.e., the typical approach used for regulatory compliance) tend to smooth out peak concentrations. Concentrations of volatile solvents also may decrease after the sample is collected if provisions are not taken to avoid volatilization after sample collection. Furthermore, the likelihood is low that a pulse will occur during the period of collection for a monthly or quarterly regulatory compliance program. Monitoring plans that require the collection of daily 24-hour composite samples could address this shortcoming, but they would be expensive, and this level of effort might be considered burdensome by operators of AWTFs. High-frequency monitoring (e.g., through the use of a total organic carbon [TOC] analyzer that examines a sample every few minutes) could provide an alternative approach for providing information about the presence of pulses of chemical contaminants, provided the device is sensitive enough to detect compounds when concentrations exceed levels of concern.

Knowledge about the occurrence of pulses of solvents comes from the small number of potable reuse projects that use high-frequency TOC analyzers. For example, the Orange County Water District of Fountain Valley, California, operates analyzers capable of high-frequency monitoring of TOC before and after reverse osmosis at the Groundwater Replenishment System. During a 2-day period starting on February 18, 2013, operators of the AWTF detected elevated concentrations of TOC (see **Figure 4-1**) in both the reverse osmosis feed water and reverse osmosis product water. A subsequent analysis of grab

and composite samples by gas chromatography/mass spectrometry (GC/MS) indicated that nearly all the organic carbon in the reverse osmosis product water during the period of elevated concentrations was attributable to acetone (Dadakis and Dunivin, 2013).

Results from this event are relevant to DPR for the following two reasons:

- Despite the implementation of an exemplary industrial source control program and use of a WWTP that has relatively few industrial dischargers, the Groundwater Replenishment System experienced conditions that resulted in over 5 mg/L of acetone present after reverse osmosis treatment for over 4 hours. Although the acetone may have been removed partially during the subsequent advanced oxidation process, the relatively low reactivity of the compound with hydroxyl radicals likely resulted in relatively high concentrations of the compound (i.e., >1 mg/L) in the final product water. The peak concentrations of acetone likely were attenuated by subsequent biotransformation and mixing in the aquifer. If this pulse occurred in a DPR system using the same treatment processes, little further attenuation would be likely.

- Without the use of a high-frequency TOC analyzer capable of detecting acetone, an AWTF of this type would not detect the acetone pulse in time to avoid introducing water with elevated acetone concentrations to a DWTF or drinking water distribution system (i.e., daily composite or grab samples typically require several days to process, and surrogate parameters other than the high-frequency TOC analyzer could not detect acetone at these concentrations).

Because only a small number of water recycling facilities employ high-frequency TOC monitoring on reverse osmosis product water, it is difficult to know how frequently DPR facilities will experience pulses of contaminants that are not rejected well by reverse osmosis membranes. Considering that the Orange County Water District's Groundwater Replenishment System receives treated wastewater from a wasrewater treatment plant (WWTP) that receives only about 5 percent of its raw wastewater from industrial and commercial sources and because considerable effort has been put into source control, it is likely that other communities in California will encounter these events at a greater frequency than the Groundwater Replenishment System, which has observed at least six such spikes of TOC between 2007 and 2012 (**Figure 4-2**).

Researchers interested in human exposure to volatile organic compounds in occupational settings and the urban environment also have detected pulses of benzene, substituted aromatic compounds, and tetrachloroethene emitted from wastewater collection systems (Quigley and Corsi, 1995). This research provides additional evidence that organic solvents are released routinely to sewers. Methods developed as part of efforts to identify the sources of these solvents (e.g., the use of photoionization detectors to continuously monitor volatile organic compounds in the headspace of sewers or within WWTPs) could provide a basis for improving industrial source control programs by detecting the release of solvents to sewer trunk lines and improving WWTP operations by alerting operators to the presence of high concentrations of solvents.

The chemical contaminants most likely to pose the greatest risks of this nature fall into the following two categories: (1) solvents and other chemicals commonly used in relatively large quantities in commercial or industrial activities; and (2) highly toxic contaminants used in small amounts or that are present as trace impurities or byproducts of another process.

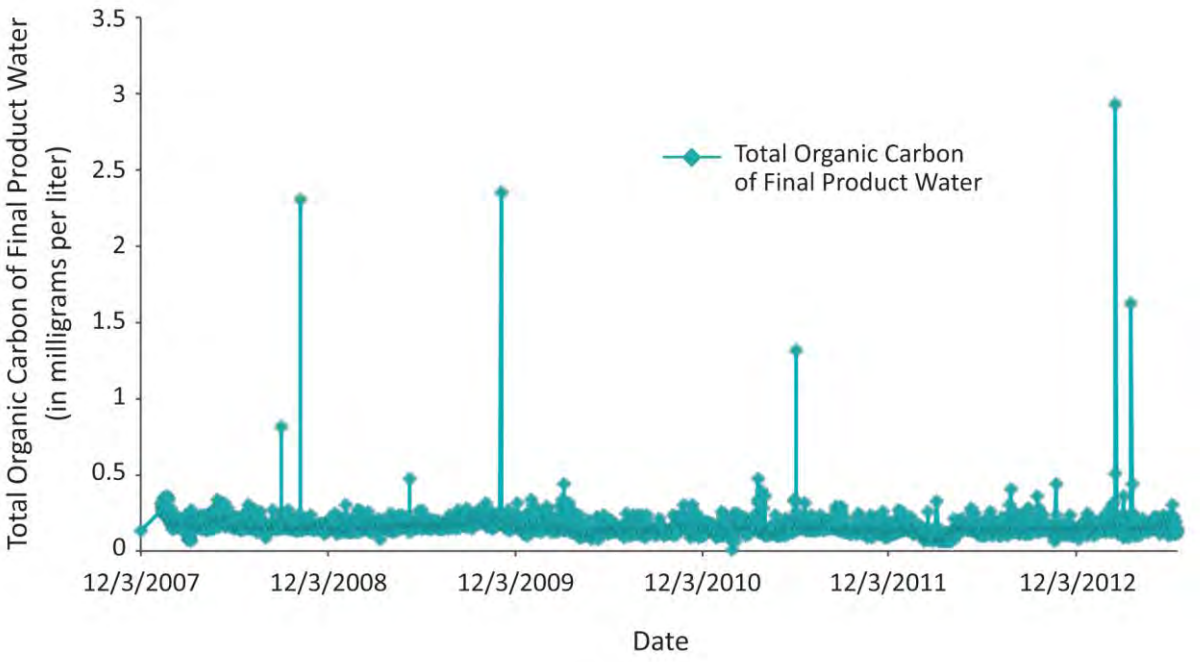


Figure 4-2: Total organic carbon measured in daily 24-hour composite samples of final product water from the Orange County Water District's Groundwater Replenishment System (Patel, 2013).

The former group includes contaminants like acetone, methyl ethyl ketone, and methanol. To minimize exposure to high concentrations of these types of chemical contaminants in drinking water, chemical monitoring plans for DPR systems should include high-frequency TOC monitoring or other surrogate parameters capable of detecting pulses of solvents and other compounds that are poorly removed in reverse osmosis and subsequent advanced oxidation processes. For these compounds, the ability of a high-frequency TOC analyzer to detect an increase in TOC over typical background levels would ensure that consumers would not be exposed to a concentration of the contaminant over approximately 100 to 500 µg/L. Based on current knowledge about the toxicity of compounds that fall into this category and the expected maximum frequency at which the public might be exposed (i.e., a few times per year), the Expert Panel believes this type of exposure would not pose unacceptable human health risks.

Highly toxic compounds that might be present as impurities or byproducts could be problematic at concentrations lower than the threshold at which the high-frequency TOC analyzer could discriminate the pulse from the background TOC level. For example, the Orange County Sanitation District has detected pulses of NDMA in its sewer trunk lines from the disposal of dithiocarbamates used for the treatment of metals in wastewater from printed circuit board manufacturing facilities. Pulses of NDMA also were traced back to the use of root control chemicals that were contaminated with trace amounts of the compound (Sedlak et al., 2005). Assuming 99 percent of NDMA is removed during the advanced oxidation process that follows reverse osmosis at many AWTFs, a pulse of NDMA with a concentration between 1 and 500 µg/L would result in the delivery of water to a DWTF or drinking water distribution system with a concentration above the notification level of 0.01 µg/L. Other highly toxic contaminants

that might pose similar concerns include 1,2,3-trichloropropane (which has a notification level of 0.005 µg/L) and nitrosodiethylamine and nitrosodi-n-propylamine (both with notification levels of 0.01 µg/L).

For most highly toxic compounds, cancer is the health concern that led to the establishment of the relatively low notification levels or drinking water MCLs. The approach used to derive drinking water guidelines for carcinogens usually assumes that risk is a function of the total dose of the chemical whether it occurs over a short period or during a lifetime. Drinking water standards generally spread this dose over a lifetime; therefore, an individual's exposure to concentrations in slight excess of drinking water standards for carcinogens a few times per year is unlikely to pose an unacceptable health risk. In some cases, chemicals may produce an irreversible health effect with short-term exposures (e.g., exposure of a fetus or a newborn child to a chemical that causes a developmental deficit). The Expert Panel is unaware of any contaminants in recycled water for which concentrations that could not be detected by a high-frequency TOC analyzer would pose an unacceptable health risk.

The Expert Panel believes that a short-duration pulse is best managed through a targeted industrial source control program and more frequent sampling for compounds in this category during the start-up phase of a new DPR project. For example, in the past 25 years, chemicals have been identified that produce developmental delays (e.g., lead, arsenic, and some pesticides). In some cases, these effects already are incorporated into regulatory limits (e.g., the focus on lead in drinking water is based on the potential to cause developmental delays rather than its carcinogenicity). It is important to recognize that such effects generally are identified from specialized studies rather than from the routine toxicological studies required for the registration of new products (furthermore, these effects are more likely to be recognized with drugs rather than with industrial chemicals). The State Water Board should actively track studies in the scientific literature on developmental impacts caused by unregulated contaminants that occur in wastewater and, potentially, DPR product water.

In addition to the breakthrough of low molecular weight compounds due to pulse discharges of industrial chemicals, the use of chemical oxidation processes (e.g., ozonation, advanced oxidation processes) prior to reverse osmosis can result in the production of byproducts such as bromate, formaldehyde, and other low molecular weight aldehydes (Weinberg et al., 1993) at detectable concentrations (i.e., >1 µg/L). The use of chlorine or chloramines in processes upstream of reverse osmosis also could result in the formation of neutral, low molecular weight disinfection byproducts (e.g., chloroform, haloacetonitriles [Huang et al., 2012]). Some of these chemicals are not removed well by reverse osmosis membranes. If the water is not subjected to an additional treatment step capable of removing them (e.g., activated carbon adsorption), then these chemicals likely would be present in drinking water produced by DPR projects employing advanced oxidation processes or chlorination prior to reverse osmosis. The State Water Board should require monitoring of these uncharged, low molecular weight oxidation or disinfection byproducts for DPR projects that use oxidants prior to treatment with reverse osmosis.

As evidenced by the data in **Figure 4.1**, high-frequency TOC analyzers also can detect pulses of contaminants in water not subjected to reverse osmosis treatment; however, DPR projects that do not employ reverse osmosis or a membrane (e.g., a tight nanofilter) capable of rejecting most charged, high molecular weight contaminants would need to consider a wider universe of contaminants that might be released to wastewater collection systems over short durations. Prior to permitting a DPR project that does not employ a treatment system capable of rejecting charged and higher molecular weight chemical contaminants, the Expert Panel believes research is needed to establish that contaminants of concern could be detected by high-frequency TOC analysis or other surrogate parameters. If high-frequency TOC

analysis is employed, additional research would be needed to assess fluctuations in baseline TOC concentrations. For example, perfluorooctanesulfonic acid (PFOS) is an organic contaminant that has been detected in municipal wastewater at elevated concentrations in communities with high densities of industrial sources (Alder and van der Voet, 2015). On the basis of the compound's limited reactivity with sulfate radicals and hydroxyl radicals (i.e., the oxidants used to convert organic compounds to carbon dioxide in commercial TOC analyzers), it is unlikely that a pulse of PFOS could be detected with a commercial high-frequency TOC analyzer.

Consequently, the Expert Panel recommends that short-duration sources of highly toxic chemical contaminants that cannot be removed by reverse osmosis treatment need to be addressed explicitly in source control programs. It is important particularly in communities where a strong source control program – designed specifically for potable reuse – is not already in place. Because the chemicals that pose challenges to DPR projects often are impurities or byproducts of chemical use, they tend not to appear in the records of products used by commercial and industrial facilities. As a result, the types of routine source control programs that currently exist for protecting conventional WWTPs from upsets due to industrial discharges are unlikely to be sufficient to control chemicals of concern to DPR systems. In addition, the Expert Panel believes that research is needed to better characterize the universe of chemicals of human health concern that are not removed well by the treatment processes employed for DPR systems. Research also is needed on high-frequency approaches for detecting pulses of NDMA and other highly toxic contaminants in water produced by DPR systems.

Currently, most DPR projects under consideration in California include reverse osmosis as part of the treatment train. In part, this decision has been driven by the success of recently constructed IPR projects (e.g., the Orange County Water District's Groundwater Replenishment Facility and West Basin Municipal Water District's Edward C. Little Water Recycling Facility) and the availability of an ocean outfall to discharge reverse osmosis concentrate. Furthermore, the ability of reverse osmosis to remove dissolved ions is viewed by most water utilities as a worthwhile benefit, especially in locations where source waters may contain total dissolved solids at concentrations near or above the secondary drinking water standard (i.e., 500 mg/L).

In the future, DPR projects might be considered in communities lacking access to an ocean outfall. Although it is prudent to include reverse osmosis in the treatment trains of the first DPR projects implemented in California due to the water quality benefits and reliability that reverse osmosis provides, the Expert Panel believes a proposal for a DPR project that does not employ reverse osmosis in the treatment train could be considered and ultimately approved by the State. With respect to chemical contaminants, the Expert Panel believes that a DPR project that does not employ reverse osmosis will require more frequent monitoring of chemical contaminants in its final product water to assess the potential presence of chemical contaminants originating from commercial and industrial sources. In addition, evidence will be needed that validates the high-frequency monitoring program for a DPR system is capable of detecting highly toxic chemicals used in the sewershed, as well as chemicals not detected by high-frequency TOC analyzers.

4.3.1.3 Monitoring Chemical Contaminants that Could Compromise Aesthetics

Some solvents discharged to wastewater collection systems have low organoleptic thresholds (i.e., they exhibit strong odors at low concentrations), so their presence could compromise the aesthetic quality of water (e.g., MTBE imparts an odor to water at concentrations as low as 5 µg/L). Although the presence of these contaminants is unlikely to pose a public health risk, operators of AWTFs should recognize that

high-frequency TOC analysis might not detect pulses of contaminants that could compromise the aesthetics of drinking water. In addition to solvents, several other chemicals present in wastewater can compromise the aesthetics of drinking water. For example, geosmin, 2-methylisoborneol, and 2,4,6-trichloroanisole were detected frequently in wastewater effluent at concentrations up to approximately 100 times higher than their organoleptic thresholds (Agus et al., 2011). 2,4,6-trichloroanisole still was present at levels above the threshold after reverse osmosis treatment, but advanced oxidation processes removed odors from the recycled water.

If water from an AWTF is stored in a reservoir or aquifer after treatment, it is likely that concentrations of these contaminants would decrease due to volatilization and biotransformation. In a DPR system that lacks these additional attenuation mechanisms, it would be possible for off-flavors and odors to be present in the finished drinking water, especially if the system does not employ unit processes capable of removing these contaminants. Although the presence of these contaminants in drinking water does not necessarily imply a health risk, a flavor profile analysis or direct measurements of taste and odor compounds should be included in the chemical monitoring programs for DPR systems. It is worth noting that some odorous compounds in wastewater also can be formed in reservoirs (e.g., geosmin) and drinking water distribution systems (e.g., 2,4,6-trichloroanisole); therefore, the control of these compounds should be a broader concern of water providers.

4.3.1.4 Monitoring Other Chemical Contaminants of Commercial or Industrial Origin

The strategy of selecting chemical contaminants to monitor on the basis of prior experience at water recycling plants and DWTFs may not result in the detection of all compounds that could compromise water quality in a new DPR system because the wastewater in some cities could contain chemicals from industrial or commercial sources not present in the wastewater from cities where monitoring had been conducted previously. For example, concentrations of opioid pharmaceuticals were up to 1,000 times higher than the national average in wastewater collected from a municipal WWTP in the city where the drug was manufactured (Phillips et al., 2010). Effective commercial and industrial source control programs, including measures to reduce or prohibit discharge to the municipal wastewater collection system (i.e., an enforcement capability), can help control this problem and identify candidate compounds for inclusion in chemical monitoring programs (see **Chapter 8**).

A complimentary approach for anticipating the presence of previously unknown contaminants is to employ the use of non-targeted screening of chemicals. Some water recycling systems already use this approach, in which samples are screened periodically for unexpected peaks in the total ion chromatographs (TIC) from gas chromatography/mass spectrometry (GC/MS) analyses of final drinking water samples. Unfortunately, this approach – which is sometimes referred to as a "TIC analysis" – is relatively insensitive and rarely detects contaminants at concentrations within the range of MCLs of most known drinking water contaminants. Water from AWTFs typically contains TOC concentrations less than 0.1 mg/L; consequently, depending upon the extent of sample pre-concentration, the TIC analysis is unlikely to detect compounds at concentrations below that level.

Starting around 2005, a new generation of more sensitive mass spectrometers became available to researchers. These instruments have the capability to use broad scans across a wide mass range to detect previously unknown contaminants (Krauss et al., 2010). For example, the muscle relaxant tizanidine, which previously had never been detected in municipal wastewater, was detected in the Rhine River below Basel, Switzerland, through the use of high-performance liquid chromatography with high-resolution mass spectrometry (Ruff et al., 2015). One important consideration is that most

progress to-date using these non-targeted approaches has been made with high-performance liquid chromatography. This technique, which is quite powerful and sensitive, only can detect compounds that are ionized (i.e., charged) readily in the inlet of the mass spectrometer. In general, compounds with dissociable protons and high molecular weight compounds are ionized readily in mass spectrometer inlets, whereas neutral compounds – especially those lower molecular compounds that have been of greatest concern for DPR projects – tend to not be ionized readily in mass spectrometer inlets.

The inability of high-performance liquid chromatography/mass spectrometry to detect many of the uncharged, low molecular weight compounds problematic for potable reuse projects (e.g., acetone, NDMA, and 1,4-dioxane) limits the application of many new approaches to the detection of unknowns that are likely to pass through reverse osmosis membranes. It may be possible to detect these types of compounds (i.e., low molecular weight, uncharged compounds) by gas chromatography interfaced with time-of-flight mass spectrometers or hydrophilic interaction liquid chromatography coupled with reversed-phase chromatography prior to triple quadrupole mass spectrometry; however, to date, these instruments have not been used successfully for this purpose. The Expert Panel believes this research topic is important and supports a more effective screening process for yet-to-be-discovered chemical contaminants, as well as supports research to facilitate the application of techniques that can be used for non-target analyses of uncharged, low molecular weight compounds in recycled water.

In addition to non-targeted chemical analyses, bioassays could provide information on the presence of toxic compounds not already included in potable reuse monitoring programs. If the monitoring of DPR projects with one or more bioassays indicates the presence of substances that elicit biological responses linked to adverse human health effects, an effort could be initiated by using bioassay-directed fractionation or some other technique to identify the chemical contaminants responsible for the activity. This type of approach led to the discovery of steroid hormones as the dominant cause of feminization of fish in rivers that received a large fraction of flow from WWTPs (Desbrow et al., 1998). Bioassay-directed fractionation also was used in the 1980s in efforts to identify mutagenic compounds in chlorinated drinking water (Kronberg et al., 1988). The compound that was identified (i.e., MX) through the bioassay was of less concern than initially thought because the bioassay overestimated the potency of *in vivo* cancer. Nonetheless, bioassay-directed fractionation is a useful research tool for identifying compounds in recycled water that merit further evaluation. For this reason, the Expert Panel supports research efforts to employ bioassays and non-targeted analysis simultaneously to discover new chemical contaminants of concern in municipal wastewater and water produced by DPR systems; however, at this time, the Expert Panel does not believe the use of bioassays in routine chemical monitoring programs is warranted. See **Chapter 5** for more information on bioassays.

4.3.1.5 Monitoring Indicator Chemicals and Surrogate Parameters

Municipal wastewater contains a diverse suite of organic contaminants that pose a variety of potential health risks. Many of the compounds that researchers have discovered in wastewater are difficult to monitor with conventional analytical methods. Even if it was possible to monitor all these chemicals simultaneously, this effort might not be justifiable because chemicals with similar properties exhibit similar behavior during treatment. For example, studies have shown that the concentrations of ionized compounds with molecular weights above approximately 200 g/mole decrease by over 99 percent during reverse osmosis (Bellona et al., 2004); therefore, monitoring one or two charged, high molecular weight compounds before and after reverse osmosis treatment would provide the same information as measuring a suite of compounds with these characteristics.

The use of an abbreviated list of contaminants as indicators for an entire class of contaminants is well established for monitoring. For example, an assessment of waterborne pathogens often relies upon monitoring readily measured microbes (e.g., fecal coliform bacteria) as indicators of the behavior of difficult-to-measure pathogenic bacteria. Another indicator compound approach is to monitor for trihalomethanes, as done at DWTFs; research conducted over the past 40 years suggests that trihalomethanes play a relatively small role in the adverse health effects associated with the consumption of chlorine-disinfected drinking water. Nonetheless, concentrations of trihalomethanes often are correlated with the health effects of chlorinated water because trihalomethanes are indicators of the presence of other disinfection byproducts that are difficult to measure or have yet to be identified.

The use of indicator compounds to assess the fate of chemicals in potable reuse projects was first proposed in 2009 (Dickenson et al., 2009). A Science Advisory Panel convened by the State Water Board to develop monitoring requirements for chemicals of emerging concern (CECs) during water recycling further developed the idea and applied it to monitoring plans, as specified in the California Recycled Water Policy (SWRCB, 2013; Anderson et al., 2010). According to the Science Advisory Panel:

> "An indicator compound is an individual [chemical of emerging concern] occurring at a quantifiable level that represents certain physicochemical and biodegradable characteristics of a family of trace organic constituents that are relevant to fate and transport during treatment. It provides a conservative assessment of removal."

Indicator compounds are useful particularly in monitoring programs for assessing performance with respect to the removal of chemical contaminants when the compounds are present routinely in municipal wastewater at concentrations significantly higher than method detection limits. The report of the Science Advisory Panel included four indicator compounds (i.e., 17 β-estradiol, NDMA, triclosan, and caffeine) chosen on the basis of data on occurrence, behavior during treatment, and (in the case of NDMA and 17β-estradiol) toxicity to humans or fish. The Science Advisory Panel did not recommend these four compounds specifically be used as indicators in future monitoring programs for potable reuse projects; rather, these compounds were used to illustrate an approach for employing indicators, recognizing that other compounds could offer similar or better information about treatment system performance.

The Expert Panel agrees with the Science Advisory Panel that indicator compounds might be appropriate tools for assessing the performance of DPR projects; however, the State Water Board should seek expert guidance when selecting indicator compounds. The selection of indicator compounds should be based on their suitability to serve as performance indicators (i.e., the expected extent of the removal of the compound during the specific unit process). The selection of indicator compounds is not a "one-size-fits-all" endeavor. As detailed in published peer-reviewed papers on the topic (e.g., Dickenson et al., 2009), the selection of an indicator compound depends upon the specific treatment process being monitored, operating conditions, and composition of water prior to treatment; therefore, each DPR project will need its own set of indicator compounds.

A surrogate parameter is a measurable physical or chemical property (such as TOC or electrical conductivity) that correlates with the effectiveness of removing trace organic compounds by a treatment process and/or provides an indication of a treatment process failure. Reverse osmosis, for example, is expected to substantially reduce the TOC or electrical conductivity of the recycled water being treated. A reduction in the concentration of the surrogate also provides an indication that

inorganic and organic compounds known to be removed by reverse osmosis actually are being removed. Example indicator chemicals and suitable surrogate parameters are listed in **Table 4-1** for advanced treatment processes commonly employed in potable reuse treatment trains.

Table 4-1: Example Indicator Chemicals and Surrogate Parameters for Select Treatment Processes Commonly Employed in Potable Reuse Treatment Trains

Treatment Process	Performance-Based Indicator Chemical	Expected Differential Removal (%)	Surrogate Parameter	Expected Differential Removal (%)
Ozonation	ΔCarbamazepine	>90	ΔUV Absorbance	>40
Reverse osmosis	ΔSucralose	>90	ΔConductivity	>90
Advanced oxidation process (ultraviolet/hydrogen peroxide)	ΔPrimidone	>70	--	--

Δ = Change in concentration before and after the treatment process.

4.3.2 Frequency and Location of Monitoring

The California Recycled Water Policy provides guidance for monitoring trace organic chemicals in recycled water as part of IPR projects (SWRCB, 2013), which also can be adopted for the design of water quality monitoring programs for DPR projects. In addition, quality assurance and quality control measures are specified for both the collection of samples and laboratory analysis. These measures are summarized in a quality assurance project plan that includes the appropriate number of field blanks, laboratory blanks, replicate samples, and matrix spikes. The analytical methods employed in chemical monitoring programs are based on methods published by the USEPA, methods certified by the State Water Board, or are obtained from peer-reviewed methods that have been reviewed by the State Water Board, including those published by voluntary consensus standards bodies (such as the Standards Methods Committee and ASTM International). If modifications are made to the published or certified methods, they are reviewed by the State Water Board and subsequently submitted to the Regional Water Quality Control Board in an updated quality assurance project plan.

Normally, regulators phase the monitoring requirements for trace organic chemicals and surrogates. The purpose of phased monitoring is to allow monitoring requirements to be refined on the basis of monitoring results (i.e., if a specific analyte is never present at concentrations approaching a health-based standard, the frequency of analysis might be reduced). An initial assessment phase followed by a baseline monitoring phase are used to determine the project-specific monitoring requirements for standard operations. The following recommendations for the design of phased chemical monitoring programs are based on the SWRCB (2013) water recycling policy for monitoring IPR projects using groundwater replenishment, but also should be adopted for developing monitoring approaches for DPR. Monitoring occurs in three different stages:

1. During the ***initial assessment phase***, performance testing is conducted and efforts are made to assess both the occurrence of unique chemicals in the wastewater collection system, as well as leaching of contaminants from new equipment (e.g., bisphenol A and phthalate esters).

   The purpose of the initial assessment phase is to identify the occurrence of relevant chemicals from industrial and commercial dischargers to the wastewater collection system and suitable project-specific performance indicator chemicals and surrogates in recycled water to be monitored during the baseline phase. In addition, it will help (1) determine the effectiveness of individual treatment processes and (2) specify expected removal percentages for performance indicator chemicals and surrogates. Surrogates shall be selected to monitor individual treatment processes or combinations of treatment processes that are capable of removing trace organic chemicals for use in chemical monitoring programs during baseline and standard operation phases.

   Following the completion of the initial assessment monitoring phase, monitoring requirements shall be re-evaluated and subsequent requirements for the baseline monitoring phase shall be determined on a project-specific basis.

2. During the ***baseline monitoring phase*****,** project-specific performance indicator chemicals and surrogates shall be selected for monitoring. The purpose of the baseline monitoring phase is to assess and refine which performance indicator chemical and surrogates are appropriate for monitoring both the removal of trace organic chemicals and treatment system performance for the standard operation of a DPR facility.

3. After the DPR system is operational, monitoring requirements for indicator chemicals and surrogates used to assess treatment performance may be refined to establish project-specific requirements for monitoring standard operating conditions ***(standard operational monitoring phase)*****.**

4.3.3 Responding to Off-Specification Water

The chemical monitoring plan needs to include provisions for responding to situations in which water quality does not meet established drinking water standards (i.e., the water is off-specification, or "off-spec"). When high-frequency monitoring (e.g., TOC analysis) indicates that water does not meet established thresholds, the water will be routed away from the engineered storage barrier, DWTF, or drinking water distribution system, as described later in **Chapter 8**. After such an event occurs or when periodic monitoring (e.g., the analysis of quarterly samples) indicates that water from the DPR system does not meet drinking water standards or guidelines, a program should be initiated to identify the underlying cause of the problem.

4.4 Findings of the Expert Panel

In regards to evaluating chemical monitoring approaches appropriate for DPR projects, the Expert Panel concludes the following:

- **Finding #4-1:** A review of the practices employed for identifying chemical contaminants to be monitored in recycled water outside of California indicates that the system employed in California is the best available approach for DPR projects. The Expert Panel notes that the

threshold of toxicity approach employed for recycled water projects in Australia is not appropriate for determining the chemicals to be included in routine monitoring for DPR projects; rather, the use of the threshold of toxicity approach should be limited to prioritizing research efforts to assess the need to monitor additional contaminants in water produced by DPR systems.

- **Finding #4-2:** The use of indicator compounds to assess the performance of DPR projects is an important element of chemical monitoring plans to ensure the performance of DPR projects. The selection of indicator compounds should be based on their suitability as performance indicators (i.e., the expected extent of removal of the compound by the specific treatment process).

- **Finding #4-3:** Although it is prudent to include reverse osmosis as a treatment technology used in the first set of DPR projects due to the water quality benefits and performance reliability that reverse osmosis provides, proposals for DPR projects that do not employ reverse osmosis could be considered and ultimately approved by the State Water Board. With respect to chemical contaminants, a DPR project that does not employ reverse osmosis will need more frequent monitoring of a broader suite of chemical contaminants in its final product water to assess the potential presence of chemical contaminants originating from commercial and industrial sources. In addition, evidence will be needed that the high-frequency monitoring program of a DPR project is capable of detecting highly toxic chemicals that could be discharged to the wastewater collection system, as well as detecting chemicals that are not detected by high-frequency TOC analyzers.

- **Finding #4-4:** Chemical monitoring plans need to be included as part of DPR projects to ensure the protection of public health and the maintenance of adequate treatment performance. At minimum, DPR systems will be required to monitor chemical contaminants specified by state and federal regulations in drinking water and in water produced by IPR systems to ensure the DPR system is in compliance with existing drinking water standards designed to protect public health. The State Water Board should require the monitoring of secondary drinking water standards for DPR projects. See **Recommendation #4-1**.

- **Finding #4-5:** AWTFs sometimes employ an oxidant (e.g., ozone, chlorine, chloramines) prior to or after treatment with reverse osmosis. This practice can result in the formation of toxic byproducts, some of which are low molecular weight compounds that are not removed well during reverse osmosis or might remain after subsequent treatment with advanced oxidation. If the water is not subjected to an additional treatment step capable of removing these byproducts, they could be present in the drinking water produced by a DPR system. See **Recommendation #4-2**.

- **Finding #4-6:** Most AWTFs currently under consideration for DPR in California include reverse osmosis as one of the treatment steps. During reverse osmosis, charged compounds and neutral compounds with molecular weights above approximately 200 g/mol are almost entirely removed. Uncharged, low molecular weight compounds tend to be poorly rejected by reverse osmosis (e.g., NDMA, chloroform, and low-molecular weight aldehydes). Under normal operating conditions, the concentrations of low molecular weight neutral compounds in water produced by DPR systems generally are below the low TOC method detection limits observed in reverse osmosis permeate (i.e., typically <0.1 mg/L); however, operators of AWTFs have

detected short-duration pulses above the TOC method detection limits of acetone and, possibly, other contaminants in reverse osmosis permeate. These contaminants are believed to originate from discharges to the wastewater collection system by commercial and industrial activities. See **Recommendation #4-3**.

- **Finding #4-7:** It is possible that highly toxic contaminants could be discharged intermittently by commercial and industrial operations. Some of these contaminants have MCLs or notification levels that are too low to be detected by high-frequency TOC analyzers. In all known cases, the low allowable drinking water concentrations were established because the contaminants were known or suspected carcinogens. In these situations, the potential health risks associated with exposure to concentrations above the allowable drinking water levels (but below levels that could be detected by high-frequency TOC analyzers) are much lower than allowable drinking water concentrations because exposure would occur infrequently, as evidenced by current groundwater replenishment operations in California. See **Recommendation #4-4**.

4.5 General Recommendations of the Expert Panel

The Expert Panel has four general recommendations on the topic of chemical monitoring (which are not listed in preferential order) for consideration by the State Water Board:

- **Recommendation #4-1 (based on Finding #4-4):** The operators of DPR systems should be required to develop programs to explain to consumers the implications of excursions of secondary drinking water standards.

- **Recommendation #4-2 (based on Finding #4-5)**: For DPR systems that employ oxidants prior to or after reverse osmosis treatment, the State Water Board should require the monitoring of low molecular weight oxidation or disinfection byproducts beyond those for which drinking water standards have already been established.

- **Recommendation #4-3 (based on Finding #4-6):** To minimize the potential for exposure to high concentrations of low molecular weight compounds in drinking water, chemical monitoring plans for DPR systems should include the high-frequency monitoring of TOC or other surrogate parameters capable of detecting pulses of chemicals that are poorly removed by reverse osmosis and subsequent treatment with advanced oxidation. Existing high-frequency TOC analyzers are capable of detecting pulses of elevated concentrations of contaminants in reverse osmosis permeate rapidly enough to allow operators to avoid introducing final product water into the drinking water supply. In the event a pulse of contaminants arrives at the AWTF that is too low to be detected by a high-frequency TOC analyzer, the Expert Panel believes that subsequent removal in later treatment processes (e.g., during AOP) would result in concentrations of contaminants that may not pose unacceptable risks to public health.

- **Recommendation #4-4 (based on Finding #4-7):** The potential risks associated with highly toxic contaminants being discharged intermittently by commercial and industrial operations, which would be an infrequent event, are best managed through both (1) a targeted industrial source control program, and (2) more frequent sampling for compounds in this category during the startup phase of new DPR projects.

4.6 Research Recommendations of the Expert Panel

The Expert Panel has two research recommendations –specific to the State of California – for consideration by the State Water Board. These recommendations are not listed in preferential order.

- **Research Recommendation #4-1:** To better inform targeted monitoring for source control and final water quality, the State Water Board should be proactive in monitoring the literature on the potential health risks that could present serious harm to health over short durations of exposure by compounds likely to be present in recycled water. Of specific concern are chemicals that adversely affect the development of fetuses and children. Other compounds that produce such effects will undoubtedly be discovered in the future. This activity could be initiated concurrently with the development of DPR regulations and continued as an ongoing effort. A formal process should be established by the State that includes: (1) an internal process to monitor the literature and (2) an external peer review process to address the results of the internal efforts to maintain a high level of awareness of these issues.

- **Research Recommendation #4-2:** It is important to focus on non-targeted analysis and, furthermore, low molecular weight compounds. For example, the inability of reverse-phase liquid chromatography/mass spectrometry to detect many uncharged, low molecular weight compounds (e.g., halogenated solvents, formaldehyde, and 1,4-dioxane) problematic for potable reuse projects demonstrates the limitations of current analytical approaches for the detection of unknowns that are likely to pass through reverse osmosis membranes. Research is needed to develop more comprehensive methods to identify low molecular weight unknown compounds. It is possible these compounds may be detected by gas chromatography interfaced with time-of-flight mass spectrometers or hydrophilic interaction liquid chromatography coupled with reversed-phase chromatography prior to triple quadrupole mass spectrometry; however, to date, these methods have not been applied to potable reuse projects to detect these compounds. These methods or others need to be developed to increase the understanding of the make-up of the remaining TOC composed of low molecular weight compounds. In addition, these methods also could address the potential vulnerability of AWTF treatment processes to unintended spills or batch releases of chemicals in the sewershed.

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CHAPTER 5: APPLICATION OF BIOANALYTICAL TOOLS TO WATER ANALYSES

- Background on bioassays in health effects testing
- Bioassays of health-related activities in water.
- Applications of bioassays to water analyses.
- Information required to relate doses producing bioassay results to those producing health effects *in vivo*.
- Application of bioassays in health effects testing.
- Approach for certifying bioassays for use in water monitoring.

5.1 Introduction

The purpose of this chapter, and the question being addressed, is whether *in vitro* bioassays should be used as tools to monitor chemicals in advanced treated water. Specifically, an evaluation is included of the use of bioassays for the analysis of water produced by potable reuse projects.

5.1.1 Interest in Bioanalytical Tools

The Expert Panel was organized to address the feasibility of developing criteria for direct potable reuse (DPR) in the State of California. One issue identified for consideration by the Expert Panel was the recommendation of the Science Advisory Panel of the State Water Resource Control Board (State Water Board) to evaluate the use of *in vitro* bioassays in the monitoring of DPR and the development of "trigger values" for such monitoring. Specifically, the Science Advisory Panel recommended that bioanalytical tools be incorporated into the ***analyses*** of waters produced during the recovery of municipal wastewater with the intent of providing a source of drinking water. This recommendation was among several made in the final report of the Science Advisory Panel (Anderson et al., 2010). The review of other recommendations made by the Science Advisory Panel is considered beyond the scope of this effort.

5.1.2 Scope of the Expert Panel Review

Biomonitoring or bioanalytical approaches are a wide collection of methods and techniques used in medicine and public health. For example, the measurement of chemicals in urine by the Centers for Disease Control and Prevention (CDC) (Aylward et al., 2013) would fall into the category of biomonitoring. Biomonitoring has been successfully employed to detect *in vivo* surrogates for particular diseases or injuries to specific organs in clinical medicine for more than 50 years; this use is rapidly expanding in both types and methods of analyses.

The term "**BIOASSAY**" refers to the use of any biological system to detect a biological (or toxicological) effect. Thus, bioassays are a subset of biomonitoring or bioanalytical tools. Bioassays have also been used by the biomedical community for more than 100 years.

Differences in Bioassays

BIOASSAY: An analytical procedure that uses live animals or plants (*in vivo*) or tissues, cells, or enzymes (*in vitro*) to identify the biological activities of chemicals.

***IN VIVO* VERSUS IN VITRO**: *In vivo* testing implies the examination of effects on biochemical and biophysical processes and tissues in intact organisms. Microorganisms can exist as single cells; therefore, study within these organisms would be considered *in vivo*, while the study of mammalian enzymes, cells, or tissues outside of the body (e.g., conducted in culture dishes) is considered *in vitro*.

THE RESULTS OF *IN VITRO* TESTING can be used to provide information about possible mechanisms of action, but many effects seen *in vitro* occur at doses that are lethal *in vivo* and are of little interest and have less relevance to the chronic development of adverse effects *in vivo*.

As understood by the Expert Panel, the goal articulated by the State Water Board's Science Advisory Panel was to explore bioassays as methods to detect chemicals of emerging concern (CECs) that might occur in municipal wastewater and potentially occur in drinking water. Although the Science Advisory Panel's interest in this topic appeared to focus on the use of high-throughput (HTP) systems rather than bioassays in general, this review by the Expert Panel applies to bioassays that detect chemical activity, irrespective of their formats. HTP bioassays have been developed that detect many different specific biological activities of potential interest toxicologically. Based on these assumptions, the Expert Panel focused on the use of *in vitro* assays, particularly those that have been put into an HTP format. In the process, the Expert Panel excluded many ***IN VIVO* BIOASSAYS** from consideration that lack some of the shortcomings of ***IN VITRO* BIOASSAYS**.

Higher frequency and a greater variety of monitoring are considered by many to be more important for DPR than for indirect potable reuse (IPR), largely because of DPR's lack of the use of an environmental buffer; therefore, the Expert Panel assumes much of the focus is on the routine biomonitoring of finished water. As will be discussed, this application would require appropriate calibration of the bioassay to risk before the bioassay could be used to make decisions for corrective action. As stated repeatedly in the ToxCast Program (Dix et al., 2007) of the U.S. Environmental Protection Agency (USEPA), the bioassay has to be "fit for purpose" (i.e., suited to the application). The question is whether *in vitro* bioassays should be part of the criteria to implement DPR projects.

The Expert Panel addressed the validity of several potential applications of bioassays for DPR. Some research applications of bioassays should not be considered as criteria to be applied to DPR or other forms of water reuse. These applications, however, will be described and discussed herein because their use could have tangible institutional benefits. The suggestion that *in vitro* bioassays should be part of the criteria used in monitoring DPR implies that *in vitro* bioassays would be employed in routine monitoring (e.g., compliance monitoring) and serve much the same purpose as maximum contaminant level goals (MCLGs), public health goals (PHGs), and maximum contaminant levels (MCLs). The shortcomings of using *in vitro* bioassays for monitoring were identified by the Expert Panel, and suggestions were made about the type of ancillary data needed to pursue this use.

The Expert Panel recognizes that the bioassays used in health effects testing by the USEPA and other federal regulatory agencies are interpreted within the context of decision matrices or decision trees. Based on the online materials provided by the USEPA's ToxCast program, it is clear that the USEPA views its current set of bioassays within the context of conventional tiered testing schemes. In such schemes, each bioassay is identified as being appropriate for screening (Tier 1), confirmation (Tier 2), or risk

assessment (Tier 3); therefore, a major focus of this evaluation by the Expert Panel was to determine which tier the HTP bioassays being considered for monitoring were suitable for application. In turn, the Expert Panel addressed the nature of each application in terms of whether a bioassay is suitable to the task that might be assigned to it in water analysis. The tasks apparently envisioned by the Science Advisory Panel's report to the State Water Board can be classified loosely as routine monitoring (rather than screening and identification). If the bioassay did not fit an "assigned" task, the Expert Panel considered whether the bioassays proposed could be used if coupled with other critical data.

5.2 Background on Bioassays in Health Effects Testing

5.2.1 Role of *In Vitro* Bioassays in Health Effects Testing

Over the past 50 years, a wide variety of approaches has been developed and used to test chemicals for human health effects. Early on, almost all these efforts were directed at whole animal testing for a variety of endpoints and routes of administration, depending largely upon the production and use of the chemical. The basic decision trees that started incorporating *in vitro* bioassays into health effects testing were established with the implementation of the Toxic Substances Control Act (TSCA) in the 1970s; however, questions of time, expense, new endpoints of concern, and animal welfare have driven progress in the development of *in vitro* systems, as well as new approaches. New approaches increasingly rely on *in silico* methods (i.e., computer modeling structure-activity relationships, toxic pathway analyses, or decision matrices) to identify those chemicals that require testing in animals, thereby increasing the efficiency of testing and reducing the number of animals used for safety evaluations.

5.2.2 USEPA Efforts to Validate *In Vitro* Methods for the Prioritization of Chemicals for Further Testing

Within the USEPA, the National Computational Toxicology Program has spearheaded an investigation into the use of *in vitro* methods by taking advantage of massive databases of *in vivo* testing data against which various *in vitro* systems can be evaluated for their ability to predict toxicological outcomes (Kavlock et al., 2012). Analyses of these data are done within the USEPA's Toxicity Forecaster (ToxCast). The approach was constructed primarily to respond to the tens of thousands of chemicals regulated under the TSCA and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) that lacked any information on hazards. The drinking water program has been charged with addressing compounds found in water sources for endocrine activity under the Food Protection Act; however, at this point in time, the USEPA's Office of Water has left testing to the TSCA and FIFRA to manage the testing of such compounds in commerce.

5.2.3 Tox21 Collaboration among Federal Agencies

In addition to the activities of the USEPA, a collaborative interagency program was instituted, referred to as Tox21, to reduce reliance on *in vivo* methods by focusing on *in vitro* methods to research and test the toxicity of chemicals (Tice et al., 2013). Tox21 is a partnership of the following four federal agencies (Collins et al., 2008; MOU, 2008):

- USEPA, Office of Research and Development, National Center for Computational Toxicology

Adverse Health Outcome

A toxic response to chemicals that leads to the development of a disease that compromises the function or survival of an individual (i.e., morbidity and mortality); also known as an "adverse health effect."

- National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), National Institutes of Health (NIH).
- National Center for Advancing Translational Sciences (NCATS)/NCATS Chemical Genomics Center (NCGC), National Institutes of Health (NIH).
- U.S. Food and Drug Administration (FDA).

5.2.4 OECD Assay Validation Program

The Organization for Economic Cooperation and Development (OECD) also has a program for validating assays, particularly in the area of endocrine disrupters (OECD, 2007; 2012a,b; 2013a,b). One goal is to develop approaches ranging from the application of bioassays for screening chemicals to the use of test results for hazard and risk assessment. Currently, the major use of these approaches is to screen chemicals for prioritization for further testing, usually *in vivo*. At present, risk assessments still rely on *in vivo* data.

5.2.5 ToxCast Bioassays

A large number of publications are available from the aforementioned federal programs that describe the specific nature of their studies, test results, and efforts to validate the ToxCast approach for preliminary screening of chemicals. Some publications are called out here to direct the reader to selected summaries of progress. These papers and others from ToxCast were drawn upon by the Expert Panel to address the key question of how well these bioassays predict **ADVERSE HEALTH OUTCOMES** in their present state of development. The topics of these publications and their citations include:

- Estrogen screening assays (Becker et al., 2014).
- Endocrine disruptor screening assays (LeBaron et al., 2014).
- HTP screening assays (Zhu et al., 2014).
- Phenotypic assays (Berg et al., 2013).
- Enzymatic and receptor signaling assays (Sipes et al., 2013).
- HTP decision support tools for risk management (Kavlock et al., 2012).
- Genotoxicity assays (Knight et al., 2009).
- Prenatal developmental toxicity studies (Knudsen et al., 2009).
- Profiling the reproductive toxicity of chemicals (Martin et al., 2009).

In addition, this selection of papers was made, in part, to highlight the evolution in thinking about how *in vitro* bioassays might eventually take a more prominent role in human health assessments for human exposures. For example, one recent statistical analysis found that the predictive accuracy relative to *in vivo* toxicities is low and is more suitable for identifying risk factors rather than for making *in vivo*

predictions of toxic effects (Thomas et al., 2012). A subsequent paper, however, proposed a more structured strategy for using these systems in a tiered framework to illustrate how *in vitro* methods can be used to (1) eliminate chemicals that do not require *in vivo* testing and (2) confine animal testing to high-value chemicals that require animal data for the purpose of risk assessment (Thomas et al., 2013). This very expensive *in vivo* testing would be confined to chemicals for which there is – or would be predicted to have – substantial human exposure (i.e., exposures that approach the minimal effect level of a chemical). In general, it is recognized that better experimental data mapping of toxicogenomic and proteomic responses to chemicals affecting **ADVERSE OUTCOME PATHWAYS (AOPathways)** should substantially improve the predictability of *in vitro* bioassays in the future (Yu et al., 2014).

Adverse Outcome Pathways (AOPathway)

The purpose of an adverse outcome pathway (AOPathway) is to provide the framework that connects the events of a chemical interaction with a biological process, starting with a molecular initiating event and leading to an adverse health outcome (USEPA, 2014).

In most cases, only a few of the more critical steps are included, allowing the pathway to be developed with incomplete knowledge of the intervening steps.

5.2.6 Use of Tiered Decision Trees

Bioassays used in the testing of chemicals are interpreted within a tiered decision tree that describes their defined roles in health effects assessments. A general diagram of a decision tree is shown in **Figure 5-1.**

It is important to note that the specifics of the decision-tree used are closely coupled to the legislative mandate of the regulatory program. In the case of programs authorized under the TSCA, the intent is to minimize the overall cost of required testing. Economy in testing occurs because it allows the manufacturer or distributor of the product to suspend the development of a product before larger expenditures on testing can be made. The producer also could choose to cease marketing or completely discard an existing product that tests positive at the screening or confirmation tiers (Tiers #1 and #2). Positives at both these levels mean that the chemical must have data generated that is considered adequate for estimating risk at real or predicted levels of human exposure if the chemical/product is to be further developed and marketed in the estimated amounts. There is no legislative mandate for the testing of chemicals under the Safe Drinking Water Act. Essentially, decisions in drinking water are made depending upon a risk assessment based on existing and accessible data.

Clearly, regulatory decisions based on health effects require sufficient data to assess the risk of a chemical that occurs in drinking water. MCLs are developed based on data considered appropriate for making risk assessments. It is presumed that the use of bioassays for routine monitoring will function equivalently to scaling the MCL of a chemical to its dose-response for producing an adverse health effect (e.g., as is done in the development of MCLGs, PHGs, or their equivalents).

5.3 Bioassays of Health-Related Activities in Water

Several advisory panels and workshops have been convened to review advancements in bioassay technology and consider whether such bioassays should have a place in water analyses and what that their role should be. These meetings and discussions are described in **Sections 5.3.1** to **5.3.3**, but the actual use of bioassays in water analyses are discussed technically in **Section 5.4.**

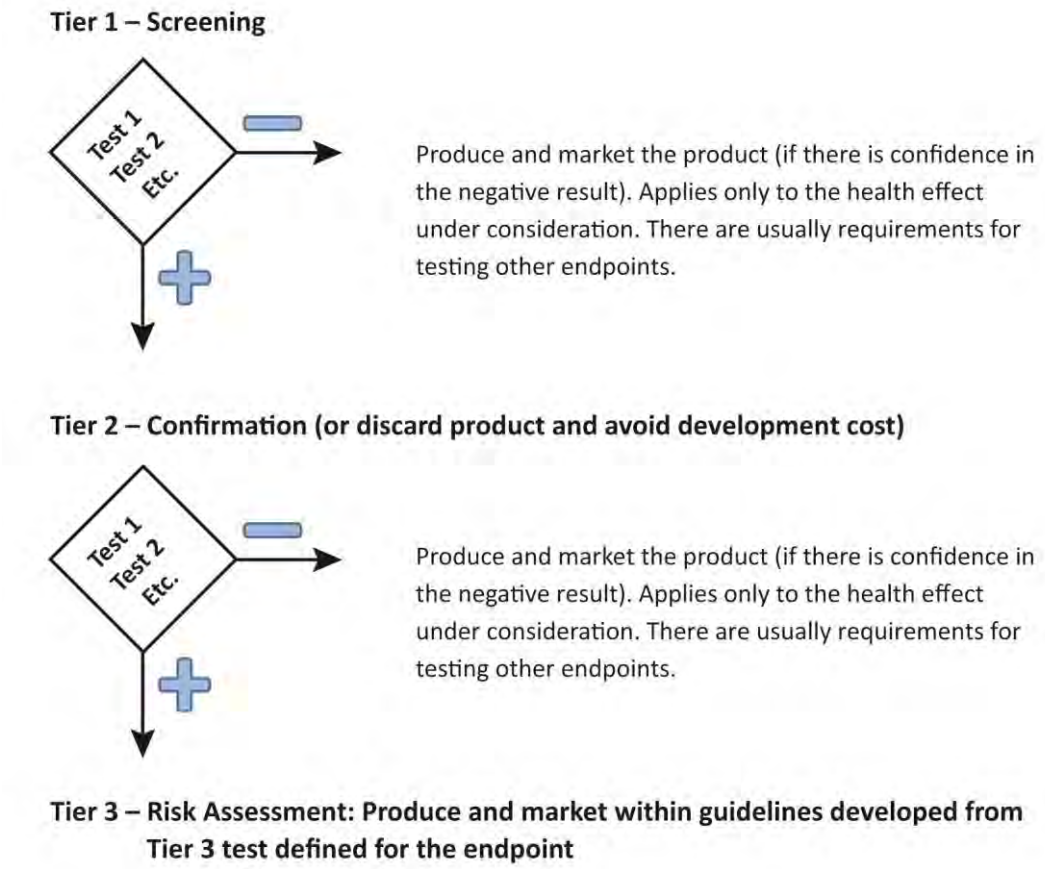

Figure 5-1: Example of a tiered decision tree used in health effects testing.

5.3.1 Science Advisory Panel for the California State Water Resources Control Board: Evaluate the Use of Bioassays for Water Analyses

A Science Advisory Panel for the State Water Board was convened in 2009 to (1) determine the current state of scientific knowledge regarding the risks of CECs to public health and the environment and (2) make recommendations to ensure all uses of recycled water meet regulatory conditions (Anderson et al., 2010). As part of their final recommendations, released on June 25, 2010, the Science Advisory Panel endorsed the following:

- The development of bioanalytical techniques (or "bioassays") to address both known and unknown CECs.
- The development of appropriate trigger levels for these techniques that correspond to a response posing a concern from a human health standpoint.
- Effect-based identification, more commonly known as "screening and identification."

High-Throughput Screening

High-throughput screening (HTS) involves robotics, data processing and control software, and sensitive detectors, allowing a researcher to quickly conduct thousands of chemical, genetic, or pharmacological tests to rapidly identify active compounds, antibodies, or genes that alter or are byproducts of a particular biomolecular pathway.

To the Expert Panel's knowledge, two activities resulted from the recommendations of the Science Advisory Panel. One activity was a Southern California Coastal Water Research Project (SCCWRP) publication that described the use of 103 bioassays and selected a group of these bioassays considered useful for benchmarking water samples (SWRCB, 2014). The second activity, undertaken by a collaboration of utilities, focused on applying a number of bioassays to wastewater, recycled water, and drinking water. The resulting reports (Escher et al., 2014a,b) described much of the work in detail and suggested a methodology for establishing effect-based target values; these reports are reviewed in **Section 5.3.2.3.**

Many bioassays have been developed and fielded in the past 20 years; more recently, many have been put into an HTP format. Those HTP bioassays used for **HIGH-THROUGHPUT SCREENING (HTS)** are largely drawn from their use in the pharmaceutical industry to identify new chemicals that might be developed into drugs. The second major incentive has been the USEPA's mandate in the Food Quality Protection Act to screen for endocrine disruptors (USEPA, 2015). Increased interest exists in developing bioassays with other targets to broaden the spectrum of important toxicologically oriented pathways, but this spectrum remains very narrow at present.

It is important to note that the emphasis placed on *in vitro* bioassays by ToxCast and other programs is stimulating the development of a framework upon which *in vitro* bioassay results may be more clearly interpreted for risk assessment, as discussed at the FutureToxIII Workshop in Washington, D.C. on November 19-20, 2015, which was attended by Expert Panel member Dr. Richard Bull. Progress in this area and potential applications for water analyses are discussed in **Section 5.4.**

5.3.2 Australian Water Recycling Center of Excellence Workshop: Identify and Define Steps to Allow the Use of Bioassays as a Basis for Risk Assessment

The Australian Water Recycling Center of Excellence convened a workshop on "Assessing Human Health Risks in Drinking Water" in Leura, New South Wales, Australia, on February 9-11, 2015, in which a number of academicians, regulatory agency representatives, scientists, and engineers participated. The workshop consensus document (Greenfield and Law, 2015) recognized three primary uses for bioanalytical tools in recycled water assessment, which include:

- Characterizing source water.
- Optimizing technology and monitoring treatment performance.
- Assessing the safety of product water for human health.

Workshop participants concluded that the first two items have been amply demonstrated, but much work is still needed to allow most bioassays to be used as a basis for risk assessment. The goal of the workshop was to identify those steps that must be defined before *in vitro* bioassays can be used for estimating risk.

The identified steps included:

Step 1: Extrapolate from *in vitro* target concentration to *in vivo* dose.

Step 2: Determine what endpoints are relevant in water quality assessments.

Step 3: Extrapolate from a cell-based response to an adverse health effect.

Step 4: Take the **TOXICOKINETICS** (Step 1) and AOPathways (Step 3) established for single compounds and extrapolate to mixtures of chemicals.

Since the workshop, participants have questioned (1) whether these steps are, in fact, appropriate and (2) whether other steps are required to reach the goal articulated at the workshop that all health effects assessments would be based on *in vitro* testing.

Toxicokinetics

A quantitative description of rates at which a chemical will enter and be distributed throughout the body, as well as identifying the route and rates of its excretion.

"Toxicokinetics" and "pharmacokinetics" are equivalent terms. Pharmacokinetics is the older, more general term and will be used in this paper. Toxicokinetics is used by some to distinguish it from work on drugs and was used in the Leura workshop report (for that reason, the term is retained in this chapter).

5.3.3 Specialty Seminar on DPR in California: Challenges Must Be Addressed to Use Bioassays as Monitoring Tools

Use of ToxCast

ToxCast is a more effective approach for identifying chemicals requiring further testing.

At present, it is not being used for making risk assessments.

The National Water Research Institute (NWRI) and the State Water Board held a Specialty Seminar on "Direct Potable Reuse in California" in collaboration with the UC Berkeley School of Public Health on September 23, 2015, where different applications of *in vitro* bioassays were the topic of three presentations. The seminar was designed to provide information to the Expert Panel on specific topics identified by the State Water Board as relating to the Expert Panel's charge to assess the feasibility of establishing health-based guidelines for DPR. The Expert Panel focused on the proposed use of *in vitro* bioassays for monitoring because this application requires the same grounding in risk assessment as monitoring for chemicals that have MCLs, health advisories, or notification levels. More detail about the information presented at the Seminar is provided in **Sections 5.3.3.1** to **5.3.3.3.**

5.3.3.1 Design of *In Vitro* Bioassays

The first presenter at the seminar, Dr. Michael Denison of the University of California, Davis, described the design of *in vitro* bioassays and illustrated the use of bioassays in identifying chemical contaminants in environmental samples. He focused on activities associated with established toxicological effects *in vivo*. Dr. Denison emphasized that HTP bioassays, in particular, are best directed at very specific targets. HTP bioassays that address pathways to toxic effects are few compared to the number of pathways known or expected to exist. He illustrated how environmental samples (e.g., water samples) can be tested with a bioassay developed for a specific biological target: the aryl-hydrocarbon receptor (e.g., a nuclear receptor), which is activated by a group of environmental compounds. Three groups of halogenated compounds are known to produce their toxic effects by this mechanism. Most importantly, the adverse health effects induced by these compounds are proportional to their dose-response interactions with this receptor; therefore, the application of this bioassay to environmental samples is straightforward. Other bioassays can be (and have been) developed that target specific steps in a range of biological processes affected by environmental chemicals; however, in most cases, the direct relationship of bioassay responses to adverse health outcomes remains to be established.

5.3.3.2 Evaluation of the Use of Bioassay Datasets for Risk Assessment

Seminar presenter Dr. Kevin Crofton of the USEPA's National Center for Computational Toxicology (NCCT) addressed the evaluation of massive datasets developed using large arrays of HTP bioassays. Dr. Crofton also reviewed the reasons why the **TOXCAST** activity was undertaken by the Office of Research and Development (ORD) of the USEPA. It had become clear that TSCA- and FIFRA-mandated testing of chemicals in commerce or under development would require hundreds of years to complete (Dix et al., 2007); therefore, the major purpose of the ToxCast program is to determine if *in vitro* methods could be more efficiently used to streamline chemical testing for both human and environmental effects. To this point, the program has focused on the detection of biological activities important to the elicitation of adverse health outcomes that can be managed in HTP formats.

The effort has focused on developing computational models that use prior *in vivo* data available on chemicals to determine the extent to which *in silico* and *in vitro* models are able to predict *in vivo*

Chemical Dose

The dose of a chemical can be expressed in several ways.

In drinking water, the focus is generally on the external dose, which is the dose used in developing Maximum Contaminant Levels.

In clinical pharmacology and toxicology, it is preferable to express the dose in terms of the concentration delivered to the targeted cell *in vivo*.

The relationship between the external dose and the concentrations at the target cell is arrived at using pharmacokinetic modeling.

Sometimes, the dose is more appropriately expressed as the area under the plasma concentration-time curve (AUC).

Key Event

A key event is one of several necessary steps in the AOPathway that must occur for the adverse health outcome to develop. A specific duration of the induced effect may be required for it to be transmitted down the AOPathway.

effects. This approach relies on historical testing data from whole animals performed under a variety of federal programs, including the Office of Pesticides Programs, TSCA, FDA, and the National Toxicology Program.

Exposure screening methods have been developed by the USEPA's ExpoCast database to generate a projected multimedia exposure estimate. To better connect these projected external exposures to *in vitro* bioassay concentrations, primary human hepatocytes are used to estimate two important pharmacokinetic variables for individual compounds: (1) metabolic clearance and (2) measures of plasma protein binding *in vitro*. ToxCast uses pharmacokinetic data to convert *in vitro* concentrations used in the bioassays to oral exposure equivalents through an approach called "reverse toxicokinetics" (Wetmore et al., 2015). Estimates of these two pharmacokinetic variables are unlikely to be dependably accurate for any individual chemical within two or three orders of magnitude and do not take into account important variables, such as reactive metabolites and excretion through the lung, gut, kidneys, or skin. Nevertheless, these data allow a crude, but more meaningful, comparison of the **DOSES** than the effective concentrations used in *in vitro* bioassays to those that produce adverse health outcomes *in vivo*. In other words, the approach provides a way of considering likely exposure to the chemical at the first tier or screening level of a decision tree and is a real innovation.

A second, critical aspect of improving the predictive capability of *in vitro* bioassays is the development of the concept of AOPathways (Allen et al., 2014) that describe the **KEY EVENTS** that must be activated along that pathway for a chemical to produce an adverse health outcome. It involves incorporating the response-response relationships between key events in the pathway into a computational model. The AOPathway is independent of the chemical. In other words, sufficient activation of the AOPathway will produce the indicated toxic effect. Notably, this type of quantitative modeling has yet to be completed for any AOPathway. Quantitative models (including pharmacokinetic models) will allow *in vitro* bioassay data to estimate risk that is consistent with current uses of *in vivo* data to estimate risk for purposes of establishing MCLs.

The AOPathway is a sequence of steps (e.g., molecular, cellular, tissue, and whole animal events) that leads to an adverse health outcome. The general structure of the AOPathways is outlined in **Figure 5-2.** It is activated by an "initiating event," usually an effect at the molecular level. The initiating event and subsequent effects known to be required for the development of the adverse health effect are referred to as "key events."

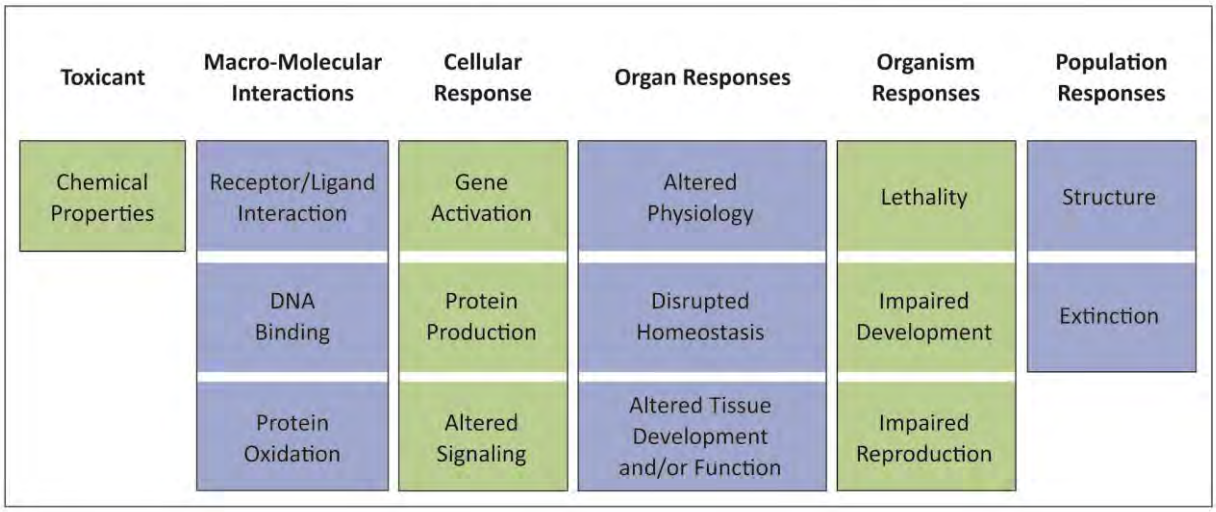

Figure 5-2: General elements of an Adverse Outcome Pathway (AOPathway) (AOP-Wiki, 2015). Please note that the descriptors under each level of effect are not coupled to the next level of biological organization (e.g., molecular, cellular, organ, organism, or population levels.

The intent of the ToxCast approach is to develop a set of bioassays that (1) are predictive of adverse health effects on a broad scale (eventually) and combine these predictions with exposure estimates for a rapid, cost-effective approximation of thresholds of "risk" and (2) helps prioritize chemicals for further testing (Dix et al., 2007). With this approach, many potentially important endpoints can be examined at the same time; therefore, it becomes more like whole animal testing in which a multitude of activities of importance to the development of adverse health outcomes can be detected as a result of conducting more bioassays.

5.3.3.3 Application and Interpretation of Bioassays for Water Quality Monitoring

Seminar presenter Dr. Richard Bull, a member of the Expert Panel, focused on the application of individual bioassays to water samples and how these data should be interpreted. Specifically, he identified challenges that need to be addressed if these bioassays are to be applied as monitoring tools. Several key concepts introduced by the previous speakers were elaborated upon in Dr. Bull's presentation.

The first point is that the use of receptor-reporter constructs incorporated in cells *in vitro* only provide a partial view of what happens *in vivo*. The activation of a pathway at the molecular level is subject to modification by interactions of **FEEDBACK LOOPS** within a cell, among cells in a tissue, between tissues, and by the neuroendocrine system. These interactions will not be consistently visible *in vitro* and may act as key events in AOPathways.

Feedback Loop

A feedback loop is an approach used for controlling a biological process through signals that arise from too little or too much activity of a pathway.

Feedback loops are characteristic of biological systems that maintain homeostasis, such as (1) systems that regulate body temperature or (2) fine adjustments of metabolic and signaling pathways within the body (for instance, the regulation of hormone levels is an example relevant to bioassays proposed for use in water).

Toxicodynamics

Toxicodynamics is the quantitative relationships among key events in an AOPathway.

Models of the interactions and final outcomes make use of selected elements of systems biology.

Frequently, activities will be observed that are not involved directly in the adverse health outcome.

More detailed models are needed to determine if these activities do play a role in the adverse health outcome.

Most of these "side" activities are not well understood and occur after the expression of genes.

Only those steps known to be involved are included in a current model that is usable, but subject to periodic updates with additional data.

AOPathways can have many steps, but most bioassays being considered for water analyses are directed at a single step within the pathway. Activity at another point in the signaling pathway may not be detected. The AOPathway can be activated or inhibited independently of the step being assayed (more detail is provided in **Section 5.4).** In some instances, the adverse health outcome occurs as a result of the need to downregulate the activity initiated by the active chemical. Because of the design of HTP bioassays, a negative result in a bioassay directed at a single point within the AOPathway does not guarantee an adverse health outcome will not occur. Adverse health outcomes can be produced by more than one AOPathway; consequently, a negative result from one assay or a limited number of assays (or AOPathways) cannot be interpreted as "no risk of developing an adverse health effect."

Dr. Bull addressed the need for the calibration of bioassays. The most important question is whether the results of an *in vitro* bioassay can be calibrated against the risk to health resulting from AOPathway activation *in vivo*. Many factors must be taken into account, but of critical importance are the processes controlling the key steps for the delivery of an external dose to the affected tissue or cells of the person consuming water; therefore, a validated pharmacokinetic model is essential. Modeling is needed of the **TOXICODYNAMIC** relationships between each pair of key events within the AOPathways (which can be looked upon simplistically as response-response relationships along the path). The question that needs to be asked is how much activation or inhibition must occur before Step 1 within an AOPathways initiates Step 2, and so on.

If health effects-directed bioassays are to be used for compliance monitoring of drinking water, the data has to be transformed to a form that relates to risk in a manner equivalent to the health underpinnings of MCLs.

5.4 Applications of Bioassays to Water Analyses

Over the last 40 years, bioassays have been used in water analysis for one of two purposes:

- Screening for a particular biological activity, followed by the subsequent identification of active compounds using chemical analyses (screening and identification).
- Sporadic use for water quality monitoring.

The first of these applications has been a common longstanding approach used in other fields, such as drug discovery (Berg et al., 2012). The sophistication of bioassays used for this purpose has increased dramatically over the past two decades (Shockley, 2015); however, bioassays used in drug discovery are not limited to measuring responses to the initiating event, and include measurement responses to map the pharmacogenomic and proteomic responses in differentiated cells, as well to allow a more finely drawn definition of the AOPathway. The availability of HTP formats in non-differentiated cells for many

in vitro bioassays based on receptor-reporter constructs has significantly increased interest in using bioassays for routine water monitoring, but they rarely capture other elements of the AOPathway represented by the approaches within the drug industry.

5.4.1 Bioassay Use to Guide Chemical Identification of Contaminants in Water

With its introduction in the late 1960s, the Ames *Salmonella*/microsome mutagenicity assay (Ames test) became and has remained an important bioassay for human health safety testing of chemicals and commercial products. At the time of its development, there was considerable debate on the mechanisms involved in chemical carcinogenesis; the Ames test provided the first clear evidence that many recognized carcinogens were mutagens.

Beginning in the 1970s, the Ames test and other mutagenesis assays were used to detect mutagenic activity in samples from drinking water (Loper et al., 1978). If mutagenic activity was detected, the sample was fractionated to identify the mutagens using mass spectroscopy coupled with various separation techniques (e.g., gas, liquid, ion chromatography). Consistent with the decision logic developed by regulatory agencies at the time, the primary aim of these studies was to identify compounds that require testing as carcinogens (*in vivo* data was then, and is now, considered necessary to make risk estimates for carcinogens).

This effort was successful in identifying many mutagenic chemicals in drinking water, most of which arose as a result of disinfection. It was especially useful in identifying many mutagens and, due to more extensive testing, several carcinogens are now known to occur in chlorinated drinking water. Unfortunately, less progress has been made in the past few decades. Although a large number of mutagens were identified, the ability to follow up with whole animal studies has been limited.

A number of short-term *in vivo* assays were conducted in the interim, including initiation/promotion assays on animals that were sensitive to certain types of cancers or were especially sensitive to carcinogens. The resulting data, however, have not been used for quantitative risk assessment because of the way the assays were manipulated to exaggerate effects (i.e., genetically or through the use of additional chemicals) so that carcinogenic responses appeared at earlier time points. Nonetheless, these *in vivo* bioassays were the first to identify acrylamide (Bull et al., 1984) and some disinfection byproducts (Bull et al. 1985; Robinson et al., 1989) as carcinogens. Bioassays for other endpoints have been used sporadically (e.g., cell transformation assays), but are considered too expensive and time consuming to be useful in screening samples of water contaminants for carcinogenic activity.

An example that illustrates the power of bioassays to direct the identification of chemicals responsible for particular health effects was the use of bioassays by Sumpter and his colleagues in the 1990s to investigate the feminization of male fish downstream from municipal wastewater discharges (Purdom et al., 1994). A systematic series of papers first established that **ESTROGENIC ACTIVITY** could be identified in chemically fractionated samples of wastewater effluent using a yeast system in which human estrogen responsive sequences were transfected with a construct that connected an estrogen receptor alpha (ERα) and an appropriate estrogen response element coupled to a *Lac-Z* reporter gene (Desbrow et al., 1998). On the activation of expression of the Lac-Z gene, the enzyme β-galactosidase was secreted into the media in which the yeast grew that contained chlorophenol-β-D-galactosopynanoside. This yellow-colored chemical was metabolized by β-galactosidase into a red compound that could be measured spectrophotometrically. Active fractions were identified in all tested wastewater treatment effluents. Subsequent chemical analysis demonstrated estrone and 17β-estradiol in all samples, with

Estrogen Example

The identification of estrogens as being responsible for the feminization of male fish is a classic example of bioassay-directed identification of toxicologically important chemicals in wastewater. Systematic steps included:

1. Identified an *in vivo* effect.
2. Formed a hypothesis of probable cause.
3. Applied an *in vitro* bioassay to detect chemicals in the wastewater.
4. Chemically identified chemicals responsible for the bioassay results.
5. Experimentally demonstrated that the identified chemicals were present in concentrations capable of producing the effect observed in fish.

ethinylestradiol being identified in one-third of the sampled wastewaters. Other chemicals that occur commonly in wastewater had estrogenic activity as well (Thorpe et al., 2003; Harris et al., 1997), but were of much lower potency and did not contribute significantly to the feminization of male fish (Routledge et al., 1998) in wastewater effluents. It was later confirmed that "response additivity" was induced by synthetic and natural estrogens (Brian et al., 2005), as some counterintuitive synergistic interactions were reported using the yeast system in investigations in the United States during this same timeframe. The strength of the studies conducted by Sumpter and colleagues (only a few of which are summarized here) is that they systematically and quantitatively worked back and forth between *in vitro* screening and *in vivo* confirmation (this degree of follow-up has generally not been employed with bioassays applied to drinking water); therefore, the conclusion that natural estrogens and (to a lesser extent) ethinylestradiol is present in municipal wastewater effluents was firmly established and broadly accepted.

The recent introduction of HTP bioassays has greatly expanded the ability to detect a variety of biological activities with health effects implications. Some excellent work is beginning to appear in the literature on the use of these methods for screening and identification, but interest in using these tools for monitoring seems to have sidelined this non-controversial use of bioassays for water research. Nevertheless, recent examples exist where HTP bioassays have been used to good effect (e.g., Wu et al., 2010, 2014).

Many studies have tested for DNA damage, mutagenesis, and clastogenesis (i.e., the loss, addition, or rearrangement of chromosomes) resulting from disinfecting drinking water. In general, the conclusions have been the same, showing chlorination (in particular) introduces almost exclusively direct acting mutagens at levels much higher than in the source water. Lesser effects are observed with chlorine dioxide, ozone, chloramine, and peracetic acid (Marabini et al., 2006).

Many past studies also have used bioassays for water analyses. A short review of some of these studies is included herein to illustrate the types of research that have been conducted since the advent of HTP bioassay formats. Most studies have been related to the evaluation of treatment processes, but some appear to focus on assessing the value of bioassays for monitoring product water on a routine basis.

5.4.2 Applications in Water Quality Monitoring

The use of bioassays for water quality monitoring requires a more thorough evaluation of the meaning attached to positive and negative bioassay results. It must be clear, qualitatively and from a dose-response standpoint, how bioassay results are linked to adverse health outcomes. The steps necessary to relate *in vitro* bioassay results to adverse health effects in humans are outlined in **Sections 5.3.2.1** to **5.3.2.3.**

Over the past decade, many papers have been published that explored the use of bioassays for the purpose of monitoring water quality. The intent of most studies was to evaluate the addition or removal of these activities with water treatment. Some studies involved using a single or small number of bioassays. A few studies contemplated the question of how these bioassay results could be calibrated against the probability of adverse health effects. The following is a selected review of papers that have seriously ventured into the realm of using bioassays for water quality monitoring.

5.4.2.1 Study by Escher et al. (2014a,b)

Escher et al. (2014a) examined the feasibility of applying 103 different bioassays to the detection of biological activity in wastewater, recycled water, and drinking water. The bioassays were broadly classified as having specific "modes of action" (in this case, meaning that the initiating event involved a specific interaction with a particular protein, such as binding to a receptor, and not the usual meaning of the term as used in pharmacology, toxicology, and risk assessment) or a reactive mode of action where there is (1) a chemical modification of DNA or protein or (2) the initiation of an activity that leads to such damage (e.g., increases in oxidative stress, lipid peroxidation). Responses were observed with five of 25 constructs that reported interactions with nuclear receptors. These five included:

- Pregnane X receptor (PXR).
- Peroxisome proliferation receptor gamma (PPARγ).
- Estrogen receptor alpha (ERα).
- Glucocorticoid receptor.
- Liver X receptor (LXR).

Five of 48 transcription factors gave rise to positive responses. The three nuclear receptors, PXR, ERα, and androgen receptor (AR), and the following other factors associated with "relevant" pathways were included in a dose-response analysis:

- Constitutive androstane receptor (CAR).
- Peroxisome proliferator activated receptor alpha (PPARα).
- AR.
- Glucocorticoid receptor (GR).
- THRα1, not defined.
- RORβ, not defined.
- HSE, not defined.
- HIFa, not defined.
- Nrf2/KEAP system (NFκB).
- p53.

Consequently, 14 bioassays of the original 103 were examined. It should be clear that the bioassays chosen were among a variety of commercially available bioassays of the same endpoint; therefore, considerably fewer than 103 distinct pathways were tested (see Table 1 in Escher et al., 2014a). The final selections were the bioassays that performed the best or had advantages over other options (e.g., common platforms with other bioassays). The criteria for performance appeared to be related to whether it gave rise to positive results with samples concentrated from wastewater.

In addition to the specific assays identified above, Escher et al. (2014a) used the micronucleus assay, SOS chromotest, and umuC assays as measures of "reactive toxicity." The authors focused on DNA damage irrespective of the biological outcome, which is confusing when coupled with the assays employed. The goal of most testing schemes in the past was to identify those interactions that are mutagenic (i.e., changes occur to the DNA sequence being expressed, whether by induction of point or frame shift mutations or by larger DNA lesions, such as deletions or reduplication that can result from errors in DNA repair synthesis) because these are the lesions that result in changes within critical sequences of proteins that modify function and lead to diseases like cancer. In other words, mutations are usually considered key events, and not simply the reaction of the chemical or its metabolite with DNA. Many reaction products with DNA do not lead to mutation. More sophisticated bioassays have been developed to address so-called "non-genotoxic" activities leading to cancer (e.g., the mouse lymphoma assay), but the Expert Panel is not aware of whether or not this assay is available in HTP format.

A heat map is reproduced in **Figure 5-3** that identifies samples from waters that gave rise to positive results from these bioassays. As can be observed in the heat map, there is a pattern of decreasing frequency of positive bioassay results as one moves from the wastewater effluents to ozone/BAC and RO-treated water.

Based largely upon the fact that the responses of these assays were observed in wastewater samples, the authors recommended going forward with the set of bioassays they selected; however, these data were not provided in terms of the equivalence to the reference compound in this graph, making it difficult to assign quantitative meaning to the data. The presentation of the data was complex, and the labeling of tables and graphs was frequently unclear. Samples were taken from wastewater treatment plant effluents and at various stages of the advanced treatment of wastewater, as well as river water and drinking water drawn from more conventional sources. It was not clear whether bioanalytical equivalents (BEQs) were calculated by reference to a dose response conducted in pure water, control media, or in the sample matrix.

Note on Trigger Values

Trigger values were not developed on principles appropriate for placing *in vitro* bioassay results on an equivalent basis with doses that produce *in vivo* effects.

Effect-based **TRIGGER VALUES** were developed for several receptor-reporter based bioassays by Escher et al. (2014b). The approach taken was complex, relying in part upon guideline values developed under the *Australian Guidelines for Water Recycling* (AGWR, 2007) and the relative equivalency of a bioassay result with that of a reference compound. The relative equivalency of the bioassay result from the sample and results with one or more compounds that are recognized as acting through that receptor (i.e., BEQs) has been used in formal analyses of bioassay data for more than 100 years and, in modern times, has been codified under the Clean Water Act in the concept of toxic equivalents (TEQs).

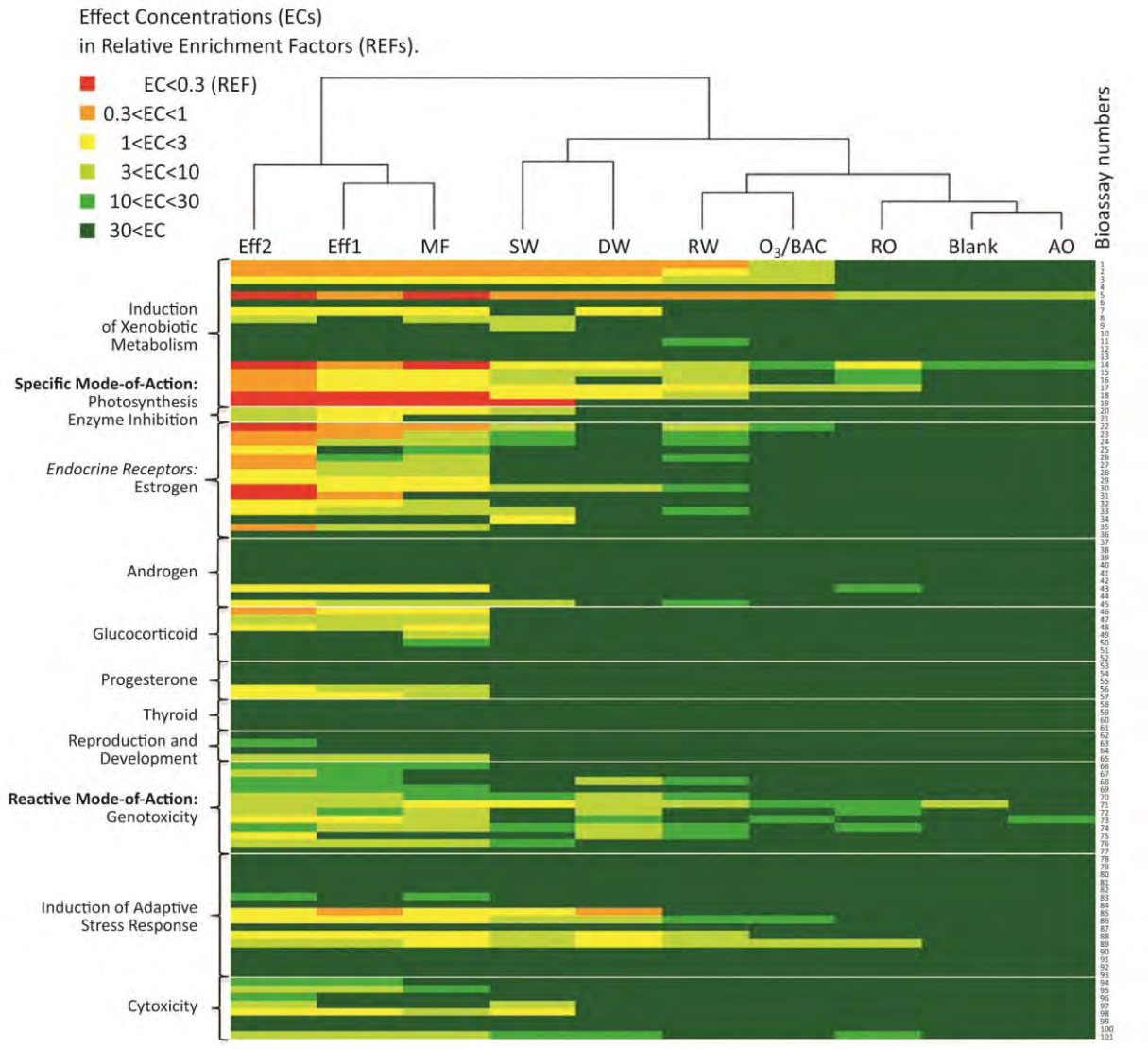

Figure 5-3: Summary of results from 103 bioassays (excluding inactive FACTORIAL and the bacterial cytotoxicity assays). Plotted are the effect concentrations (ECs) (i.e., EC10, ECIR1.5, or ECSR0.2) in units of relative enrichment factors (REFs). The colors encode for the magnitude of the EC. Green (or dark black) stands for high-effect concentrations (low potency) and transitions though the range to red (dark grey) for low-effect concentrations (high potency). Dark green are EC values that were >30 REF (which means that the sample was enriched 30 times and still did not show an effect), green from 10 to 30 REF, and light green from 3 to 10 REF. A sample that has its EC at concentrations of the native sample up to three times enriched is denoted in yellow. Samples that must be diluted for the EC include: orange was diluted up to 3 times (REF 1 to 0.3) and red was diluted over 3 times (from Escher et al., 2014b). The names of the bioassays provided in Escher et al. (2014b) are presented in this report in Table 5A-1 of Appendix 5A. Eff = Effluent. MF = Microfiltration. SW = Surface water. DW = Drinking Water. RW = Recycled water. O_3/BAC = Ozone and biological activated carbon. RO = Reverse osmosis. AO = Advanced oxidation.

Note on Reactive Chemicals

The varying mechanism and widely differing metabolism and pharmacokinetics of "reactive" chemicals make it difficult to determine whether the non-specific nature of the results of the bioassays applied to water samples to identify such activity will have any probability of producing adverse health effects *in vivo*.

The use of guidance values such as MCLs as the "standard" regardless of how they are established is not scientifically acceptable. There are many considerations other than the probability of health effects that are embedded in guidelines, no matter their source (e.g., the ability to measure, cost of compliance, etc.) that make this approach problematic. In the specific case of the AGWR, the inconsistent methods used for the development of guidelines further confuse the issue. Some Australian guidelines were developed by a triage process that is only applied to chemicals for which there are insufficient data to assess risk, called the threshold of toxicological concern (TTC).

Essentially, this methodology places a chemical in a group based loosely on its structure and determines the probable "no observed adverse effect level" (NOAEL) that can be translated to a concentration in water below which the chemical is unlikely to produce an effect (Kroes et al., 2004). The intent of this process is not to set MCLs or the equivalent, but to indicate that the concentration of the chemical (usually a minor contaminant) in a product is so low that there is no need to spend the resources required to develop toxicological data appropriate to use in risk assessment.

It was appropriately noted that pharmacokinetic differences among compounds associated with these receptor-mediated assays were not taken into account. Pharmacokinetic modeling is a critical step needed for bioassays to be used in risk assessment and to derive a valid target value for monitoring.

More problematic were the trigger values termed "effect-based trigger effect concentrations" (EBT-ECs). Bioassays in this group included Microtox (or other cytotoxicity assays) and the activation of biological responses to oxidative stress or the alkylation of sulfhydryl groups that are detected in the AREc32 bioassay (identified as the Nrf2/KEAP system *in vivo*). Here, the trigger value appeared to be arbitrarily related to measures of "activity" following different water treatment processes (from secondary-treated effluent to advanced wastewater treatment). The authors recommend the continued use of this bioassay largely because it was consistently positive; however, the Expert Panel feels the following factors make it extremely difficult to apply and interpret when used for complex mixtures of unknown composition:

- There is a broad range of chemicals that produce oxidative stress.
- There are many different mechanisms that produce oxidative stress.
- There is a constant high level of endogenous production of oxidants in the body.
- There are frequently competing activities produced by many of the same chemicals are at least as likely, if not more likely, to be involved in adverse health outcomes associated with the chemicals.

Because the natures of these chemicals vary so widely and this variety might be anticipated in water samples, there seems to be no way to represent the group of chemicals that would activate this activity in a single pharmacokinetic model. It is probable that the bioavailability of these chemicals could vary

by several orders of magnitude and, as a consequence, the relevance of *in vitro* dose-response data will not easily be related to *in vivo* dose-response.

The relevance of *in vitro* or bacterial "cytotoxicity" assays relative to cytotoxic mechanisms induced *in vivo* are tenuous (Garle et al., 1994), mostly because chemicals that induce systemic toxicity of this type are interacting at a much more specific target and the active form (i.e., metabolite) of the chemical as it is often produced locally. Concentrations achieved in plasma that produce cytotoxicity *in vivo* are usually much lower than those required to produce cytotoxicity *in vitro* screening. Excellent examples include (1) liver toxicity produced by various halogenated solvents and (2) trihalomethanes in which the *in vitro* cytotoxicity appears to be attributable to membrane solvation, but the toxicities are produced by much more subtle interactions of metabolites.

Activity was frequently observed with the AREc32 assay, which detects, among other things, oxidants *in vitro*. The effects of such a compound *in vivo* depend upon whether it is sufficiently stable to be absorbed into the systemic circulation. The compounds that generate oxidative stress as a byproduct of their metabolism will generate reactive oxygen species (ROS) or reactive nitrogen species (RNS) at the site of their metabolism. It seems unlikely that very many of these compounds would be detected *in vitro*. It is incumbent on the researchers using such bioassays to confirm that representatives of diverse set of chemicals do in fact detect these chemicals *in vitro*. Finally, it has to be realized that the dose produced by these latter compounds in the context of the amount of ROS (Oshino et al., 1975) and RNS (Hrudey et al., 2012) produced *in vivo* as a result of normal and abnormal physiological function. Moreover, the robustness of the antioxidant capacity within the body has to be taken into account. Nevertheless, when uncontrolled, endogenous oxidative stress does contribute to disease (Woods et al., 2009; Zhu et al., 2008; Gao et al., 2014). It generally takes very much larger doses of exogenously oxidants to overwhelm the antioxidant capacity of the body. There are exceptions, but chemicals that are potent oxidants *in vivo* involve localized delivery of the oxidant to cells secondary to metabolism.

Activating the Nrf2/KEAP pathway (the basis of the AREc32 bioassay) has been shown to inhibit the effects of a variety of toxicants, including carcinogens (Lau et al., 2008), and has been pursued as a chemoprevention target by the pharmaceutical industry. The skin-sensitization AOPathway employs this bioassay and is easily justified by the fact that this AOPathway focuses on topical exposure. No AOPathway has been proposed for systemic toxicity based on this initiating event. A problem specific to drinking water is that the AREc32 assay will easily detect commonly employed residual disinfectant levels in drinking water (Woods et al., 2009; Wei et al., 2009; Zhu et al., 2008; Jang et al., 2009) in the absence of identifiable systemic health effects in animals at much higher concentrations. Chlorine does give rise to effects in skin, but so far that effect has not been associated with the Nrf2/KEAP system, to the knowledge of the Expert Panel. In part, the problem with interpreting this bioassay to systemic toxicity is a pharmacokinetic one, but it is also difficult to identify a set point of activity *in vivo* above which an adverse health outcome would be anticipated because no mode of action or AOPathway has been assembled and peer-reviewed.

The EBT-EC derivation (Escher et al., 2014b) appears to have no clear and consistent linkage to adverse health effect outcomes *in vivo*. Nevertheless, the bioassays appeared to detect activities in wastewater effluents, which persist through microfiltration, but were undetectable after treatment with reverse osmosis (RO), ozone (O_3)/biological activated carbon (BAC), or advanced oxidation processes (how general this pattern will be for advanced oxidation is not clear as it follows RO in most, if not all, systems).

The other reactive mode of action that was evaluated was mutagenicity using several different bioassays (Escher et al., 2014b). In this case, mutagens requiring metabolic activation were consistently detected in wastewater effluent and the activity was reduced after RO, advanced oxidation, and O_3/BAC. These compounds were not detected at an REF >30 in river water or surface water. Some activity was observed in drinking water derived from a conventional source, but it was less than that observed in source water. Direct acting mutagens were also detected in wastewater at similar REFs as the mutagens requiring metabolic activation; they were less effectively removed by RO, but were decreased by advanced oxidation and O_3/BAC.

5.4.2.2 Additional Studies

Two additional papers published by the same authors address issues related to the use of *in vitro* bioassays for water monitoring. The first paper provides a more focused look at demonstrating the ability of 36 bioassays to detect 18 "biological" endpoints (Jia et al., 2015) in a water recycling plant that was ultimately subject to soil aquifer treatment as the water was introduced into an aquifer. Several bioassays detected activity and identified treatments of the water that reduced or increased activity. Significant differences in the sensitivity among bioassays putatively addressing the same endpoint were observed. No explanation of these differences was provided, nor were the bases of these differences investigated or explained. While those that addressed the activation of nuclear receptor-constructs could be associated with a draft AOPathway, the selection criteria for other assays were vague and appeared primarily based on sensitivity rather than relevance to adverse health outcomes. The data were not presented with background as to how these results would be used to impute a hazard to health, as no reference was made to AOPathways (previously known as "mode of action"; see USEPA, 2005) and the relationship of these results with probable risk.

Bioanalytical Equivalents

Data reporting for receptor-mediated responses should be expressed in terms of the bioanalytical equivalents (BEQs) of a potent, known ligand for the receptor with acceptable statistical treatment of the confidence interval of the estimated response.

Presenting data in terms of REFs, removals, or $EC_{IR1.5}$ does not provide a basis for judging the validity of the data or a basis for estimating the potential health risks that might ensue.

Mehinto et al. (2015) reported on a small study of inter-laboratory comparisons of *in vitro* bioassays applied to recycled water. The calibration of the bioassays based on estrogen receptor (ER), glucocorticoid receptor (GR), and progesterone receptor (PR) constructs (GeneBlazer) with standard compounds was more or less consistent among the five participating laboratories, although two laboratories reported EC_{50} values below the expected range for the PR and ER bioassays. A relatively large inter-laboratory error variation was reported for two wastewater samples (out of five) in the ER assay. The bioactivity of samples was found in parallel samples used for chemical analyses. These waters had the highest concentrations of steroid hormones measured chemically; however, it was indicated that chemical analyses accounted for less than 5 percent of ER activity and less than 1 percent of glucocorticoid activity measured with the bioassays. One reason given for the discrepancy was that chemical analysis was attempted for only four GR agonists and two ER agonists (i.e., drugs that mimic natural hormones). The authors did not attempt to determine whether these activities may have arisen from factors other than direct agonists in the sample. The authors of this paper are to be complemented as they were among the few who **PRESENTED DATA IN BEQS**, which provides context to the issue that is not provided by REFs, BEQ reductions, or $EC_{IR1.5}$. In this case, the BEQs were 6.5 and

1.5 as nanograms (ng) of 17β-estradiol/L in the plant influent and 2.6 BEQ in the plant effluent. Similar values for BEQs were reported for the GR and PR bioassays.

The question of whether the levels of activity at these BEQs are significant from a health effects standpoint was not addressed in Mehinto et al. (2015). This question is critical if these bioassays are to be used for the routine monitoring of water. The Australian guideline for 17β-estradiol is 175 nanograms per liter (ng/L). It would be compared to the Australian guideline value for ethinylestradiol of 1.5 ng/L. The relative binding affinities for the estrogen receptor is 100,000 and 190,000 respectively (Blair et al., 2000). Their minimum therapeutic doses are 0.5 milligrams per day (mg/day) for estradiol (used as a treatment for vasomotor symptoms of menopause) and 0.02 mg/day for ethinyl estradiol (used as a contraceptive) (Lacy et al., 2007). These doses would be closer if estradiol were used as a contraceptive. This example shows that there should not be two orders of magnitude differences in the guidelines for these two estrogens as their *in vivo* activity is within an order of magnitude of one another in terms of dose. The health implications of measured bioassay results should be discussed in papers of this kind and to communicate these implications to the public.

5.5 Data and/or Information Required to Relate Doses Producing Bioassay Results to Those Producing Health Effects *In Vivo*

5.5.1 Establish the Adverse Outcome Pathway(s)

AOPathways are a construct in which a measured biological activity is placed within a pathway that results in functional and pathological effects leading to an overt toxic effect (Tollefsen et al., 2014). This pharmacology concept dates back to the early twentieth century, but was first codified as mode-of-action (MOA) in the USEPA (2005) *Cancer Risk Assessment Guidelines*. The Hill criteria used to evaluate epidemiological data for causal relationships were adapted to evaluate an MOA. The steps of this process will not be iterated here, but they still apply to the establishment of an AOPathway. These pathways are not necessarily activated in linear fashion with dose. For example, the chemical might have to be metabolized by a secondary, lower affinity pathway than the main metabolic pathway (e.g., trihalomethanes; see Bull, 2012) or the adverse health outcome could be the result of downregulation of an over-activated normal signaling pathway by feedback regulation or by activating a compensating pathway (e.g., Kolisetty et al., 2013). One mode of action is provided in **Figure 5-4** to illustrate the latter point.

Two examples of AOPathways that have been introduced into final comment in the ToxCast program are shown in **Figures 5-5** and **5-6.** The first AOPathway, shown in **Figure 5-5**, identifies the initiating event as an inhibition of the estrogen receptor and indicates what will happen if there is a significant inhibition of endogenous estrogen *in vivo*. The second AOPathway, shown in **Figure 5-6**, has essentially the same elements, except that the chemical inhibits the enzyme aromatase, which will decrease the availability of estrogen. The outcomes are the same, but initiating events are different.

Notably, because the HTP bioassays currently in use generally focus on an initiating event, the bioassay measuring aromatase inhibition would not be recognized by the bioassay using the ER-receptor reporter gene. Chemicals affecting one or the other bioassay would have very similar anti-estrogenic effects *in vivo*. In these specific cases, one bioassay would detect tamoxifen, but not anastrozole, letrozole, and exemstane, which are aromatase inhibitors used for many of the same therapeutic purposes as tamoxifen (Buzdar et al., 2002). Some pesticides have been shown to inhibit aromatase (Sanderson et al., 2002), so this is not an AOPathway that is only responsive to drugs.

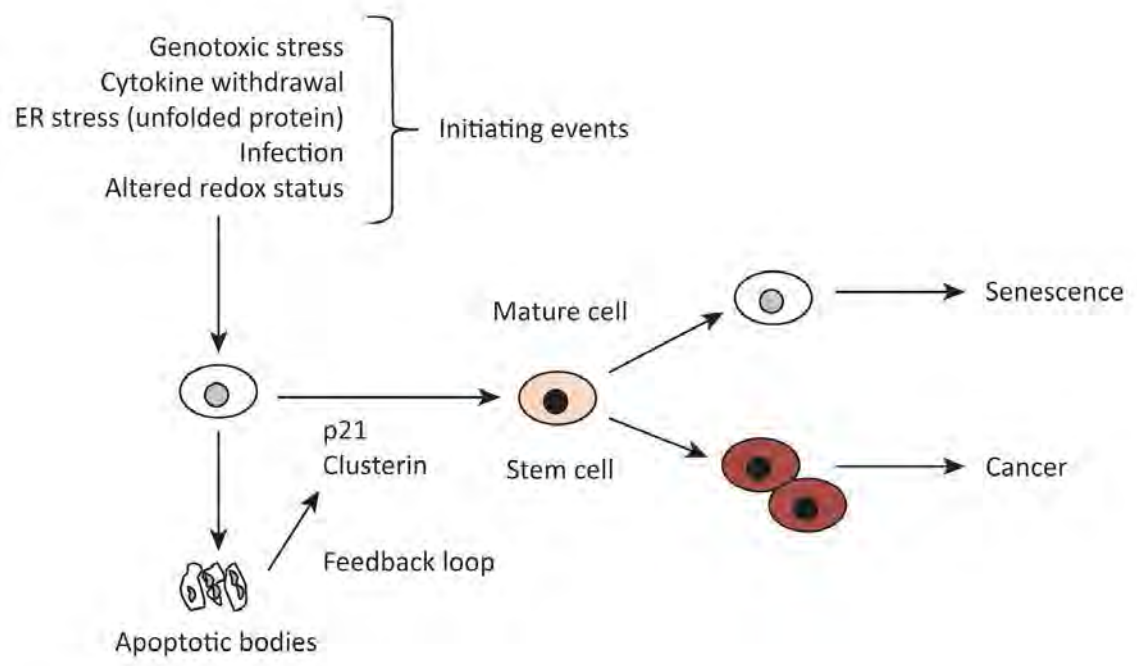

Figure 5-4: **The role of suppressed apoptosis in carcinogenesis. Apoptosis is a process that eliminates old or damaged cells from an organ. A variety of effects from chemicals can trigger this response, illustrated by the list of activities displayed in the upper left hand corner of this figure. The apoptotic cells break up in a characteristic way that give rise to apoptotic bodies and eventually are scavenged from the system. Cells destined for apoptosis can be rescued. Generally, the rescue occurs because of cellular feedback loops, which in this case involves increases in the cellular concentrations of two transcription factors (i.e., p21 and clusterin) that suppress apoptosis. It occurs when the rates of apoptosis become excessive to the point that tissue/organ function is impaired. Some rescued cells can continue to function as normal cells; however, some of these cells may have damage to their DNA that would give rise to mutations upon division, which can put their progeny on the path to develop cancer. Adapted from Bull and Cotruvo (2013).**

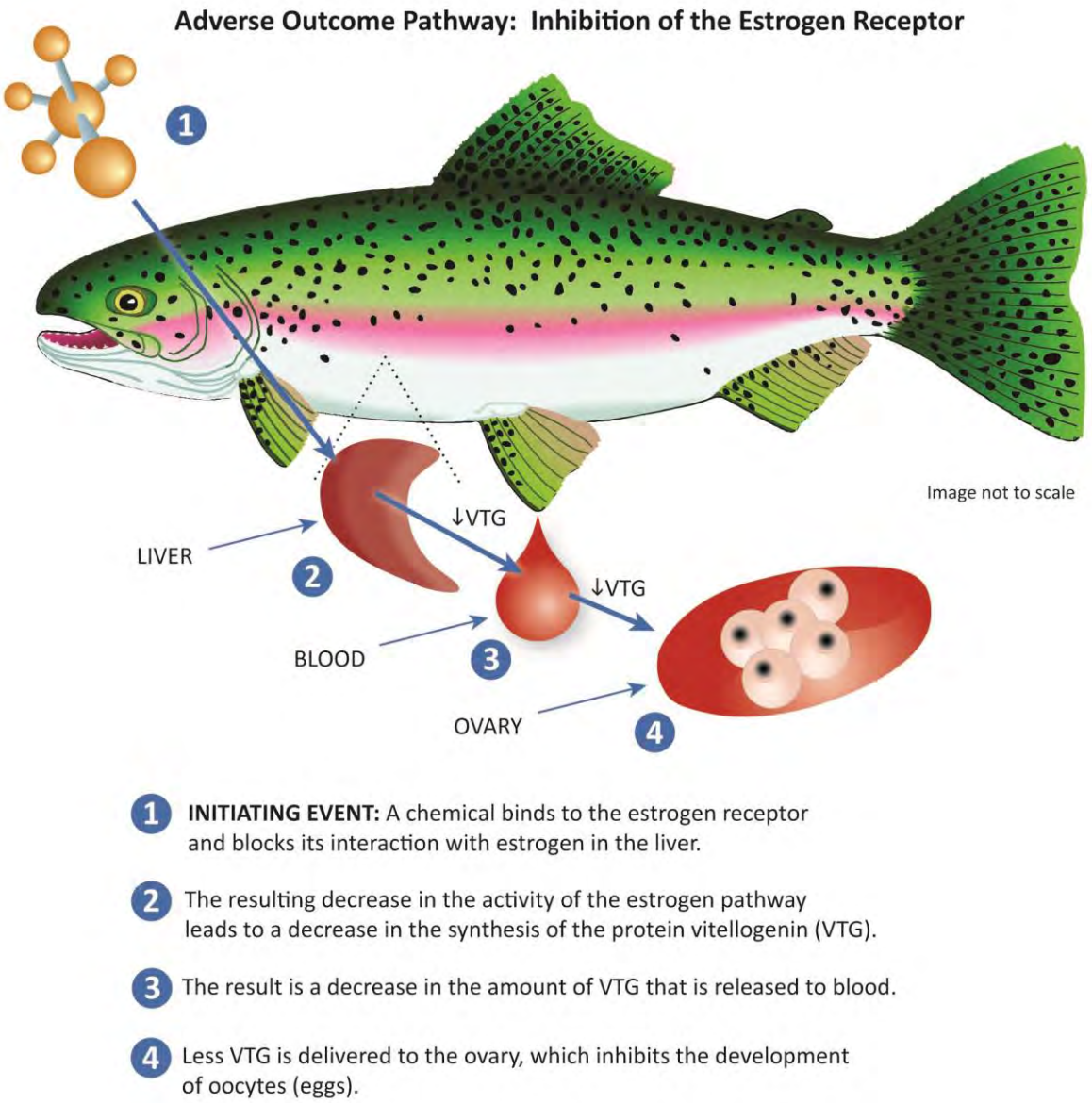

Figure 5-5: First example of an Adverse Outcome Pathway (AOPathway) that leads to reproductive dysfunction in fish. The AOPathway identifies a chemical action that leads to the dysfunction by inhibiting the ability of endogenous estrogen to interact with its receptor (an initiating event). An example of such a chemical is the drug Tamoxifen, which is used in the treatment of breast cancer. The liver protein whose synthesis is depressed is vitellogenin (VTG) by decreased estrogen activity. Reduced VTG in the liver results in less VTG being released to the blood, which in turn, delivers less VTG to the ovary and inhibits the development of oocytes (eggs). If the inhibition of estrogen activity is sufficient, depressed spawning results.

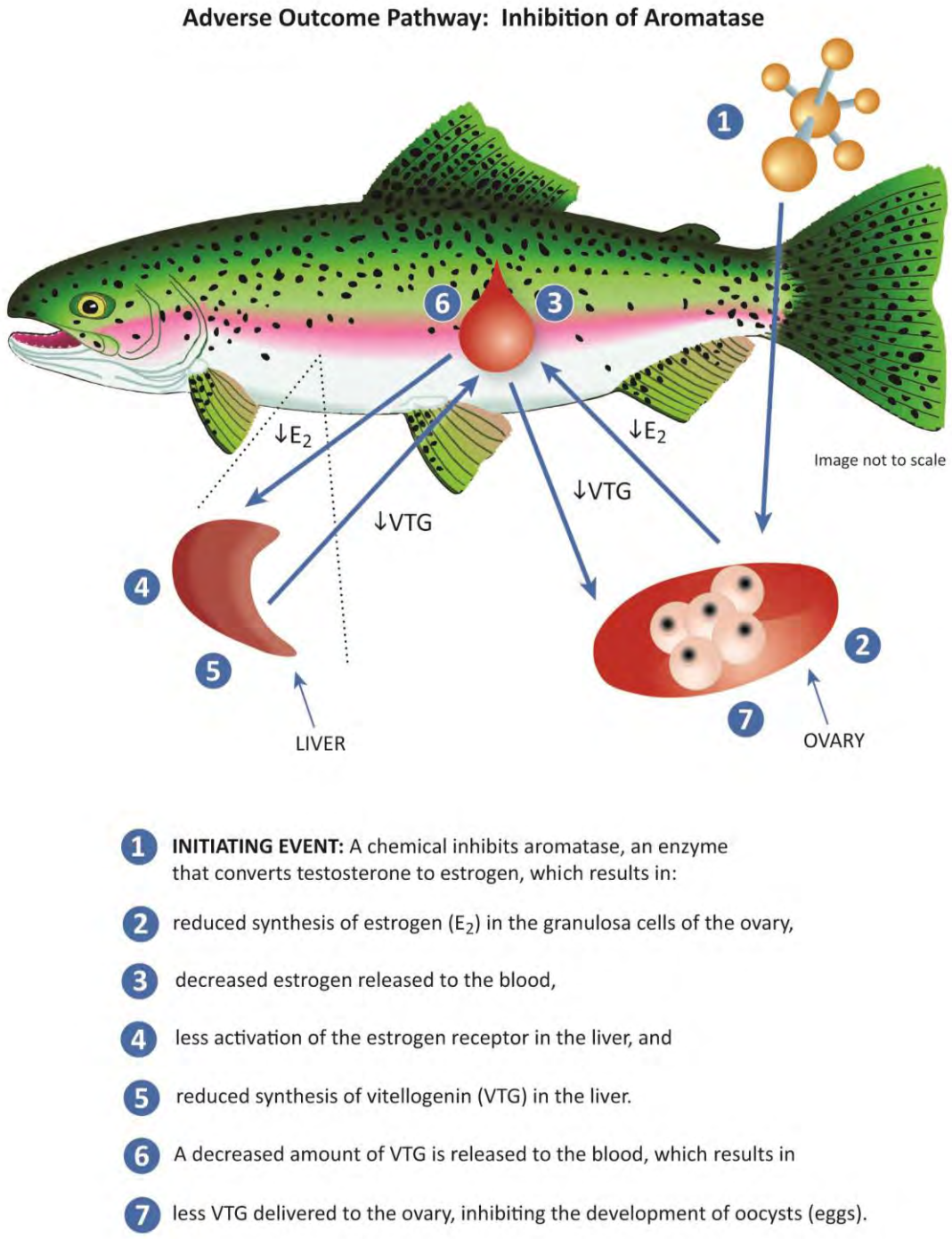

Figure 5-6: Second example of an Adverse Outcome Pathway involving estrogen and reproductive dysfunction in fish. In this case, a chemical inhibits the enzyme aromatase, which converts testosterone to estrogen in the ovaries. An example of such as chemical is the drug Anastrozole, used to treat postmenopausal women with breast cancer. Note that the initiating event in this case is quite different, but that the effect is still to decrease estrogen activity and the downstream elements of the pathway are the same. Different *in vitro* assays would be required to detect these two different ways of affecting the estrogen signaling pathway.

At present, there is only one AOPathway for skin sensitization that has been approved by OECD. According to AOPathway-Wiki (2015), 114 AOPathways are in development, 14 of which are in the process of internal and external peer review by the OECD. Modeling of the response-response relationships within AOPathways has not progressed far, but there are data in the literature that should enable this process for a few bioassays based upon pathways activated by nuclear receptors where the key events are relatively well understood.

In the absence of validated and quantitatively described AOPathways, the remainder of the discussion will focus on pharmacokinetic models used to associate human exposures with the concentrations (i.e., dose) of a chemical needed *in vivo* to produce an effect. Pharmacokinetic models are developed using datasets on the absorption, distribution, metabolism, and excretion of chemicals in humans or surrogate animals. Concentrations of chemicals in the tissue where the AOPathway is initiated generally will be much lower than those concentrations consumed in drinking water because few chemicals in drinking water bioaccumulate. Consequently, the sensitivity of the bioassay needs to be judged against concentrations that would occur in extracellular fluid after the chemical is consumed.

Reverse Toxicokinetics

The Expert Panel is of the opinion that the "reverse toxicokinetic" method employed for screening in the ToxCast program is not an adequate model for adjusting *in vitro* concentrations active in a bioassay to an *in vivo* dose for the purposes of risk assessment equivalent to that employed for the development of maximum contaminant level goals (MCLGs) and public health goals (PHGs).

Addressing such questions is relatively straightforward for single chemicals, but becomes problematic for complex mixtures of chemicals where:

- The identities of the chemicals are unknown unless the assay is coupled with a chemical analysis.
- The range of the physical and chemical properties of the chemicals that elicit the response is too broad to assume their likely metabolic and pharmacokinetic properties are sufficiently similar to use a common pharmacokinetic model to estimate their concentrations in blood plasma after exposure.

In the opinion of the Expert Panel, the **REVERSE TOXICOKINETIC APPROACH** employed for approximating human pharmacokinetic variables using *in vitro* screening procedures in the ToxCast screening program ***is not*** appropriate for estimating risk in a way that is consistent with the risk assessments that underlie the development of MCLs. The reverse toxicokinetic approach does not address absorption, reactive metabolites, or clearance via urine, bile, or sweat. Moreover, genuine pharmacokinetic models are available for most drugs, including those that are endocrine active. In these cases, there is no reason for a screening method to replace a model developed based on human data.

Notably, the ToxCast approach is fundamentally different from the application of individual assays for screening specific endpoints or biological activities. ToxCast is designed for screening a wide variety of biological activities that could be important to public health and is not restricted to HTP bioassays (Dix et al., 2007). HTP approaches were the main focus of questions raised by the Science Advisory Panel

report to the State Water Board because HTP bioassays are used more easily for monitoring complex mixtures, but address very targeted endpoints rather than actual adverse health outcomes.

As noted in **Section 5.2.3.3.,** although HTP bioassays are usually directed towards molecular initiating events, AOPathways also include key events at the cellular, tissue, and whole body levels, as well as compensatory processes (which are frequently involved in the development of pathology). It is important to know (with some confidence) the identified key events that play a role in producing an adverse health outcome, as it helps to establish the relationship of bioassay results to adverse health outcomes.

5.5.2 Scaling of Dose-Response *in Vitro* to Dose-Response *in Vivo*

To properly scale a chemical's *in vitro* dose-response to its *in vivo* dose-response, it is necessary to model the pharmacokinetic variables that determine the dose delivered to the affected tissue subsequent to a given external exposure. The principles involved are described by Yoon et al. (2012). It also is useful to have a way of estimating the effective dose to the molecular target *in vivo*, which necessarily requires quantitative measures of the metabolism of the chemical and an understanding as to whether it is active itself or that a metabolite serves as the active toxicant (e.g., acrylamide; see Fennell et al., 2015).

For practical reasons, it often is assumed that the concentration of the metabolite in the tissue is a function of chemical concentration in the medium that delivers the parent chemical to the cell (usually assumed to be blood plasma). It is widely recognized that the relationship of a metabolite concentration *in vivo* can be non-linear relative to the external dose based on the kinetics of the process forming the metabolite and those factors that inactivate it. At the low doses generally experienced by humans, these relationships tend to be in the linear range; however, in whole animal testing (where maximum tolerated doses are administered), non-linearity is common.

Many examples exist to illustrate the application of pharmacokinetic modelling to chemicals. The following drinking water contaminants have had pharmacokinetic models developed:

- Chloroform (Corley et al., 1990).
- Bromodichloromethane (Lilly et al., 1997)
- Bromate (Bull et al., 2012; Campbell et al., 2013; Bull and Cotruvo, 2013).
- Dichloroacetic acid (Li et al., 2008) and brominated analogs (Schultz et al., 1999).
- Trichloroacetic acid and brominated analogs (Merdink et al., 2001)
- Trichloroethylene (Kim et al., 2009)
- Formaldehyde (Schroeter et al., 2014).

Most drugs, including natural and synthetic hormones used in medicine, have pharmacokinetic data that can be easily adapted to the task of determining whether a bioassay result can be adjusted to *in vivo* exposures of these compounds. More problematic in this respect would be phytoestrogens, most disinfection byproducts, and industrial chemicals that might occur in water.

The practical consequences of the processes that control the dose delivered to tissue usually require that chemicals in drinking water are active *in vitro* in low micromolar (µM) to low picomolar (pM) concentrations to be relevant to the *in vivo* doses to animals or humans that initiate adverse health effects. Even lower *in vivo* concentrations would be anticipated for human exposure to chemicals in drinking water.

To address this problem for chemicals in which pharmacokinetic data do not exist, the USEPA has used **METABOLIC CLEARANCE** by **PRIMARY HEPATOCYTES** and **PLASMA PROTEIN BINDING** *in vitro* to partially rectify the bioassay doses to human exposures from the environment. This crude approximation provides a basis for comparing the concentrations required to activate a bioassay to those concentrations of chemicals required in plasma that produce adverse health effects (e.g., see Wetmore et al., 2015). Thus, the method allows a better assessment of exposure for screening purposes, although a better characterization of the pharmacokinetics of chemicals will be needed if bioassays are to be used for risk assessment, as is done for the development of MCLGs and PHGs.

A second common use of pharmacokinetic analyses in toxicology is to determine which of several candidate modes of action attributed to a chemical based upon *in vitro* data are consistent with the doses relevant to the induction of adverse health effects by the chemical *in vivo* (e.g., bromate; see Bull and Cotruvo, 2013).

Dose Response Terminology

METABOLIC CLEARANCE: Volume of biological fluid completely cleared of drug metabolites as measured in unit time. It is a measure of rate at which a compound is removed from the body by metabolism.

PROTEIN BINDING: Generally refers to the portion of the concentration in blood that is bound to plasma proteins. The fraction of the chemical bound to these proteins is not directly available to tissues or eliminated in urine.

HEPATOCYTE: The cell in the liver responsible for its specialized functions. Primary hepatocytes are commonly used to simulate in vivo metabolism of chemicals. Continuous cell lines used in bioassays typically do not have this capability.

5.6 Application of Bioassays in Health Effects Testing

5.6.1 Batteries of Assays Directed at Predicting a Single Adverse Health Outcome

Many studies have been undertaken to determine if the predictive ability of assays targeting a single adverse health outcome is improved when employed in groups ("assay batteries"). Different combinations were evaluated over time to determine whether a group of genotoxicity assays better predicts the likelihood that a chemical is a carcinogen, given the high rate of apparent false negatives and false positives of individual assays. A recent set of papers came to the same conclusions as previous publications, namely that results indicate the predictivity of assay batteries did not improve significantly upon a single genotoxicity assay, regardless of whether the assays were based on bacteria or mammalian cell lines (Kirkland et al., 2005, 2006).

Prior to these analyses, it had become apparent that a significant fraction of rodent carcinogens did not act by genotoxic mechanisms, which led to investigations of test systems to detect both non-genotoxic (epigenetic) and genotoxic carcinogens (Benigni and Boassa, 2011; Cunningham et al., 2012; Benigni et al., 2013). The use of a combination of assays recognizing different mechanisms of action resulted in an estimated sensitivity of 90 to 95 percent, demonstrating that alternative AOPathways (modes of action) to mutagenicity are involved in carcinogenesis (or, the production of cancerous cells).

Judson et al. (2015) described an integrated application of 18 HTP bioassays directed at the detection of estrogen receptor activation by 118 of 1,182 chemicals tested. The Judson et al. (2015) approach provides an improved logic for developing and evaluating batteries of bioassays targeting various steps in an initiating event. These assays included the following steps:
Reporter gene constructs for both estrogen receptors ERα and ERβ in several formats.

- Cell-free estrogen receptor binding assays.
- Protein dimerization.
- Transcription factor activity by reverse transcription polymerase chain reaction (RT-PCR).
- Cell proliferation.
- Pseudoreceptor activity [which detects low-level activation by Bisphenol A (BPA)].

In effect, the performance of these bioassays could be evaluated at various stages in the signaling pathway activated through the estrogen receptors. Finally, the Judson et al. (2015) approach initiated a systematic study of assay interferences (finding cytotoxicity to be the most common) and also provided a generic model for evaluating AOPathways that can be conceptually transferred to other systems.

5.6.2 A Collection of Bioassays that Addresses Multiple Initiating Events and Associated AOPathways

Applying a large number of bioassays to different waters based on their degree of treatment would substantially increase the sophistication of screening and identification applications of bioassays. A **COLLECTION OF BIOASSAYS** addressing different AOPathways provides much broader coverage of potential health effects than a single bioassay addressing only one AOPathway. The breadth of endpoints covered by the USEPA's ToxCast and Tox21 efforts has an inherent advantage over applying single targeted bioassays. For example, as collections of bioassays begin to deal with an ever-expanding number of defined targets that address a larger set of AOPathways, public confidence will increase in the predictive ability of this approach to screen for potential adverse health effects. In the interest of initiating the ToxCast program, the USEPA has made some shortcuts in the selection of bioassays. The selection was based largely on the availability of HTP assays, most of which were of interest to the pharmaceutical industry; therefore, a large fraction of the bioassays applied so far are used in screening for known drug targets. In other words, in this initial period, bioassays were not selected by ToxCast to represent known toxicological pathways or even a large fraction of them; however, the current set does include some pathways of toxicological importance that are of interest for DPR and drinking water.

Advantage of a Collection of Bioassays

Collections of bioassays addressing multiple advanced oxidation pathways (AOPathways) have an advantage over a small number of bioassays directed at the same AOPathway or a smaller number of AOPathways.

The advantage is that they identify more distinct activities of toxicological interest at the screening step.

In themselves, however, they do not provide a basis for risk assessment consistent with USEPA practice in the development of maximum contaminant levels (MCLs).

While the selection includes some AOPathways of interest in toxicology, there are many other important targets that are not represented by the bioassays available at present. Many currently used assays are difficult to connect to an AOPathway, in part because most pathways are still poorly understood; however, because of the potential savings in costs for testing new products, time savings, and animal welfare considerations, there is strong incentive in the private and public sectors to develop additional assays to address these shortcomings.

From a scientific point-of-view, the application of the ToxCast bioassays would not address the risk assessment questions that are highlighted in this chapter; therefore, it is premature to consider using this approach for routine monitoring. Second, it is much too expensive for this purpose. A rough estimate provided at the Specialty Seminar on "DPR in California" was around $35,000 to $40,000 per sample for about 200 assays; however, these costs will decrease significantly as sample numbers increase (due to the economies of scale resulting from the use of robotic technology).

Cost/Benefit Analysis of Drugs

It is important to recognize that cost/benefit evaluations for the approval of pharmaceuticals are different than evaluations for protecting humans from environmental hazards.

Patients are willing to accept some risk of harm if pharmacological activities of the drugs are effective in treating their diseases.

The costs and benefits relative to environmental standards generally address benefits to one party and costs to another, and the tolerance for harm is much less.

While the ToxCast group of bioassays has many of the same shortcomings in predicting adverse health outcomes *in vivo,* as discussed previously (Thomas et al., 2012), this approach can further expand and define a range of biological activities present in various sources of water and finished drinking water. It would also improve the "benchmarking" of different water sources available to date. Aside from a larger collection of bioassays, the ToxCast program would allow appropriate pharmacokinetic models to be applied where available and use "reverse toxicokinetics" where models do not exist, but only when a specific chemical has been shown to be responsible for the bioassay result. The use of an established pharmacokinetic model would place exposures to these activities into a better context, but this collection of assays will remain screening assays (i.e., Tier 1). To be clear, the Expert Panel is not recommending this approach for routine monitoring of water; if it is undertaken, it should be applied only once, perhaps focusing on samples collected over four seasons of a single year.

The benefits of this approach include:

- A more thorough benchmarking of water sources, with the added benefit of including consideration of the actual pharmacokinetics of chemicals where models have been published and the reverse toxicokinetic screening of those for which models do not exist. A broader benchmark would provide a better indication of whether exposure through water needs to be investigated further and whether or not the results of the bioassays were positive or negative.

- It could identify additional bioassays that are sufficiently sensitive to chemicals in wastewater to warrant pursuit with targeted screening. Such findings would identify a research need for developing AOPathways for those bioassays that appear sensitive (presumably, ToxCast would pursue such a clearly identified research need).

- The Expert Panel is of the opinion that partial financial support from the USEPA might be available for such a project because it would point to activities of potential importance in regulating water reuse as a drinking water source in the future.

- The study would provide the National Center for Computational Toxicology with a platform for demonstrating the performance of their bioassays with samples of complex and variable composition that could gather support from programs other than the TSCA and FIFRA.

The State Water Board might also be interested in such a project. There could be significant cost savings if participating utilities would take on the costs of the sampling program; however, it is important that the collection of samples be done consistently with good quality control (see **Section 5.7.2**). USEPA's Duluth Laboratory should be consulted as it has experience in obtaining samples from water compatible with the bioassay systems employed by ToxCast.

Finally, ToxCast's current collection of available bioassays includes many receptor assays meant to screen for the pharmacological activity of chemicals. Thus, the ToxCast bioassays could provide a broader assessment of whether pharmacologically-active compounds (i.e., drugs) are present in wastewater and/or advanced treated water at effective concentrations. Fortunately, data obtained with this study could be adjusted easily to measures of human dosimetry because most modern drugs and endocrine disruptors (and many older ones) have existing pharmacokinetic models. Based on the low concentrations of pharmacologically-active agents in water investigated to date, there is little reason to believe pharmacologically-active compounds represent a risk (Bull et al., 2012); this effort would provide a more definitive answer.

5.6.3 Bioassays Applied Individually for Monitoring

At present, the bioassays that appear suitable for monitoring are confined to a subset of nuclear receptor-activated reporter assays. These assays are activated by the very specific association of a ligand within the binding pocket of a receptor. Ligands bind to specific agonists and antagonists will bind with high affinity; however, there are regulated binding sites on receptors or associated cofactors that can enhance or reduce the activity of receptors via feedback loops, which could be a problem with a complex mixture of unknown composition. Aside from sources of interference of this type, the specificity of assays can approach the specificity of analyses for specific chemicals. It should be noted that non-receptor bioassays (e.g., aromatase inhibition) could be linked to an adverse outcome as described in **Figure 5-6**.

In cases where "reactive" modes of action trigger a bioassay response [e.g., the Nrf2/KEAP system used by Escher et al. (2014a), designated as the AREc32 assay], there is not a clear way for normalizing *in vitro* doses and those doses that cause adverse health effects *in vivo*, especially in the complex mixtures that will be recovered from water using solid phase extraction protocols. The difficulty is that the ranges of chemical and physical properties of the chemicals and metabolites that induce these responses could be very broad. Some chemicals will be highly reactive with the KEAP protein at low doses *in vitro*. Drinking water disinfectants activate this system *in vitro* at residual concentrations that are required in drinking water (Woods et al., 2009; Wei et al., 2009; Zhu et al., 2008; Jang et al., 2009), but generally will not be absorbed intact. Some of their oxyhalide anion byproducts are absorbed (e.g., chlorite, bromate) and may produce effects via this mechanism, but they will be much less potent in this assay than the disinfectants *in vitro*. Organic chemicals and metals (Wang et al., 2010) will interact with endogenous molecules to generate the reactive oxygen species (ROS) as part of their metabolism. In some cases, the

interaction resulting in ROS has been postulated as being responsible for the toxicity, but alternate explanations exist that are, in many cases, more convincing modes of action (e.g., covalent binding to macromolecules). Most importantly, there is a tremendous amount of such activity generated by normal intermediary metabolism (Oshino et al., 1975) that overshadows that which might be produced by most exogenous chemical exposures, unless the doses were extreme.

It is essential that the interpretation of bioassay results used in compliance monitoring of water be clearly embedded in the science that relates these results to risk. The description of the process used to arrive at risk equivalents must be well documented (e.g., Crump et al., 2010; Wetmore et al., 2011, 2013, 2014; Zhang et al., 2014). "Target levels" derived from risk assessments must be as firm as those that underlie the derivations of MCLGs and PHGs for individual chemicals.

The complexity of the issues of applying bioassays to the routine monitoring of water quality suggests that bioassays should not be applied to this purpose without undergoing a review process that establishes the conditions under which the results of these bioassays might be used and the results accepted and acted upon by the utility and/or State Water Board.

5.7 Approach Recommended for Certifying Bioassays for Use in Water Monitoring

5.7.1 What Process Should Be Used to Validate a Health Effects Bioassay for Application to Water?

Most bioassays, especially HTP assays, measure the activity of a discrete point within a single AOPathway (usually at the point of "initiation"), but may not detect the activation of the same AOPathway initiated at another point in the same system (see Figures 5-5 and 5-6). For reasons of this type, bioassays that are proposed for use in water quality monitoring should undergo a review process that involves the following:

- Identifies the principles of the bioassay.
- Certifies the technical description of the application of the bioassay.
- Provides a clear description as to how the results will be interpreted, precisely defining what has and has not been measured.
- Clearly documents and clarifies claims that a bioassay will detect interactions among chemicals in the sample. Vague claims unsupported by evidence need to be avoided.

Considering the rapid pace at which bioassays currently are being developed, the review process must also:

- Provide for determinations of whether new and/or improved bioassays deliver equivalent or superior results.
- Be time-responsive.

5.7.2 Validation of Assay Results

The need to identify the expectations for a particular *in vitro* bioassay is an essential part of its validation. The usual requirements for calibration and quality control that applies to any analytical method also should be required of bioassays. The specifics of these requirements will need to go

somewhat beyond those of chemical analyses. Examples include quality control issues that arise as the result of using intact cells as the platform on which many bioassays are based and issues of non-specific interference with receptor binding assays. Expectations may include:

- The bioassay actually detects the compounds it is designed to detect.
- Dose-response characteristics are kinetically consistent with the interaction of standard compounds with a receptor.
- The usual dynamic range of the bioassay is identified in which consistent data can be expected with standard compounds. Likely causes of false negative and false positive results should be identified.
- Conditions are identified where there may be interference with the bioassay.
- Application to water analyses is standardized, including methods for concentrating chemicals from water.
- Consensus is reached regarding the units by which results are to be expressed. The preferred units would be BEQ with a known active compound.
- Procedures are developed for ruling out false positive or false negative results.

More detail is provided in **Sections 5.7.2.1** to **5.7.2.5.**

5.7.2.1 Sample Preparation Is a Critical Issue in the Application of Bioassays for Monitoring

Although the methods of sample preparation were described in some detail in the literature reviewed by the Expert Panel, the actual sample recovery was dealt with vaguely. The term "relative enrichment factor (REF)" has been used, but the parameters of this factor are not described in the publications reviewed. It is assumed REF means that it is a nominal concentration factor without any reference to the recovery of chemicals that produce the measured activity. Recoveries of selected chemicals targeted by the bioassay should be examined by the addition of standards to the water sample before it is concentrated and chemical recovery is quantified. In this instance, the bioassay should be the measure of recovery, as the Expert Panel noted that some additional clean-up of samples was generally used for chemical analysis relative to that for bioassays. Poor recovery could also reflect some interference with the bioassay by the sample matrix.

5.7.2.2 Performance Evaluation of Bioassays Should Be a Routine Practice in the Applications of Bioassays for Water Monitoring

A list of affinities of chemicals (reciprocal of the K_m) that are known to interact with each receptor should be assembled and made available. The K_m for interactions (i.e., agonist or antagonist) and the dynamic range of the bioassay should be criteria used to evaluate various engineered *in vitro* bioassay systems. Operationally, these constants should be compared with the same variables in the sample and process controls. The fact that there will be cross-reactivity with ligands that target other nuclear receptors should be noted. These ligands generally will have much lower affinity for the target receptor-reporter construct being used; therefore, this type of interference is likely to be observable only at high concentrations of other nuclear receptor ligands, but ligand cross-reactivity should be considered if interference with the bioassay is suspected. In normal cells and cancer cells, the receptors can be activated by other mechanisms (Campbell et al., 2001); if activity cannot be accounted for by chemical

analyses, the possibility of bioassay responses due to other forms of action than receptor-ligand binding must be explored. It was noted that high concentrations of thiosulfate were used to quench disinfectant residuals in some studies. Thiosulfate will also reduce many direct-acting mutagens; consequently, if mutagenesis assays are included, this type of effect must be ruled out. Potential complications must be recognized, and a process must be developed to determine whether the bioassay result is valid.

5.7.2.3 False Positives and False Negatives are an Important Issue with Bioassays

Provisions must be made to address **FALSE POSITIVE** and **FALSE NEGATIVE** results. There are a variety of ways these false results might arise and can possibly be addressed; however, because the Expert Panel had little time to research the issue, this discussion is limited and incomplete.

ToxCast has identified cytotoxicity as the most commonly encountered false negative in the testing of individual chemicals (Judson et al., 2015). If the cell containing the receptor-reporter construct is killed, it cannot respond. The problem is exacerbated for water quality monitoring, where it is likely that cytotoxicity will be caused by contaminants other than the ones capable of inducing the receptor-mediated response. Sub-cytotoxic doses may also contribute to false negatives, and the possibilities are diverse. The depletion of cofactors and inhibition of protein synthesis are two examples that have received little attention in water quality analyses.

The most straightforward approach to address this type of false negative is to add a recognized agonist to a replicate sample at an effective concentration (e.g., the EC_{50}). If an agonist bioassay is being used, the lack of a positive response with the added standard agonist indicates that the negative response may be due to cytotoxicity or other causes identified above. In an antagonism assay, this result could be interpreted as a false positive, but since the cells have to respond to an agonist to detect an antagonist, false negatives of this type should not be possible.

False Negatives

False negatives suggest safety when there is no assurance of safety.

False Positives

False positives suggest a hazard when there is none.

Ensuring that there are no false positives is most easily dealt with by measuring active compounds in the sample chemically. If the active chemical can be identified in the sample and its concentration is consistent with the activity observed, the result is not a false positive. A more difficult problem is arises if the chemical(s) responsible for the activity cannot be identified.

5.7.2.4 Activation of Secondary Pathways within Cells Can Modify Bioassay Results

Bioassays based upon receptor-reporter constructs using intact cells can have interferences that fall into two general categories: specific and non-specific.

All cells contain a network of signaling pathways that interact with one another, and interactions of the cell at this level would be called “specific.” If a chemical in the sample activates or inhibits one of these other pathways, the response of an agonist can be inhibited or enhanced. This problem can be evaluated by looking for evidence in the kinetics of receptor activation with a standard agonist at various concentration factors of the sample. So-called “mixed” or “uncompetitive” kinetics should be observed if there is a chemical in the sample that is acting directly by activating another pathway that

Dose Response Curve

The dose-response curve produced by chemicals known to affect these systems (*in vitro* or *in vivo* assays) is analogous to a standard curve used in chemical analysis. The activities so measured are frequently expressed in equivalents of the standard compounds.

perturbs receptor sensitivity. This type of interference could vary by the cell type that is used to express the receptor-reporter construct. It depends upon which signaling pathways are expressed in the undifferentiated cells commonly used in these bioassays.

Although it would not be as definitive, one could begin with an analysis of the shape of the **DOSE-RESPONSE CURVES** in response to agonists in the water sample extract with that observed in the control medium.

An example of a specific problem that can arise in the evaluation of complex mixtures is that the estrogen pathway can be activated in the absence of a ligand of the estrogen receptor. One mechanism capable of doing this is the activation of the receptor by phosphatidylinositol 3-kinase/AKT (PI3K/AKT) by activating the estrogen-independent activation function 1 (AF-1) (Campbell et al., 2001; Sun et al., 2001). The PI3K/AKT system is activated by a variety of stimuli, including matrix adhesion (Khwaja et al., 1997), and a variety of survival factors. An abbreviated diagram is provided in **Figure 5-7** that outlines some of the cellular signaling pathways that activate PI3K/AKT (e.g., Kim et al., 2015). This type of interference could be addressed by the use of specific PI3K inhibitors, such as Wortmannin and LY294002 (Kim et al., 2015), but it should be cautioned that 17β-estradiol also induces PI3K (Stirone et al., 2005) and this pathway is involved in some of its effects. Consequently, it appears that the only way to deal with false positives in a bioassay based upon an ER-reporter construct is to analyze the samples and determine if there is activity that cannot be accounted for by compounds thought likely to occur.

Strictly speaking, the activation of the PI3I/AKT pathway is not a false positive, but identifying the compound(s) responsible in the mixture that act in this manner may be very difficult. If it occurs, it becomes a potentially difficult problem to deal with in compliance monitoring.

Non-specific interferences would affect the energetic and biosynthetic pathways, receptor trafficking, and availability of co-factors of the cell. Non-specific interferences might be detected by a change in the performance characteristics of the bioassay, most likely by its dynamic range when a dose-response curve is run in the sample matrix.

5.7.2.5 Suppliers of Bioassay Systems Need to Identify the Types of Interferences with Their Systems

At this stage in the development of bioassays for biomonitoring complex mixtures of variable composition, it is unlikely that suppliers of bioassays will have pursued problems as subtle as those described in **Section 5.7.2.4** in any detail because it is not a problem if one is applying the bioassay to screen pure compounds. Nevertheless, the expectation of the suppliers should be stated from the beginning because the intent is to apply these bioassays to concentrated samples of a very complex mixture; however, it will eventually be up to the analyst to rule out these interferences in the performance of the bioassays, if the activity measured cannot be accounted for by chemical analyses of the sample.

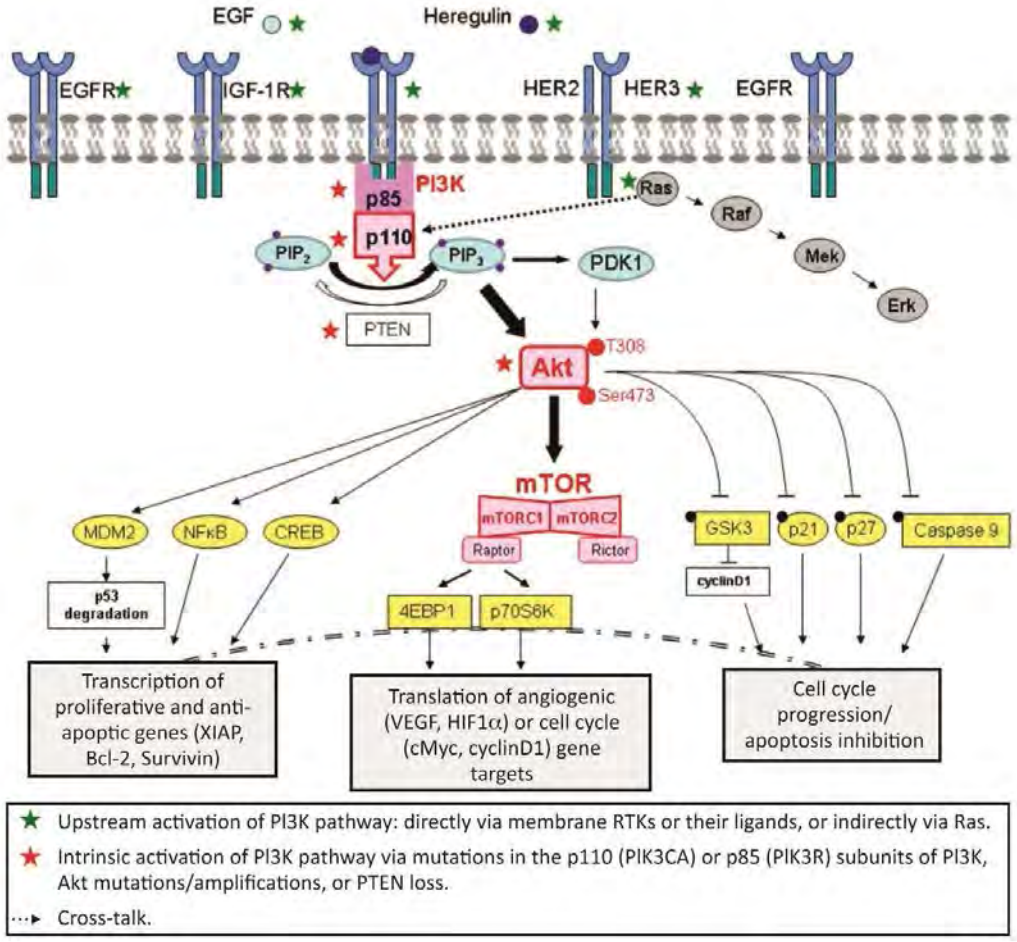

Figure 5-7: Selected signaling mechanisms that activate the PI3K/AKT/mTOR pathway, which modifies estrogen receptor activity (see Section 4.7.2.4 for discussion). From Leary et al. (2013).

5.7.3 Specific Issues to Address in Certifying Bioassays for Routine Monitoring

The following list outlines the essential features for an evaluation of bioassay results. It is important to note that answers to all these questions are not required prior to data use, but rather need to be explored to provide a "state-of-the-science" description to regulators that explicitly deals with the interpretation of the data and provides information on uncertainties in the information they provide. The Expert Panel suggests that the following issues be addressed by an applicant and during the review of the proposed application:

- How are the data to be interpreted and what actions will be triggered if a target value is exceeded?
- What is the AOPathway in which the proposed bioassay operates?
- What perturbation of the biological events in an AOPathway could result in an adverse health outcome in an average person (or to sensitive individuals)?
- The *in vitro* doses of a recognized activator of the AOPathway need to be compared to blood/tissue concentrations produced by exposure to *in vivo* doses of the same compound that activates or inhibits that receptor *in vivo*. If there is a range of chemical and physical properties among chemicals that can activate the receptor (generally, fairly restrained among steroid hormones, but some weaker analogs may vary), that variation should be represented in pharmacokinetic models by a number of activators spanning that range. It can be done directly using an appropriate pharmacokinetic model for activators/inhibitors of steroid hormone receptors because an adequate amount of data exists and many pharmacokinetic models are available for this purpose in the biomedical literature.
- Identify an excursion in the activation of the receptor that is needed to cause an adverse health outcome. As an avenue to associated bioassay results to health effects, the data should be expressed as BEQs. In the past, the USEPA has assumed a threshold activity above background as a point of departure. As an example, see the NRC (2005) report on the inhibition of the sodium iodide symporter by perchlorate.
- Establish appropriate uncertainty factors to apply to the point of departure developed to arrive at a target value that has an equivalent health effects backing as an MCLG, PHG, or MCL.
- Identify other mechanisms by which the AOPathway can be activated and/or modified by the interaction of chemicals in the water sample at other sites in the pathway or associated pathways.
- Identify other AOPathways that will produce or contribute to the same adverse health effect.

5.7.4 Guidance for the Communication of Bioassay Results

Guidance is needed to help communicate bioassay results, whether positive or negative, to the public and regulators. At minimum:

- Recognize and make available both the intent and limitations of the bioassays being considered for water quality monitoring to regulators and the public.

- Scientifically justify claims that bioassays can account for interactions among chemicals within a complex mixture to cause a specific adverse health outcome.

- Describe in scientific terms the limits of what interactions within the AOPathway will be measured by a bioassay (that is, do not rely on vague claims that appear in background statements in published papers).

- Provide an accurate interpretation of "negative result," avoiding broad and unsubstantiated claims of safety. In other words, this result indicates a particular adverse health outcome that can be associated with the AOPathway of the chemical, but it does not mean that the same adverse health outcome cannot be caused by other chemicals working through other AOPathways.

5.7.5 Additional Factors to Consider in Establishing Target Values for Routine Monitoring

Arriving at a target value for compliance monitoring must involve consideration of the reversibility of effects on key events once activated. The reversibility of the initiating event substantially influences health risk assessments. If an initiating event activates a response that is reversible with suspension or reduction of the exposure, the adverse health effect is considered to have a threshold dose, and MCLGs or their equivalents are addressed by the application of uncertainty factors for across-species and within-species variability in sensitivity. For reversibility, the duration of exposure may have been an important variable. A duration of a response of minutes may have very different implications for an outcome than one lasting a few hours, and will differ again if the response is maintained for days to weeks. For example, there are normal variations in endogenous estrogen (and other hormones) during the menstrual cycle and pregnancy. These changes occur over a short time span and express normal physiological functions of the endocrine system. As a consequence, small sporadic exposures are unlikely to disturb this pattern in adults; however, a continuous administration of estrogen at relatively low doses will effectively interfere with conception (although considered a desirable effect by many, it would not be welcomed by others, and probably by no one if it is received involuntarily), but with relatively few complications. In contrast, exposure to higher doses on a continuous period of time does lead to a variety of pathologies, including cancer.

Issues of reversibility can be addressed by reviewing biomedical literature (and not just toxicology literature). A larger question for which there may be inadequate data to resolve is how long does an endocrine effect (i.e., not limited to estrogen) have to be sustained before it affects development. These issues have yet to be consistently addressed in the development of standards around the world. Where standards have been published, such effects have occasionally been taken into account (an example was the development of an MCLG for perchlorate based on the inhibition of iodine uptake for the synthesis of thyroid hormones; see NRC, 2005).

An irreversible initiating event implies that the damage accumulates (although some damage may be repaired, if some consistent fraction of the effect is permanent, like a fixed mutation, the effect will be cumulative with time and is considered irreversible). Linear low-dose extrapolation ordinarily is used to estimate risk in such circumstances. The clearest examples of this approach are carcinogens that act through direct modification of DNA and, thereby, induce a mutation. Normal cell replication also results

in mutation, but small increases in the rate of cell replication by chemical treatment are treated as a threshold effect. There are legitimate scientific debates over which mode of action is involved in a specific case, but the principle is clear in the regulatory assessments of some state and federal regulatory agencies. To be conservative, in cases of inadequate data to establish a non-mutagenic mode of action, regulatory agencies usually assume that mutation is the mode of action.

Irreversibility of effects, however, can extend to health effects other than cancer. No-threshold arguments have been put forward for other effects (e.g., peripheral neuropathy produced by acrylamide and related toxicants). Another example would be if an effective exposure is reached in a small window of susceptibility and induces a developmental delay that impacts the ability of an individual to function throughout his or her lifetime. In this instance, it might be appropriate to apply linear extrapolation from the effective dose to lower doses, but it is likely that some minimum dose would be required to induce an adverse health effect with a single isolated dose; therefore, any AOPathway needs to be considered in terms of whether its effect is reversible or will present an adverse health outcome despite the fact that the exposure is removed.

5.8 Findings of the Expert Panel

The Expert Panel notes that bioassays of different types perform specific functions in health effects testing. In general, there are three recognized tiers in the decision matrix: screening, confirmation, and risk assessment. It is not necessary, however, to have three tiers if all three functions are addressed by a single bioassay or a collection of data that allows modelling of the likely *in vivo* outcomes with a high degree of confidence (which will require ancillary human/animal data). The three tiers include:

- **Tier 1 (Screening):** The Expert Panel emphasizes that broad application of *in vitro* bioassays in the ToxCast and related programs are directed at the first screening level of health effects testing. Generally, such tests are used to prioritize chemicals or products for more extensive testing.

- **Tier 2 (Confirmation)**: ToxCast and the regulatory programs it supports also address Tier 2 bioassays. Some may be *in vitro* bioassays, but they are often assays applied in intact animals and even alternative species, such as zebrafish or *C. elegans*. The ToxCast program has improved the relationship between bioassay results and likely human exposures to a chemical by introducing an element of exposure assessment not used before in evaluating screening data. The exposure estimates, however, are crude and based on a limited number of pharmacokinetic variables; consequently, they should not be applied in place of actual pharmacokinetic data published on compounds of interest.

- **Tier 3 (Risk Assessment)**: Bioassays (also referred to as “apical tests”) are those deemed appropriate for conventional risk assessment as employed under the Safe Drinking Water Act to develop MCLGs and PHGs. Generally, they are *in vivo* tests, but do not need to be, if a combination of other studies could provide the essential data elements needed for risk assessment. For example, the following should be sufficient for risk assessment: a firmly established “structure activity relationship” model for a chemical class (such as aromatic amines); an established AOPathway that has been amenable to pharmacodynamic modelling; and a validated pharmacokinetic model.

The following is a summary of the Expert Panel's findings, which are not listed in priority order.

- **Finding #5-1:** Introducing bioassays as routine monitoring tools into IPR/DPR water analysis programs at this time provides little, if any, advantage over the current chemical-specific monitoring using methods of analytical chemistry. *In vitro* bioassays should not be broadly applied to routine water monitoring in the absence of a clear and quantifiable risk relationship between the bioassay result and adverse health effects through an established AOPathway that is consistent with that used in developing MCLGs and PHGs (i.e., consistent with the California drinking water regulatory process) for individual chemicals. At present, the only worldwide "approved AOPathway" is for skin sensitization, which has been approved by OECD. In the ToxCast system, approximately 114 AOPathways are in various stages of development. See **Recommendations #5-1** and **#5-2.**

- **Finding #5-2:** Bioassays can be used to guide the chemical identification of unknown contaminants that have activities of concern. The Expert Panel recognizes the legitimate use of *in vitro* bioassays to identify contaminants of potential toxicological interest that might be found in various water sources and finished drinking water. Such efforts have been successfully applied in the past; however, it does require dedicated resources and time. An exploration of this type could be conducted as a screening tool at the start-up of a DPR project, but such a requirement must have specific objectives rather than diffuse questions of "activity." If activity is detected, the effort should be directed at identifying the chemicals responsible for this activity (see further discussion in **Finding #5-4** and **Recommendation #5-3**).

- **Finding #5-3:** The Expert Panel emphasizes that if *in vitro* bioassays are to be considered for use in routine water monitoring, then the results must be understood by risk assessors and clearly interpreted by regulators and the public in terms of health risk. It will be difficult to address this concern without instituting an approval process for bioassays to be applied to routine monitoring. At this time, although there may be some value in using selected bioassays for routine water monitoring, potential use should be confined to those bioassays that can be coupled to the probability of adverse health effects. The meaningful employment of these bioassays must depend upon using currently available data to first develop an AOPathway that can be coupled to *in vivo* dose metrics via an appropriate pharmacokinetic model (and not the reverse toxicokinetic screening that is used in ToxCast as a crude estimate). It should not be a problem for many CECs because multiple pharmacokinetic models exist for most endocrine active and pharmaceutical chemicals commonly used in medicine. Pharmacokinetic models also exist for many industrial chemicals and pesticides with endocrine activity that would suit this purpose. Pharmacokinetic models have not been generally developed for chemicals that are not controversial.

  In addition, the selection of AOPathways represented in the *in vitro* bioassays by ToxCast includes some of toxicological interest; there are many other important targets and AOPathways that produce adverse health outcomes that are not represented in the current collection of bioassays. In large part, it is because most AOPathways are poorly understood and HTP assays are lacking for many biologically important targets.

  Further, if bioassays are to be considered for use in water monitoring, the methods used for concentrating samples from water need to be optimized for the bioassay employed. In addition,

recoveries of representative target compounds from the sampled water need to be provided with the results of bioassay. See **Recommendation #5-4**.

- **Finding #5-4:** The Expert Panel emphasizes that if *in vitro* bioassays are to be considered for use in screening type monitoring to assess the performance of DPR operations and final product waters, a certification process should be established that would require the standardization of bioassays, methods of concentrating samples from water, and identifying methods for minimizing and/or eliminating false positives and false negatives, as well as describing the methods and normal quality control. The Expert Panel considered a broad range of data, addressing not only the capabilities of bioassays, but also requirements that would arise either as dictated by regulation or as rules that are considered in the routine monitoring of DPR unit processes and final product water. This need is absolute if these tools are to be employed in compliance monitoring. The Expert Panel cautions that any monitoring related to screening type trigger-values could still inappropriately and/or inadvertently become real standards even if they are not linked to risk and become industry practice.

  While the use of bioassays as a simple screening tool requires a less rigorous calibration, it remains important that strict attention be paid to the same issues not only as a matter of good practice, but also because these results are likely to become public and may need to be explained to the public; therefore, at this time, the Expert Panel has included the potential for use of bioassays as a screening tool as a research recommendation. See **Recommendations #5-5** and **#5-6**.

5.9 Recommendations of the Expert Panel

Much more research is needed to validate the use of *in vitro* bioassays for health hazard assessment and dose-response evaluations in drinking water treatment. Research of this type is more properly done by the USEPA, FDA, and NIEHS/NTP than the State Water Board. There are several areas that do require investigation of a more immediate concern for the use of these tools on water samples from potable reuse schemes. Most problems identified relate to the application and interpretation of the bioassays and their results to samples of complex mixtures of varying composition. The Expert Panel has the following recommendations, which are not listed in priority order:

- **Recommendation #5-1** (based on Finding #5-1): Define a clear and quantifiable relationship between bioassay results and adverse health outcomes *in vivo*. Research has shown that high-throughput (HTP) single endpoint assays can be applied to testing water (nuclear receptor-activated reporter assays appear the most suitable); however, the challenge is in interpreting the data in terms of the risk of adverse health outcomes in a manner used in the development of MCLGs and PHGs. The use of bioassays in routine monitoring should be considered analogous to the monitoring of specific chemicals with identified health risks; therefore, as stated previously, the use of bioassays for routine water quality monitoring requires a more thorough evaluation of the dose-response evaluation of the data in the context of *in vivo* health effects. There needs to be clear descriptions of the meanings that will be attached to positive and negative bioassay results. This issue comes to the fore if the intent is to monitor water intended for human consumption. It must be clear – qualitatively and from a dose-response standpoint – how bioassay results are linked to adverse health outcomes. Before any *in vitro* bioassay is used in the field for this purpose, guidance should be developed for the appropriate technical interpretation of these data relative to health risk and the communication of the

results of each bioassay in light of its specific application. The Expert Panel recognizes this very broad task is too large to be addressed by the State of California or the water industry. It is best left to the Federal Programs pursuing these issues.

- **Recommendation #5-2** (based on Finding #5-1): Develop AOPathways for chemicals with established modes of action, but no formal AOPathway. If a bioassay appears that it might be usefully employed for monitoring, but lacks an "approved AOPathway," it may be possible to develop an appropriate AOPathway. This process would require a review of *in vivo* data in the literature, for which – if sufficient – an AOPathway could be developed by an expert familiar with the use of modes of action in risk assessment. An example would be bioassays based upon an estrogen-receptor/reporter construct (that is, an AOPathway could be developed and subjected to appropriate scientific peer-review in the open risk assessment literature or by a regulatory agency). There is a huge amount of both qualitative and quantitative human and animal data in the biomedical literature on several estrogens that would provide all that is needed to develop an AOPathway. In these limited cases, an effort focused on the modes of action of chemicals that are known to act primarily by the identified initiating event could be assembled into an AOPathway and coupled with an appropriate pharmacokinetic model to validate the use of the bioassay for monitoring. This work, however, is not an area for amateurs. Expertise within the Office of Environmental Health Hazard Assessment (OEHHA) is available to address this problem, and there are qualified researchers who have specialized in research on modes of action. A technical document for this approach would need to be developed, as well as peer-reviewed in an appropriate journal or by a panel of appropriate experts.

- **Recommendation #5-3** (based on Finding #5-2): Develop guidance on the interpretation of bioassay data. Over the last 40 years, bioassays have been used in water analyses to screen for particular biological activities, followed by subsequent identification of the active compounds by chemical analyses (screening and identification). The introduction of HTP bioassays in recent years has greatly expanded the ability to detect biological activities that might contribute to adverse health effects. There are some excellent examples of work of this type and some new efforts are appearing in the literature. The Expert Panel previously concluded that *in vitro* bioassays could be used for the purposes of guiding the identification of chemicals with biological activities of potential health concern in various drinking water sources and finished water. If properly done, such efforts could help to develop public confidence in DPR. Although it is considered less important to validate these assays to the extent required for using them in compliance monitoring, there would be advantages to standardizing methods across the water industry and developing guidelines for presenting the results to the public. Having a group of accepted bioassays for this purpose would help diffuse the potential impact of random reports in the literature using bioassays that have not undergone this level of vetting.

- **Recommendation #5-4** (based on Finding #5-3): Undertake research on methods of concentrating organic chemicals in water. It is unlikely that current methods for sampling water are optimal for all bioassays. Currently, little effort has been expended to determine the recovery of compounds in water samples that are active in a specific assay. The compounds that activate one bioassay are likely to have significantly different chemical/physical properties than those that interact with another. Activators of most nuclear receptors are fairly non-polar, whereas compounds that activate the Nrf/KEAP system will have widely varying chemical/physical properties; however, only one concentration technique has been commonly

used in published studies of water. The same can be said for many of the so-called non-specific assays. These problems can be addressed by more carefully targeting chemicals of interest and making it standard operating procedure to report the recovery of members of that group of chemicals.

- **Recommendation #5-5** (based on Finding #5-4): Conduct investigation(s) on the use of bioassays for screening and identification of chemicals of potential health concern in water. As previously concluded by the Expert Panel, the use of bioassays for routine monitoring is not recommended primarily because the data generated by bioassays are not themselves clearly relatable to health risk. The standard should be that the bioassay data be linked in a way that allows for risk assessments of the same quality used in the development of MCLGs, PHGs, and MCLs; however, single bioassays or a collection of bioassays could be assembled to guide the identification of chemicals of health concern. This effort is easily within the reach of the water industry.

- **Recommendation #5-6** (based on Finding #5-4): Identify chemicals in water that activate cell-based assays. If cell-based assays continue to be applied in water quality monitoring, it will be necessary to confirm that bioassay results are in fact produced by the intended target analytes and not indirectly by other components in these complex mixtures. It is a particular problem when positive responses are accepted with relatively low thresholds (e.g., $EC_{IR1.5}$). Cells have very intricate signaling pathways, and there can be a high degree of interaction among these pathways. Some pathways have the potential of producing a response over background in the absence of the targeted agonists and create a situation that could be difficult to resolve. Utilities need to recognize that the above concerns are a possibility and that measuring of this sort of activity could trigger a significant effort in the need to identify such compounds. Chemicals that act via other pathways are unlikely to have the same chemical and physical properties as the known agonists for a receptor.

5.10 References

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CHAPTER 6: TRADITIONAL AND MOLECULAR METHODS FOR ASSESSING MICROBIAL WATER QUALITY

- Background on the microbial water quality of wastewater, water microbiome, and drinking water distribution system.
- Monitoring approaches used in wastewater and advanced water treatment, including current and new methods, molecular tools, online and real-time technologies, and process control monitoring.
- Efficacy of treatment processes to remove key pathogens, and assignment of $\log_{10}$ reduction value credits to treatment processes.

6.1 Introduction

Untreated wastewater contains hundreds of potential microbial agents capable of causing disease in humans via exposure to contaminated water through inhalation or ingestion. The variety of microorganisms and concentrations are not easy to ascertain and, unlike chemical constituents, there are no obvious ways to undertake source control of microorganisms in the sewershed. For the last 150 years, the water industry has used fecal indicator bacteria to assess pollution levels in water and to evaluate the performance of treatment processes to control waterborne disease (Geldreich, 1978). Monitoring indicators remains the regulatory approach within the water industry for drinking water and wastewater, as well as water reuse. In addition, online measurements of surrogate parameters (e.g., turbidity for filtration and chlorine residual for disinfection) commonly are used to monitor process performance. Yet scientific and technological advances now provide insight into the microbial world like never before, allowing for the detection, characterization, and quantification of any and almost all microorganisms. These advances have transformed medicine, clinical diagnostics, and food safety procedures. The use of these technologies has provided insight into gut microbiology and the microbes found in feces (and, hence, found in municipal wastewater). Understanding these naturally occurring populations also has improved our understanding of the healthy human microbiome.

The application of modern techniques to the water environment is providing unprecedented insight into the microbiome of source waters and is being applied to the study of engineered systems, including the impacts of various treatment processes and environmental pressures (e.g., disinfectant residual) on the water microbiome. Piped distribution networks recently have been of particular interest, with a number of research efforts focused on the microbiome of the drinking water distribution system and premise plumbing; however, unlike the food industry, the water industry has not adopted these new technologies and applied them for routine pathogenic diagnostics, as has been done for a wide array of chemical contaminants. One barrier is the inability to distinguish between viable and non-viable organisms when using only molecular tools; however, this limitation applies mainly to disinfection processes, and there is much to be learned about the microbial ecology and efficiency of the removal of particular pathogens by the current physical barriers used in advanced water treatment. Nonetheless, several challenges need to be addressed before such methods can be applied for the routine monitoring of pathogens by water utilities. Furthermore, in most cases, the laboratories that service wastewater

Metagenomics

The study of genetic material occurring in environmental (e.g., water) samples.

Next Generation Sequencing Technologies

Methods that rely on high throughput processes to sequence nucleic acids. These methods facilitate detecting large numbers of different genes in a single sample.

treatment plants (WWTPs), drinking water treatment facilities (DWTFs), and advanced water treatment facilities (AWTFs) do not have the necessary instrumentation or trained personnel to do so.

The purpose of this chapter is to provide an overview of how emerging techniques could be applied to the analyses of waters, such as those from all stages of a direct potable reuse (DPR) system, and to briefly discuss monitoring tools and strategies that are appropriate for ensuring public health is adequately protected from exposure to pathogens due to the use of DPR.

6.1.1 Interest in Methods for Monitoring Microbials

Recent advancements in **NEXT GENERATION SEQUENCING (NGS)** technology coupled with **METAGENOMICS** offer an opportunity to identify human pathogens in various environmental samples without *a priori* knowledge. In addition, the use of quantitative polymerase chain reaction (qPCR) and digital droplet PCR (ddPCR) methods is moving forward concurrently with the development of standard protocols for use in water, resulting in the quantification of a whole array of pathogens at specificities and sensitivities that are dramatically improved.

Regulations have focused on compliance monitoring, which uses indicator organisms as aggregate measures of water quality. Often, the result of compliance monitoring is the accumulation of large numbers of non-detects ("zeros"), especially in finished water, which provides limited information. Alternatively, performance-based policies have provided guidance and/or standards on the use of multiple treatment barriers to achieve high $\log_{10}$ reductions of bacteria, protozoan cysts, and viruses; however, these approaches do not provide direct knowledge about the types and concentrations of these pathogens nor the variabilities among treatment processes to reduce pathogen concentrations. Consequently, indicators have been used as the basis to estimate the pathogen removal needed to reduce the risk of exposure to pathogens in water to acceptable levels. In the water industry, the use of performance indicators remains a key approach for addressing treatment variabilities and process failures.

Contaminated water remains an important transmission route for a number of different pathogens. Hundreds of different etiological agents are excreted in feces (and a few in urine); hence, they are found in wastewater. As the water industry moves forward with potable reuse, particularly DPR, tremendous interest exists in how to improve the evaluation of the microbial quality of water and improve the efficacy of treatment processes to reduce pathogens to levels that ensure public health is adequately protected from exposure to pathogens.

6.1.2 Scope of the Review of Microbial Water Quality

The objectives of this chapter include:

- Summarize the current microbial monitoring methods used in wastewater treatment and advanced water treatment.

- Describe new monitoring methods and their applications.
- Address the assessment and efficacy of processes to remove key pathogens from water.
- Develop recommendations for research and future data needs.

6.2 Background

6.2.1 Pathogens Removal Targets

The ability to monitor and remove contaminants of concern has been instrumental in ensuring the safety of potable reuse. IPR standards have been developed in California that include performance-based criteria for microbial contaminants, suggesting a 12-$\log_{10}$ reduction of virus, 10-$\log_{10}$ reduction of *Cryptosporidium*, and 10-$\log_{10}$ reduction of *Giardia*. These targets are based upon pathogen occurrence data in wastewater and drinking water public health goals using quantitative microbial risk assessment (QMRA). An acceptable public health goal of 10^{-4} per person per year annual risk of infection from the consumption of potable water has been established by the USEPA and is discussed further in **Chapter 8**.

6.2.1.1 Brief History of Waterborne Disease and the Identification of Pathogens

Waterborne outbreaks of typhoid fever, caused by *Salmonella typhi* bacteria, was a serious health threat in the United States prior to the early-twentieth century. But as advances in sanitation and vaccines curbed the rate of typhoid outbreaks, it was recognized that the majority of waterborne outbreaks actually were being caused by unknown etiological agents. The public health community took action to improve its diagnostic capabilities, which allowed new agents of waterborne disease to be observed and documented. Risks were identified in sources of groundwater and surface water, and while disinfection seemed effective in controlling bacteria, protecting the public from (mostly unknown) viruses and parasites remained a challenge. Over the past 50 years, research has been undertaken to identify a number of new waterborne pathogens. For example, *Giardia* and noroviruses were identified in the 1970s, and *Cryptosporidium* and *E.coli* 0157:H7 were identified in the 1980s. Even today, about 10 percent of waterborne disease outbreaks are of an unknown etiology (Beer et al., 2015).

6.2.1.2 Emerging Microbial Concerns and Quantitative Microbial Risk Assessment

The discovery of new waterborne pathogens sparked the investigation and development of methods for their isolation and characterization from water. Examples include:

- *Legionella* (McDade et al., 1977).
- *Helicobacter* (Goodwin et al., 1989; Nayak and Rose, 2007).
- *Arcobacter* (Fong et al., 2012).
- Norovirus (Kapikian et al., 1972).
- The toxins of cyanobacteria (Svrcek and Smith, 2004).

Consequently, advancements in these new molecular tools and techniques have simplified the evaluation and characterization of novel waterborne pathogens. For instance, the QMRA framework has promoted the interpretation of pathogen concentrations into risk levels. This approach has been

accepted widely as a formal process for estimating human health risks from microbial pathogens and infectious disease processes related to drinking and recreational water exposure pathways (Regli et al., 1991, Haas et al., 2014; USEPA, 2014). In addition, QMRA has been used to examine the risks from biosolids (Gurian, 2012) and water reuse (WRRF, 2014). The QMRA process assumes that (1) hazards are identified appropriately, (2) dose-response functions have been mathematically described, and (3) exposure pathways (including concentrations and distributions, particularly temporal changes) can be described. All QMRA analyses have assumptions, uncertainties, and variabilities that affect risk output, which is described as a probability of infection or disease.

Hundreds of different pathogens can be identified in wastewater, depending on the assays used and the health status of the contributing population. Some of these pathogens are summarized in **Table 6-1**. The use of qPCR and other new methods makes it possible to characterize a broad spectrum of potential pathogens that need to be controlled, refine the exact level of treatment needed, and evaluate control strategies.

Table 6-1: Examples of Important Waterborne Pathogens Associated with Wastewater

Groups of Pathogens	Examples of Pathogens	Diseases
Viruses	Achiviurses, adenoviruses, astroviruses, enteroviruses, hepatitis viruses, noroviruses, papillomaviruses, polyomaviruses, rotaviruses	Cancer, gastrointestinal illnesses, liver impairment, myocarditis, neurological impacts, respiratory infections, warts
Protozoa	*Cryptosporidium, Cyclospora, Giardia*, Microspordia	Gastrointestinal illness, chronic infections, failure to thrive
Bacteria	*Campylobacter*, *E.coli* 0157:H7, other pathogenic *E. coli*, *Helicobacter, Salmonella*	Gastrointestinal illness, Guillain-Barré syndrome, reactive arthritis, Uremic Hemolytic Syndrome

6.2.2 Measurement of Indicator Organisms and Pathogens

The detection of indicator organisms and pathogens in water has relied on culture-based methods. A major limitation of culture-based methods is that the majority of microorganisms are not culturable or are difficult to culture. For example, it is estimated that less than 1 percent of all bacteria are culturable in the laboratory using current techniques. Specific waterborne pathogens for which culture-based methods do not exist include many viruses (e.g., norovirus) and *Giardia*. Culture-based methods for organisms like *Campylobacter, Cryptosporidium*, and *Legionella* are complex and time consuming. Due to the limitations of culture-based methods, alternative strategies have been developed. For example, the USEPA culture-based methods for *Giardia* cysts and *Cryptosporidium* oocysts rely on direct microscopy and the observation of internal life stages (i.e., trophozoities and sporozoites), which does not always correlate with viability or infectivity. **QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)** – which is based on the ability to detect a target nucleic acid sequence – has been developed for many waterborne pathogens; however, qPCR does not distinguish between viable and non-viable organisms.

Although some modifications to qPCR have been made to better distinguish only viable organisms [e.g., PMA (propidium monoazide)-qPCR or ICC (integrated cell culture)-PCR], these approaches are not in widespread use and have limitations when applied to certain processes, like chlorination (Srinivasan et al., 2011).

Although culture-based methods are used for *E.coli*, enterococci, clostridia, and coliphage (all of which have been monitored in wastewater and water reuse systems) (Rose et al., 2001; USEPA, 2015), qPCR methods also are available for this purpose. For example, qPCR has been sanctioned for the evaluation of enterococci in recreational waters (USEPA, 2015).

6.2.3 Water Microbiome

The advances in technologies and instruments for studying what is now termed the "microbiome" began with the discovery of DNA and RNA (i.e., nucleic acids), the building blocks that carry the code for informing all of life. The basic understanding of how DNA is replicated spurred technology to study this phenomenon. Twenty years later, the first technology – known as Sanger sequencing – became available (**Figure 6-1**) and, by 1985, PCR was developed based on the thermal stability of an enzyme that can replicate DNA in the test tube. The development of PCR has allowed for methods specific to the investigation of any microorganism of interest as long as there is some prior knowledge of the genetic sequence.

In the last 30 years, the instrumentation and development of high-throughput NGS technologies (in some cases, focused on whole genome sequencing) have allowed for major research initiatives around the human genome and human microbiome. Current research is focused on the **MICROBIOME OF THE WATER ENVIRONMENT**. Initial work focused mostly on bacteria using 454 instrumentation and targeted 16S ribosomal databases for identifying the bacteria; however, the use of this instrument was costly and now almost all current research uses Illumina instruments.

Quantitative Polymerase Chain Reaction (qPCR)

An enzymatic process for rapidly replicating a target nucleic acid sequence (e.g., section of DNA) under controlled laboratory conditions. qPCR detects the rate at which this multiplication occurs and allows quantification of the original number of target nucleic acid sequences present in a sample.

Water Microbiome

The community of microorganisms (bacteria, viruses, protozoans, algae and fungi) that are present in a particular water environment. This community often is assessed by genetic analysis of environmental nucleic acid.

In a field now known as "metagenomics," whole communities or mixtures of genetic material (which, for microbes, corresponds to algae, bacteria, fungi, protozoa ,and viruses) can be recovered directly from environmental samples and assessed using NGS (Edwards and Rohwer, 2005; Mokili et al., 2012). Metagenomic analysis has become a powerful tool for characterizing microorganisms without requiring *a priori* knowledge of these communities (Roux et al., 2012; Holmfeldt et al., 2013). This advancement has provided a novel opportunity to assess microbial water and wastewater quality characteristics by using genetic data rather than focusing on a few specific, well-known groups that are cultivatable. A review of methods used for assessing microbes in the water environment (including those for wastewater and sludge) is provided in Aw and Rose (2012).

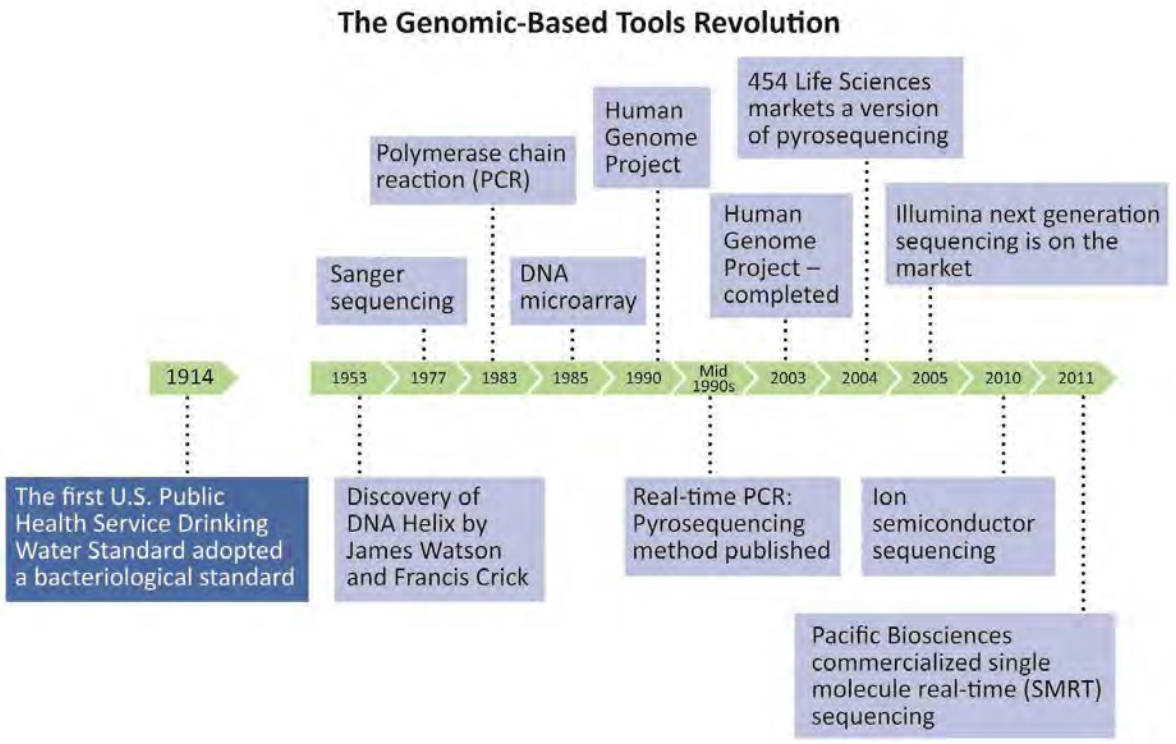

Figure 6-1: Advances in genomic technology. Image provided by Tiong Aw and Yiseul Kim of Michigan State University.

During the last decade of development, most metagenomic studies on water and wastewater were focused on bacteria or were limited to the lower sequencing yield of earlier technologies (e.g., 454 pyrosequencing) (Cantalupo et al., 2011; Kristiansson et al., 2011; Ye and Zhang, 2011; Tamaki et al., 2012). Pathogenic bacteria sequences belonged to the genera *Aeromonas, Clostridium,* and *Mycobacterium*, and several classes of antibiotic resistance genes were identified. Those bacteria that were not pathogenic belonged to the *Bacteroides* group now commonly used for source tracking. Although the rapid development of metagenomics has provided a new perspective of microbial communities in different environments, only a few studies have used metagenomic approaches to characterize viruses in wastewater environments (Cantalupo et al., 2011; Tamaki et al., 2012; Bibby and Peccia, 2013a,b; Aw et al., 2014). Eukaryotes are even less well characterized in wastewater or various water environments. There are a number of pathogenic eukaryotes, including protozoa (e.g., parasites such as *Giardia* and *Cryptosporidium,* and free-living protozoa such as *Acanthamoeba*), toxin-producing diatoms, and fungi.

These technologies promise to provide information useful for identifying new pathogens in water, as well as can lead to a better understanding of microbial ecology, including the interactions between beneficial organisms and pathogens (e.g., bacteria and free-living protozoa) and how these interactions influence pathogen die-off/inactivation or growth. Ultimately, this knowledge can be used to develop improved monitoring strategies and, eventually, new instruments for monitoring microbes in the water environment.

6.2.4 Drinking Water Distribution System

In the United States, there are 1-million miles of drinking water distribution pipes and an estimated 5- to 10-million miles of small-diameter premise plumbing pipes (Loganathan and Lee, 2005). The recent discovery of drinking water contamination (particularly, lead) in Flint, Michigan (referred to as the "Flint Water Crisis"), has brought new attention to water quality in drinking water distribution systems. One goal for safe and wholesome water is microbial and chemical stability in the drinking water distribution system. Current issues associated with the distribution system include:

- Pathogens like *Legionella sp., Mycobacterium sp. (M. avium), P. aeurigonosa*, and *N. fowleri* grow in drinking water distribution systems and premise plumbing (Falkinham et al., 2001; Falkinham et al., 2015; Falkinham, 2015; Thomas and Ashbolt, 2011).
- *Legionella* has been found in 50 percent of the potable water systems used for commercial buildings and 10 to 30 percent of residential water systems (Stout and Muder, 2004).
- The chemistry of water influences microbes by providing growth-promoting nutrients (e.g., calcium, manganese, and assimilable organic carbon) and inhibitors/inactivating agents, such as copper and disinfectants (Bargellini et al., 2011; Baron et al., 2011; Borella et al., 2003; Liu et al., 2015).
- The microbiome in the drinking water distribution system is influenced by pH, phosphorus, sulfate, and magnesium (Li et al., 2014).

Although many studies have documented correlations between certain water quality parameters and the presence of opportunistic pathogens, our current understanding is insufficient to determine *a priori* whether introducing a new water source will increase or decrease the risk of opportunistic pathogens being present in the drinking water distribution system.

DPR-treated water will differ from most conventional water sources because the advanced treatment trains may modify microbial and chemical water quality in ways that could change the stability of the water in the drinking water distribution system. Furthermore, a unique feature of DPR (as compared to IPR) is that DPR-treated water may go directly into a drinking water distribution system (though it likely will be blended with a conventional water source). Consequently, a unique concern with DPR is understanding whether the introduction of a new source of water will alter the microbial stability of existing water in the drinking water distribution system. As with the blending of any waters, blending DPR-treated water with conventional water is likely to change the microbial ecology in the drinking water distribution system. This change may occur due to different amounts and proportions of nutrients that cause population shifts or physical changes in the biofilm matrix on pipe walls. Although it is common practice to blend waters from different sources and of different qualities in conventional drinking water supplies, it is important to anticipate any changes that might be stimulated by DPR-treated water. For example, DPR-treated water may be lower in assimilable organic carbon than conventional water sources, which could lead to the starvation and sloughing of biofilms in the drinking water distribution system. Also, the impacts of changing the blending ratio need to be better understood. For example, DPR-treated water may be used as the base supply and augmented with conventional source(s) to meet demand, which could result in constant changes (or fluctuations) in water quality.

Pilot testing is one approach that can be used to assess the impacts of DPR water on water distribution systems and premise plumbing. For instance, studies can be conducted via pipe-loop testing. In addition to testing the impacts on chemical water quality (e.g., an increase in corrosion), testing the impacts on total bacterial counts and the biofilm could provide useful insights. Additional monitoring is recommended prior to operating the DPR system (to establish a baseline) and during the start-up phase when the highly treated water is introduced into the drinking water distribution system and premise plumbing. Suggested parameters include assimilable organic carbon, Heterotrophic Plate Count (HPC) bacteria, total bacterial counts, and opportunistic pathogens.

6.3 Monitoring Approaches

6.3.1 Current Objectives for Microbial Monitoring in Conventional Drinking Water Treatment

Currently, there are four main objectives to monitoring conventional drinking water for microbial contaminants.

The first objective is ***characterizing pathogens in source water*****.** The Information Collection Rule (effective from 1996 to 2000) required specific extended monitoring of *Cryptosporidium* oocysts, *Giardia* cysts, and total cultivatable viruses (measured on Buffalo Green Monkey kidney cell lines) in surface waters for a period of several years. These data were used to address the necessary $\log_{10}$ reductions for these pathogens by drinking water treatment (for surface waters) to achieve an acceptable risk level in treated drinking water (Regli et al., 1991). In groundwater, monitoring has focused on viruses as the microbial pathogens of concern. Recently, the USEPA has developed national monitoring guidelines for adenoviruses and enteroviruses to ensure 99.99-percent (4-$\log_{10}$) inactivation or reduction of viruses from groundwater used as a source of drinking water (USEPA, 2015).

The second objective is ***evaluating the efficiency of the DWTF*****.** Because it is not possible to measure the expected low concentrations of target pathogens with high frequency, surrogate parameters are used in their place (such as turbidity for filtration and chlorine residual for disinfection). These parameters can be used for process control (e.g., maximum allowable turbidity and minimum allowable chlorine residual) and to assign $\log_{10}$ reduction credits. In addition, the relationship between the surrogate and pathogen needs to be established. For example, disinfection conditions to achieve specific levels of inactivation for specific bacteria, protozoa, and viruses were established by experimental research and investment by the USEPA.

The third objective is ***monitoring the quality of final treated water***, and the fourth objective is ***monitoring the quality of water in the drinking water distribution system***. Objectives three and four rely on the use of total coliform and *E. coli* bacteria, indicators that are monitored daily in small sample volumes (i.e., 100 mL) at large water treatment plants.

6.3.2 Objectives for Microbial Monitoring in Direct Potable Reuse

Similar to the current approach used for drinking water, there should be four main objectives for monitoring the microbial quality of DPR-treated water. For each objective, it is necessary to consider whether the monitoring approach requires the measurement of actual pathogens or if indicators or surrogates can be used, and whether high-frequency, real-time measurement is required or periodic measurements are sufficient.

- **DPR Objective #1: Evaluate the concentrations of pathogens in raw wastewater.** The purpose of this monitoring is to determine the necessary levels of treatment to achieve an acceptable risk level of 10^{-4} per person per year. As such, it is necessary to measure the actual pathogens of concern. New molecular approaches for characterizing microbial communities show promise for expanding the ability to detect pathogens previously unrecognized in water, and qPCR and ddPCR are available for quantifying known pathogens. Although high-frequency, real-time measurement would be ideal (e.g., to identify pathogens in sewersheds during waterborne outbreaks), such technologies are not readily available for routine use and, although in development, are unlikely to be tested thoroughly for use in water in the near future; however, large volume sampling and composite sampling can be used to improve the efficiency of sample collection.

- **DPR Objective #2: Evaluate the performance of treatment processes (e.g., process monitoring).** Monitoring locations in an AWTF should be determined to verify the performance of each unit treatment process for which an LRV is assigned. For each location, the best monitoring parameter is established, as well as the frequency of monitoring. For process monitoring, it is necessary to have high-frequency, real-time results. Consequently, monitoring actual pathogens currently is not feasible and surrogate parameters must be used; however, appropriate surrogates often are established using pilot tests involving the direct comparison of surrogates and target pathogens. Although surrogate parameters have been established for most advanced water treatment processes, promise exists for the continued development of surrogates that provide more accurate and reliable measurements of treatment performance with respect to pathogen removal (see **Sections 6.3.4** and **6.3.5**). The acceptable limits for the value of the parameter must be determined, as well as the set-points for alarms and what actions are taken when parameter limits are exceeded. For example, it is important to identify when an alarm simply is an alert for an operator to investigate a process versus a trigger to divert off-spec water.

- **DPR Objective #3: Monitor the water quality of final effluent.** Concentrations of indicator organisms and actual pathogens in adequately treated water will be orders-of-magnitude lower than what is possible to measure without adequate concentration, temporal composites, and large volume sampling (e.g., see the target concentrations in **Table 6-2**). The measurement of total coliforms is used to maintain consistency with historical approaches, but will be useful only to indicate a catastrophic treatment failure (if the total coliform bacteria originated from the source water) or substantial regrowth within the AWTF. Alternative measures for monitoring the quality of final effluent should be considered, such as total cell counts (e.g., using flow cytometry; see **Section 6.4.2**). In addition, periodic analyses of actual pathogens could be undertaken to maintain public confidence.

- **DPR Objective #4: Evaluate water quality in the drinking water distribution system.** If DPR is practiced such that advanced treated water serves as the influent to a DWTF, then existing approaches likely are sufficient for monitoring drinking water distribution systems. For DPR in which finished drinking water is sent directly into a drinking water distribution system, however, it is recommended that additional monitoring be undertaken because the finished drinking water may affect the microbial and chemical stability of the water in the distribution system (see **Section 6.2.4**). In either case, because maintaining public confidence in the DPR system is essential, it may be worthwhile to implement monitoring for opportunistic pathogens.

Table 6-2: Surrogate Online, High-Frequency Parameters Currently Used to Monitor the Pathogen Reduction Performance of Unit Treatment Processes

Process/Critical Control Point	Surrogate Parameters Currently Used	Surrogates Parameters Under Development
Microfiltration (MF)/ ultrafiltration (UF)	Online Pressure Decay Test determines membrane integrity Online turbidity, recorded frequently	Filtrate particle counts (bench-top) and reduction of particles across MF membranes Online bacteria monitoring
Reverse osmosis (RO)	Reduction of electrical conductivity (online) across RO Reduction of total organic carbon (online) for RO permeate	UV absorbance (online) Online or periodic injection and monitoring of fluorescent dye
Ultraviolet (UV) disinfection	UV delivered dose, which is a function of online UV transmittance, flow, and UV sensor intensity	None
Ozonation (O_3)	Online O_3 residual	None
Engineered storage barrier with chlorine	Online chlorine residual	None

Meeting all four DPR objectives will require higher frequency monitoring during the start-up of a DPR system. For ongoing monitoring, it is important to have a program in place to analyze monitoring data and look for the deterioration of process performance over time. An additional use of process monitoring data (see **DPR Objective #2**) is to assign $\log_{10}$ reduction values (LRVs) to individual unit treatment processes. Current approaches for assigning LRVs are discussed in **Section 6.6.**

6.3.3 New Methods for Pathogen Characterization

6.3.3.1 Ultrafiltration

Ultrafiltration (UF) now can be used as an efficient concentration method. Unlike other filtration methods, ultrafiltration has several characteristics that allow for processing large volumes of water without clogging, while capturing viruses, protozoa, and bacteria to produce a concentrate that can be used for culture-based methods, PCR, and metagenomics. There have been a number of different UF systems used over the years (Paul et al., 1997; Schroeder et al., 2002; Baudoux and Brussaard, 2005; Attoui et al., 2006; Brussaard et al., 2007; Kim et al., 2015a). The main type of UF used today is tangential-flow (cross-flow) UF, which does not require solutions (e.g., hydrochloric acid, skim milk solution) that may inhibit infectivity or alter microbial community structure (Prata et al., 2012); however, in some cases, filters were found to have selective loss, depending on the type of microorganism (particularly viruses), volume filtered, duration of filtration, and organic load of the sample. Furthermore, the pre-filtration of water samples often was necessary as the systems clogged due to turbidity and suspended solids, resulting in the underestimation of microbial concentrations (Paul et al., 1991).

Viruses are very difficult to concentrate. Most filtration methods have relied on adsorption-elution methods (such as the 1 MDS, which was used for the Information Collection Rule). Adsorption and elution methods tend be biased towards concentrating viruses that adsorb well. In addition, the beef extract elution and further re-concentrate interferes with molecular approaches.

6.3.3.2 Advancements in Polymerase Chain Reaction

PCR technology has the ability to detect microbes in the environment and has a number of advantages. Conventional PCR determines the presence or absence of a target sequence (qualitative) and can be used in a "most probable number" format, but dilution is necessary. Alternatively, real-time qPCR is most commonly used for environmental studies (e.g., fluorescent probes or fluorescent DNA-binding dyes and primers to quantify products generated on each amplification of cycle SYBR® Green DNA-binding dye and fluorogenic TaqMan probes [Goyer and Dandie, 2012]).

Advantages of PCR include the following:

- Less time is needed to detect pathogens as compared to culture-based methods.
- Can design primers to be highly specific to target organisms.
- Can detect viable, but non-cultivatable and stressed microbes.
- Can be used with culture-based methods to decrease the assay time and improve specificity.
- Can assess microbial particle mass balances and removals by key physical processes.

Disadvantages of PCR include the following:

- Can detect non-infectious and infectious microbes, but may overestimate viability after disinfection processes that do not destroy nucleic acids.
- Must generate a standard curve to provide accurate quantification.
- Is susceptible to inhibitory compounds, such as humic acids found in environmental samples, which leads to false-negative results.
- Must have the appropriate specificity and sensitivity of the primers and probes used for PCR.

In most wastewater studies, viruses are targeted and tested by qPCR. For protozoa, qPCR primarily has been used to address genotypes (Li et al., 2012). Reported concentrations of adenoviruses, aichiviruses, astroviruses, HAV, HEV, norovirus genotype II, and rotavirus in wastewater using qPCR are listed in **Table 6-3** (Hellmér et al., 2014). The assumption was all these viruses were infectious. These values in sewage were used to predict the number of infected individuals in the population based on excretion and 10-percent recovery rate, and outbreaks in the population were then identified as virus concentrations increased. The results are in contrast to cell culture, which has determined total cultivatable enteric viruses on Buffalo Green Monkey (BGM) cells ranging from 10 to 10,000 Most Probable Number per liter (MPN/L), without considering a recovery efficiency of about 1 to 2 $\log_{10}$ lower.

Table 6-3: Reported Concentration Ranges of Viruses in Raw Wastewater Using Quantitative Polymerase Chain Reaction (qPCR)

Virus	Concentrations in Raw Wastewater (Per Liter)
Adenovirus	5.7×10^6 - 3.3×10^6
Aichivirus	6.0×10^2 - 3.5×10^4
Astrovirus	4.0×10^5 - 4.3×10^6
Hepatitis A Virus	1.2×10^2 - 1.4×10^4
Hepatitis E Virus	4.0×10^2 - 2.2×10^3
Norovirus Genotype II	1.2×10^5 - 3.2×10^6
Rotavirus	5.0×10^3 - 3.8×10^4

Source: Hellmér et al. (2014).

In another study, 11 types of virus were assessed by qPCR in untreated and secondary wastewater effluents from two WWTPs (Kitajima et al., 2014). On average, the sapovirus (related to norovirus), enteroviruses, group A rotavirus, adenovirus, and aichivirus were found at similar levels (~5×10^5/L gene copies), with the highest concentration being 5×10^7/L gene copies found at one WWTP. Polyomaviruses – which have been proposed as human source tracking markers – were highest. Noroviruses were removed between 1.6 and 2.8 $\log_{10}$, while the viruses that were not removed as well by secondary wastewater treatment included aichiviruses, group A rotaviruses, adenoviruses, and polyomaviruses (approximately 0.6 to 0.9 $\log_{10}$). This result may represent the differences in the detection of noroviruses between the United States and other countries where norovirus is found to be more prevalent in ambient waters

6.3.3.3 Digital Droplet Polymerase Chain Reaction

ddPCR is a sensitive and accurate quantification approach that enables the determination of target copy numbers without the need for a standard curve. These improvements are achieved by partitioning the sample onto micro-fluidic chips or micro-droplets into individual reactions so that each reaction contains at least one, or zero, copies of the nucleic acid target. The signal in ddPCR is measured after completing amplification, and the absolute number of target nucleic acid molecules in the sample is calculated directly from the ratio of positive to total partitions using binomial Poisson statistics (Pinheiro et al., 2012). Additionally, this approach may reduce the difficulty of quantifying microbes in the presence of inhibitors linked to matrix-type components analyzed in food or environmental samples (Rački et al., 2014).

The potential for ddPCR to be automated allows for an easier and less cumbersome method for the detection and quantification of microbes (Rački et al., 2014). This novel method also reduces the quantitative variability currently seen when using real-time qPCR (Rački et al., 2014a). The direct quantitation approach may be useful in standardizing the quantification of enteric pathogens in wastewater and other environmental samples.

The use of ddPCR has been shown to be more tolerant to inhibitory substances that could be present in wastewaters, particularly for RNA viruses (e.g., Pepper mild mottle virus), as compared to reverse transcription qPCR (Rački et al., 2014a). ddPCR now has been used for viruses like adenoviruses and parasites like *Cryptosporidium*, as well as source tracking markers (Kishida et al., 2014; Yang et al., 2014; Cao et al., 2015). Various studies have shown that the ddPCR system is more precise, but produces numbers with lower concentrations than estimated with qPCR. In addition, compared to qPCR, the current cost for ddPCR is slightly higher and receiving the results takes slightly more time.

In summary, ddPCR has advantages similar to qPCR, but also has the following additional benefits:

- A standard curve is not required.
- Greater precision is achievable.
- Inhibitory substances are less influential.

Disadvantages are similar to qPCR, including the detection of non-infectious and infectious microbes, thereby limiting the ability of the method to be used to address disinfection processes.

6.3.3.4 Metagenomics and Whole Genome Sequencing

The ability to explore the wastewater microbiome using metagenomics and NGS promises to provide additional insights for potable reuse. The wastewater industry has addressed a few pathogens and indicators and has focused on engineering controls to achieve high $\log_{10}$ reductions of microbial populations; however, the full benefits to the industry – and society at large – cannot be achieved without more basic work on microbiological sciences and the ecology of wastewater systems. Recent research has found that the bacterial microbiomes in raw wastewater from 71 cities across the United States were represented by a core set of 27 human fecal oligotypes, and a more stable bacterial population was found when comparing cities versus comparing individuals (Newton et al., 2015).[21] Li et al. (2015) examined bacterial pathogens from WWTP influent, as well as effluent from activated sludge, biofilm, and anaerobically digested sludge; altogether, they identified 113 bacterial pathogens. This team also reported an accumulation of bacterial pathogens in the upper foaming layer of the activated sludge. The team demonstrated a 98-percent (almost 2-$\log_{10}$) reduction by secondary wastewater treatment. Based on these results, pathogenic enterococci and *Mycobacterium* warrant further consideration.

The wastewater virome (i.e., the collection of all viruses in wastewater) is intriguing, as 70 percent of the viral sequences detected could not be characterized (Aw et al., 2014). The wastewater virome was dominated by bacteriophages, which could serve as new indicators and help further our understanding of population dynamics associated with nutrient removal. The virome also contained sequences related to known human pathogenic viruses, such as adenoviruses (species B, C, and F), polyomaviruses JC and BK, and enteroviruses (type B). An array of animal viruses was detected, suggesting zoonotic transmission that has yet to be understood.

For the wastewater and treated water environments, methodologies (e.g., a sample flow processing pathway known as a "pipeline") need to be further perfected. This need broadly encompasses sample

[21] Oligotyping of high-throughput 16S rRNA gene sequence data was used for the analysis in Newton et al. (2015).

collection (e.g., flow weighted composites), sample volume, concentration, preprocessing, sequencing, and software to undertake the bioinformatics analysis. Eukaryotes (e.g., protozoa and fungi) and viruses are under-represented in the scientific literature and should be studied further.

Concentration is a necessary element of the sampling strategy, and better methods are needed. Except for raw wastewater, it is encouraged that UF methods be used at present (Aw et al., 2014; Kim et al., 2015; Hill et al., 2013).

Instruments and technology will continue to evolve. Portable instruments that fit in a suitcase now have been used to undertake real-time surveillance with results obtained in 24 hours (Quick et al., 2016). One novel approach used the MinION (Oxford Nanopore Technologies, Oxford, UK) high-throughput instrument whereby a single strand of DNA passes through a protein nanopore at 30 bases per second. The use for targets in lower concentrations found in water compared to clinical samples needs to be explored.

Advantages of the future use of whole genome sequencing and metagenomics in the wastewater and water reuse industries include the following:

- Discovery of emerging pathogens.
- Improved understanding and fine-tuning of disinfection processes for a wide array of pathogens found in wastewater.
- Development and use of new rapid field instrumentation for sequencing to elicit pathogen-specific information and to better inform risk assessment.
- Use of designed (or a better understanding of how to optimize) microbial populations to enhance the efficacy of biological water treatment processes for the reduction of pathogens and chemicals.
- Progress toward designer biofilms used to recover energy in addition to water and nutrients.
- Monitoring wastewater to screen the health of communities and to detect excursions of pathogen inputs.

Disadvantages of whole genome sequencing and metagenomics for the wastewater and water reuse industries include the following:

- Inability to differentiate between viable and non-viable microorganisms as long as inactivation does not alter nucleic acids.
- Determining individual species is not possible with high similarity indices without the use of very long sequence reads.
- The technology requires specialized training and knowledge to deploy.
- Standardized protocols for the water environment remain under development.

6.4 Online and Rapid Techniques

6.4.1 Rapid Methods

Rapid methods are defined in microbiology as those that produce results in 3 to 18 hours. Those methods that give results in minutes without sample preparation generally have poor specificity and sensitivity. Examples of rapid methods that have been used include ATP bioluminescence (i.e., the generation of light by a biological process), which is highly non-specific, and endotoxin testing (e.g., *Limulus* amebocyte lysate [LAL]). Other methods that provide identification require highly trained personnel to run large instrumentation, including fatty acid analysis (i.e., methods that use fatty acid profiles to provide a fingerprint for microorganism identification) and Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (i.e., microbial identification), and have limited sensitivity.

6.4.2 Flow Cytometry

A flow cytometer is used as a fast, automated technique by which particles in solution individually flow through a small channel interacting with multiple lasers, allowing the detection and discrimination of different types of particles, including biological cells. Early flow cytometers were used to excite the natural pigments found in phytoplankton, which resulted in the phytoplankton fluorescing and enabled enumeration (Marie et al., 1999). Later, flow cytometer assays were developed whereby nucleic acid-specific fluorescent dyes were used to allow staining and the enumeration of bacteria (Boye et al., 1983; Noble and Fuhrman, 1998). By the late 1990s, advances in techniques by Marie et al. (1999) facilitated the enumeration of viral particles from aquatic environments by using the nucleic acid-specific dye SYBR Green I. Flow cytometry now is a routine technique used in aquatic microbiology studies. Three approaches are available, as follows:

- Direct labeling of individual cells with (1) viability stains or (2) fluorescent markers with no requirement for cellular growth.
- Flow cytometry of individual particles, which are counted as they pass through a laser beam.
- Solid-phase cytometry (i.e., staining and laser excitation method).

Although it is not possible to ascertain the precise morphological characteristics of individual nano-sized biologicals like viruses, it is possible to distinguish between different cell types based on their flow cytometric characteristics, which include size and differential fluorescence staining for DNA and RNA, to generate "fingerprints." For example, the contamination of drinking water with wastewater could be detected by comparing fingerprints (Prest et al., 2013). After samples are collected and stained, flow cytometry allows high-throughput (i.e., higher replication); studies that focus on higher spatial and temporal coverage are feasible because the analysis time per sample is much shorter (i.e., around tenfold) as compared to other counting methods. An automated online format has been demonstrated that allows for continuous monitoring in near real-time (Besmer et al., 2014). Both intact and non-intact cells can be distinguished by staining with propidium iodide, which only can pass through cells with damaged membranes (Berney et al., 2007); this approach provides a conservative estimate of non-viable cells (noting the actual number of non-viable cells will be greater).

The following issues are important when processing samples for use with flow cytometry:

- A specific pH for optimal staining is needed that requires the use of a buffer.
- The low microbial abundance samples expected from highly treated wastewater can be optimized to reduce background noise and enhance staining.
- Detection limits can be increased by analyzing flow rates that are higher than recommended (the standard is around 30 to 40 µL/min, while most benchtop flow cytometer machines can be used up to 100 to 140 µL/min) and using longer running times (the standard analysis time considered sufficient is 1 minute).

Advantages of flow cytometry include the following:

- Ability for rapid analysis.
- Potential for automation and online instruments.

Disadvantages of flow cytometry include the following:

- Use of only vital stains to assess viability.
- High detection limit without further sample concentration.

6.5 Process Monitoring

Because real-time continuous monitoring for target pathogens currently is not possible, it is necessary to use surrogate parameters to measure the performance of treatment processes. Even if real-time continuous monitoring of pathogens was possible, it still would be desirable to use surrogate measures to monitor performance because pathogen concentrations vary dramatically over time, whereas a constant high level of treatment performance is desired to ensure safe water quality. There is a long history of the use of surrogates, which is employed in current regulations for drinking water treatment (e.g., turbidity as a surrogate for pathogen removal by granular media filtration) and wastewater treatment and reuse (e.g., chlorine residual as a surrogate for the inactivation of pathogens during disinfection). Commonly used surrogates for monitoring advanced unit processes that are likely to be employed in DPR are summarized in **Table 6-2.** Each unit process that is attributed a LRV credit is defined as a critical control point (CCP), and a surrogate is identified to provide continuous performance monitoring. For example, for microfiltration (MF) or UF, online turbidity is monitored as a surrogate for the removal of *Giardia* or *Cryptosporidium*. A maximum turbidity value is established; if that value is exceeded, a valve closes and off-spec water is diverted from the treatment train. As an additional assessment of membrane integrity, a daily Pressure Decay Test is performed.

Research projects are underway to develop and evaluate improved surrogates; several examples are provided in **Table 6-2**. In some cases, currently used surrogates are believed to underestimate the pathogen removal actually achieved by treatment processes. For example, LRVs for reverse osmosis (RO) currently are based on monitoring electrical conductivity (EC) or total organic carbon (TOC), but the removal mechanism of these parameters is fundamentally different than that of pathogens. Theoretically, perfectly intact RO membranes could achieve complete rejection of pathogens, whereas some ions and small organic molecules are transported across an intact membrane by diffusion; therefore, ions and TOC are expected to be present in RO permeate even if the RO membranes are perfectly intact, and their removal efficiencies are not expected to be as high as pathogens. RO

membrane materials, however, have imperfections and can be damaged over time, allowing the passage of pathogens. In addition, o-rings that provide a seal to separate the feed and permeate streams can become damaged and crack over time, allowing feed water to short-circuit into the permeate. Much effort has been invested in developing surrogate measures that have higher removal rates by RO membranes (i.e., more similar to pathogens) and also provide a reliable and sensitive signal when small breaches occur in membrane integrity that could allow the passage of pathogens. To date, the most promising approach appears to be the periodic injection and monitoring of fluorescent dyes, such as Trasar™ (Nalco).[22] Ongoing studies aim to correlate the removal of Trasar™ with model viruses for intact and compromised membranes. If a reliable correlation is demonstrated, it may be possible to attribute higher removal values to RO membranes.

6.6 Recommendations of the Expert Panel

The Expert Panel finds that existing knowledge is sufficient for setting microbial performance reduction levels for DPR criteria; however, additional investigations are recommended to further contribute to developing a stronger evidence base for DPR. The following recommendations of the Expert Panel are not listed by priority.

- **Recommendation #6-1**: To reduce uncertainty, a major initiative to characterize pathogens in wastewater is encouraged. This effort can be viewed as analogous to the Information Collection Rule for surface waters to characterize risks from virus, *Giardia*, and *Cryptosporidium*, or the Groundwater Treatment Rule to better characterize risks from viruses. Data should be collected from multiple facilities for several years to capture the variability associated with (1) the disease status of the population in the sewershed, (2) process variability, and (3) seasonal effects. These results can be used to inform the engineering reports, permitting, and start-up of DPR projects, as well as for probabilistic QMRA (also see **Research Recommendation #8-2** in **Chapter 8**).

- **Recommendation #6-2:** Research and technology transfer workshops are needed to modernize the approach that DPR uses for microbiological pathogen, indicator, and surrogate monitoring in the future. This effort will facilitate the advancement and use of new microbiological methods, datasets, bioinformatics, and instrumentation within the water industry. Technology transfer workshops are one pathway forward, and the Expert Panel encourages the State Water Board to further this effort.

- **Recommendation #6-3:** Large volume samples (i.e., greater than 100 liters using efficient concentration methods) and qPCR or ddPCR should be used to assess the removal of actual pathogens by membrane technologies at full-scale installations and to understand the impact of o-ring leaks and membrane integrity. These diagnostic investigations would provide valuable information and assurances in regard to removals. In general, it is now feasible to address the physical barriers to provide better information on the efficacy of these barriers in the treatment train.

- **Recommendation #6-4:** For a DPR system in which the final treated water will directly enter the drinking water distribution system, the impacts on the drinking water distribution system need

[22] Work is in progress under Water Environment & Reuse Foundation project WRRF-14-10, titled "Enhanced Pathogen and Pollutant Monitoring of the Colorado River Municipal Water District Raw Water Production Facility at Big Spring, Texas." The principal investigator is Dr. Eva Steinle-Darling of Carollo Engineers.

to be evaluated explicitly. The analysis should explain how the microbial and chemical stability of the water is maintained (or evaluated) with the introduction of the new source of water. The results and analysis should be included in the project's engineering report(s) prepared consistent with California regulations.

- **Recommendation #6-5:** A monitoring program on the drinking water distribution system should be put in place to provide information on any changes that occur once the DPR facility goes online. One year of an enhanced monitoring program (i.e., more samples, more locations, and more parameters compared to the Total Coliform Rule) for specific data should be collected as a baseline before the introduction of the new water source. This effort would be followed by one year of enhanced monitoring after the introduction of the new source of water. The following microbial parameters should be considered for analysis (in addition to total coliform bacteria and disinfectant residual): HPC, assimilable organic carbon, and specific opportunistic pathogens. Total bacterial counts also may be insightful. For chemical water quality, lead and disinfection byproducts should be included as part of the enhanced monitoring program. The monitoring of premise plumbing also should be considered.

- **Recommendation #6-6:** To the extent possible, microbiology monitoring data should become part of the public record in DPR project proposals, engineering reports, and annual reports.

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CHAPTER 7: ANTIBIOTIC RESISTANT BACTERIA AND ANTIBIOTIC RESISTANCE GENES

- Background on antibiotic resistance.
- Sources and exposure routes within communities and the environment.
- Significance of the sources for antibiotic resistance.
- Methods for assessing antibiotic resistance in water matrices.
- Occurrence and removal of antibiotic resistant bacteria and antibiotic resistance genes through water and wastewater treatment.

7.1 Introduction

7.1.1 Antibiotic Resistance

The development of antibiotic resistance is a significant worldwide public health problem. The level of concern is evidenced by the issuance of global (WHO, 2015) and national (White House, 2014; White House, 2015) action plans for dealing with antibiotic resistance. The Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria was recently established as an inter-agency group to address implementing the national strategy and action plans (HHS, 2015).

Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) are known to be in wastewater. For example:

- A World Health Organization (WHO) briefing note on antimicrobial resistance as an emerging issue in water, sanitation, and hygiene indicated that the lack of consideration of ARB and ARG in WHO guidelines for drinking water, recreational water, and wastewater in agriculture and aquaculture was a major gap in the coverage of contaminants of concern to human health (WHO, 2014).
- A Science Advisory Panel convened by the California State Water Resources Control Board (State Water Board) to address chemicals of emerging concern (CECs) in recycled water concluded that reuse practices did not cause or add to antibiotic resistance (Anderson et al., 2010).
- Another State Water Board panel addressed CECs in aquatic ecosystems and suggested gathering additional information on the occurrence of antibiotic resistance in the effluents of wastewater treatment plants (WWTPs) (SWRCB, 2012).
- A waterborne outbreak of *E. coli* O157:H7 in an unchlorinated supply in Missouri in 1989-90 was the first outbreak of a multiple resistant organism shown to be transmitted by water (Swerdlow et al., 1992; Geldreich et al., 1992).

Addressed in this chapter is the potential for direct potable reuse (DPR) water to be a disseminator of antibiotic resistance. The advanced water treatment processes used to produce DPR water are

expected to remove all detectable bacteria, including those that might be antibiotic resistant; therefore, the primary concern might be the efficacy of treatment to remove ARG.

The objectives of this chapter include:

- Explore the scope of the antibiotic resistance issue.
- Describe how antibiotics work and how resistance is developed.
- Assemble and evaluate available data on the occurrence of ARB and ARG in the environment.
- Determine the effectiveness of wastewater and drinking water treatment processes for reducing/inactivating ARB and ARG.
- Identify significant data gaps and research needs.

7.1.2 Antimicrobials and Antibiotics

Antimicrobials are chemicals that kill or limit the growth of microorganisms, including viruses, bacteria, protozoa, and fungi. Antimicrobials can be pharmaceuticals, chemical disinfectants (e.g., chlorine, triclosan and quaternary ammonium compounds), and some metal ions (e.g., silver and copper). With respect to the widespread usage of triclosan in cleaning products, the U.S. Environmental Protection Agency (USEPA) recently concluded that there is no evidence "…of a causal relationship between bacterial resistance in humans and either triclosan 'body burden' or residential exposures to triclosan residues resulting from the use of consumer products" (USEPA, 2015). Yet, there is convincing evidence for ARB from wastewater and/or manure reaching drinking water (Ashbolt et al., 2013) and the sharing of ARG between the gut microbiome of animals and humans (Ma et al., 2016). Although triclosan does not appear to impact the rate of horizontal gene transfer (Rensch et al., 2013), other stressors (such as chlorine disinfection) may do so if target cells are not killed (Guo et al., 2015; Lin et al., 2016). The U.S. Food and Drug Administration (FDA) is scheduled to release a final monograph on the use of triclosan in cleaning products by mid-September 2016 (Chemical Watch, n.d.).

Antibiotics are naturally occurring antibacterial compounds or synthetic analogs that generally are used as pharmaceuticals in humans and agriculturally for disease or prophylaxis, or as growth promoters in animals. Antibiotics are the only class of drugs for which resistance is transmissible.

Antibiotic resistance is the ability of bacteria that cause infections to survive and grow in the presence of a compound that would normally kill them or limit their growth (WHO, 2015a). Although antibiotics as drugs have only been in use for about 75 years, antibiotic resistance is not a modern phenomenon. Metagenomic analyses of ancient deoxyribonucleic acid (DNA) from 30,000-year-old permafrost sediments have identified β-lactam, tetracycline, and glycopeptide ARGs, demonstrating that antibiotic resistance is naturally occurring, as well as anthropomorphically driven (D'Costa et al., 2011).

The ARBs of primary concern in drinking water are enteric bacteria, specifically those that have been frequently reported as etiologic agents in foodborne and waterborne outbreaks. As shown in **Table 7-1**, the Centers for Disease Control and Prevention (CDC) published a report listing the top 18 drug-resistant threats to the United States and categorized these threats as urgent, serious, and of concern (CDC, 2013).

Table 7-1: Drug-Resistant Microorganism Threats in the United States[a]

Hazard Level	Microorganism
Urgent	*Clostridium difficile* (CDIFF)
	Carbapenem-Resistant Enterobacteriaceae (CRE)
	Neisseria gonorrhoeae
Serious	Multidrug-Resistant *Acinetobacter*
	Drug-Resistant *Campylobacter*
	Fluconazole-Resistant *Candida*
	Extended Spectrum beta-lactamase (ESBL) Enterobacteriaceae
	Vancomycin-Resistant *Enterococcus*
	Multidrug-Resistant *Pseudomonas aeruginosa*
	Drug-Resistant Non-Typhoidal *Salmonella enterica*
	Drug-Resistant *Salmonella typhi*
	Drug-Resistant *Shigella*
	Methicillin-Resistant *Staphylococcus aureus* (MRSA)
	Drug-Resistant *Streptococcus pneumonia*
	Drug-Resistant Tuberculosis
Concerning	Vancomycin-Resistant *Staphylococcus aureus*
	Erythromycin-Resistant Group A *Streptococcus*
	Clindamycin-Resistant Group B *Streptococcus*

[a] Adapted from CDC (2013).

7.1.3 Transfer of Antibiotic Resistance

Antibiotics work by interfering with critical enzyme systems within the cell, thereby killing the bacteria or interfering with bacteria growth and reproduction. Antibiotic resistance can occur either through vertical or horizontal transfer. In vertical transfer, the bacterial cell passes inherent resistance to its progeny. Inherent resistance could be due to the following:

- Ability of the cell to destroy or inactivate the antibiotic.
- An efflux pump that channels the antibiotic out of the cell and into the environment.
- A mutation of the bacterial DNA that confers resistance.

In horizontal or lateral transfer, ARG can be taken up by bacteria belonging to different species than the one from which the gene came. Horizontal gene transfer is largely responsible for the development of resistance in bacteria that cause infection in man and animals (Bennett, 2008). ARG may be transferred by **CONJUGATION, TRANSFORMATION**, or **TRANSDUCTION** processes (Giedratienè et al., 2011).

The movement of ARG from one bacterial cell to another by conjugation involves mobile genetic elements (MGEs) that can move around the genome. These elements can transfer resistance from one cell to another or from one location to another within the same cell. MGEs include **PLASMIDS, INTEGRONS**, **TRANSPOSONS**, and **GENE CASSETTES** (Bennett, 2008; Mazel, 2006).

7.1.4 Global and National Burden of Antibiotic Resistance

The Centers for Disease Dynamics, Economics & Policy recently indicated that resistance to all first-line and last-resort antibiotics is rising overall around the world, with patterns of ARB to specific antibiotics differing regionally and by country (CDDEP, 2015). For example, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) has declined over the last 8 years in Europe, the United States, and Canada. While it has begun to decline in South Africa, it is rising in sub-Saharan Africa, India, Latin America, and Australia. This variability, as well as differences in the quality of data and reporting among countries, makes it difficult to accurately estimate global disease and economic burdens. The burden has been estimated to be 25,000 deaths due to antibiotic-resistant infections in Europe at an annual direct and indirect cost of 1.5 billion Euros (ECDC/EMEA, 2009).

Transfer Terminology

CONJUGATION: The union of two bacterial cells by cell-to-cell contact during which genetic (most often plasmid or transposon) material is transferred from the donor to the recipient cell (Giedratienè et al., 2011).

TRANSFORMATION: A process by which bacteria are able to take up and incorporate DNA from the external milieu (Chen and Dubnau, 2004).

TRANSDUCTION: The movement of genes from one cell to another by bacteriophage mediation (Griffiths et al., 2000).

PLASMIDS: Most are circular, double-stranded DNA molecules ranging in size from two to 400 genes and promote their own transfer and the transfer of other plasmids from one bacterial cell to another.

TRANSPOSONS: Elements that can move from one site to another within and among DNA molecules.

INTEGRONS: Genetic assembly platforms that can capture and incorporate genes (in cassettes) by site-specific recombination.

GENE CASSETTES: An element containing a gene and a recombination site. They can be part of an integron or free as circular DNA.

In the United States, an estimated 2-million antibiotic-resistant infections occur annually and result in 23,000 deaths at a direct cost of $20 billion plus productivity losses of $30 billion.

7.1.5 Antibiotic Usage in the United States

Most antibiotics used in the United States are for therapeutic and nontherapeutic purposes in chickens, cattle, and swine (Landers et al., 2012). The FDA recently reported sales and distribution data for antimicrobial drugs approved for use in food-producing animals (FDA, 2015). More than 33-million pounds (15,358,210 kilograms [kg]) of antibiotics for animal use were sold and distributed in 2014, with tetracyclines accounting for almost 70 percent of the medically important class (**Table 7-2**). With the exception of sulfa products, the sale and distribution of all drug classes of antibiotics increased from 2009-2014, with percentage increases ranging from 11 to 150 percent (FDA, 2015). While the link between antibiotic use in food animals and the spread of antimicrobial resistance has been established, there is no system to precisely track the amount of antibiotics used in food animals in the United States (CDC/NARMS, 2015). The most restrictive law in the country on antibiotic use in food animals, California Senate Bill 27, Chapter 758, prohibits administering medically important antimicrobial drugs to livestock solely for the purpose of promoting weight gain or improving feed efficiency. The bill also requires gathering information on antimicrobial drug sales and usage. It was signed into law on October 10, 2015, and takes effect on January 1, 2018.

The FDA reported that more than 7.25-million pounds (around 3.29-million kilograms) of antibiotics were sold for human consumption in 2011 (FDA, 2012). Taking into consideration the quantity of antibiotics sold or distributed for food-producing animals in 2011 (FDA, 2015), about 20 percent of the antibiotics sold or distributed in the United States were for human consumption. The penicillin, cephalosporin, sulfa/trimethoprim, quinolone, and macrolide classes accounted for about 87 percent of this total (FDA, 2012). While the macrolide class only accounted for about 5 percent of total sales in both 2010 and 2011, azithromycin (a macrolide antibiotic) was the most frequently prescribed antibiotic agent in 2010 for humans (Hicks et al., 2015) (**Table 7-3**).

7.2 Community and Environmental Sources/Exposure Routes for Antibiotic Resistant Bacteria and Antibiotic Resistance Genes

As a result of the use of antibiotics for therapeutic, prophylactic, and non-therapeutic purposes in conventional industrial animal production, antibiotic-resistant and multi-drug resistant strains of bacteria, including *E. coli, Salmonella*, and *Campylobacter,* are becoming increasingly prevalent (Silbergeld et al., 2008). Both water and land can be directly affected by the industrial, agricultural, and wastewater input of antibiotics, which impose selection pressure and enable the amplification, maintenance, and spread of ARBs (Pruden et al., 2013). In 2011, 21 percent of all *Salmonella* serotypes recovered from tested retail chicken products in the United States were multi-drug resistant to six or seven antimicrobial classes (FDA/NARMS, 2015). Meanwhile, reports of highly drug-resistant infections among humans in different regions of the world have appeared in the scientific literature (Dutil et al., 2010; Le Hello et al., 2011; Le Hello et al., 2013; Mulvey et al., 2013). Also, it only takes one ARB human carrier from overseas to transport a novel strain into our wastewater treatment system, where it could be spread via horizontal gene transfer and amplify – as seen with extended spectrum beta-lactamase-producing *E. coli* that originated in India (Blaak et al., 2015).

Table 7-2: Domestic Sales and Distribution Data of Antimicrobial Drugs Approved for Use in Food-Producing Animals[a] (Source: FDA, 2015)

Importance	Drug Class	2014 Annual Totals (kg)[b]	% Change 2009-2014
Medically important[c]	Aminoglycosides[a]	304,160	36
	Cephalosporins[a]	31,722	57
	Lincosamides[a]	233,681	150
	Macrolides[a]	621,769	11
	Penicillins[a]	885,975	28
	Sulfas[a]	452,224	-11
	Tetracyclines[a]	6,600,849	25
	NIR[a,e]	345,609	5
	Subtotal	**9,475,989**	**23**
Not currently medically important[d]	Ionophores	4,718,650	26
	NIR[f]	1,163,571	<1
	Subtotal	5,882,221	20
	Grand Total	**15,358,210**	**22**

[a] Includes antimicrobial drug applications that are approved and labeled for use in both food-producing animals (e.g., cattle and swine) and nonfood-producing animals (e.g., dogs and cats).

[b] kg = Kilogram of active ingredient. Antimicrobials that were reported in International Units (IU) (e.g., Penicillins) were converted to kg. Antimicrobial class includes drugs of different molecular weights, with some drugs reported in different salt forms.

[c] Guidance for Industry #213 states that all antimicrobial drugs and their associated classes listed in Appendix A of the FDA's Guidance for Industry #152 are considered "medically important" in human medical therapy.

[d] **"**Not currently medically important" refers to any antimicrobial class not currently listed in Appendix A of the FDA's Guidance for Industry #152.

[e] NIR = Not independently reported. Antimicrobial classes for which there were fewer than three distinct sponsors actively marketing products domestically are not independently reported. These classes include the following: Amphenicols, Diaminopyrimidines, Fluoroquinolones, Polymyxins (excluding 2012 and 2013), and Streptogramins.

[f] NIR = Not independently reported. Antimicrobial classes for which there were fewer than three distinct sponsors are not independently reported. These classes include the following: Aminocoumarins, Glycolipids, Pleuromutilins, Polypeptides, and Quinoxalines.

Table 7-3: Outpatient Antibiotic Prescriptions by Antibiotic Category, Antibiotic, and Geographic Region[a]

Characteristic	Number of Prescriptions in Millions (%)	Prescriptions per 1,000 Persons
Antibiotic Category		
Penicillins	60.0 (23)	194
Macrolides	57.4 (22)	185
Cephalosporins	36.2(14)	117
Quinolones	32.7 (13)	105
ß-lactams, increased activity	22.1 (9)	71
Trimethoprim-sulfamethoxazole	20.7 (8)	67
Tetracyclines	20.7 (8)	67
Lincosamides	7.8 (3)	25
Other	0.6 (0.2)	2
Total	258.0	833
Antibiotic Agent (Top 5)		
Azithromycin	51.5	166
Amoxicillin	51.4	166
Amoxicillin/clavulanate	21.5	70
Ciproflaxin	20.4	66
Cephalexin	20.1	65
Geographic Region		
South	107.4 (42)	936
Midwest	58.2 (23)	868
Northeast	46.3 (18)	830
West	46.1 (18)	638

[a] Adapted from Hicks et al. (2013).

While the sources of these infections are not easily discernible, these data point towards a worrisome progression where more cases of ARB may be potentially untreatable (Collignon, 2013). Land application of animal waste and sewage sludge can result in antibiotics and ARB persisting in soil with subsequent runoff into source waters (Fletcher, 2015; Verlicchi and Zambello, 2015). In addition, hospital waste has been identified as a potential source for antibiotic resistance (Kovalova et al., 2012), as is animal manure (Pruden et al., 2013). Using soils archived since 1923, Graham et al. (2016) suggested that ARG in animal manure and clinical isolates in humans historically were interconnected. Banning non-therapeutic antibiotic use resulted in declining levels of ARG in manured soils.

7.3 Significance of Sources for Antibiotic Resistant Bacteria and Antibiotic Resistance Genes Exposure

The possible ARB transport routes between animals and humans are numerous. Probable ways of interaction include: (1) transmission through the food chain; (2) through direct or indirect contact with people with companion animals or working in close contact with animals, such as farmers and animal health workers; and (3) through manure-contaminated environments and aquaculture (Economou and Gousia, 2015; Levy et al., 1976; Petersen et al., 2002). In particular, the role of the environment is extremely important, as it can serve as the reservoir of ARGs (D'Costa et al., 2006). There are emerging concerns that anthropogenic impacts are changing environmental reservoirs of resistance genes, "the resistome," which will increase the probability of recruitment of resistance genes into clinically relevant pathogens (Finley et al., 2013). For example, municipal wastewater, drug manufacturing, and agricultural effluents release massive quantities of antibiotic residues and ARB, selected in the digestive tracts of people or animals by antibiotic use (Finley et al., 2013; Peak et al., 2007).

7.4 Methods for Assessing Antibiotic Resistance in Water Matrices

7.4.1 Culture-Based Methods

Culture-based approaches test the susceptibility of a bacterium or specific group of bacteria, which can be propagated in liquid or on solid media, to one or more antibiotics. There are three basic variations: **DISK-DIFFUSION, LIMITING DILUTION**, and **SELECTIVE MEDIA AUGMENTATION**. The first two have been standardized for use in clinical laboratories. The methods are well established, easy to perform,

Methods Terminology

DISK DIFFUSION: A procedure in which resistance to an antibiotic can be determined by placing paper disks infused with different dilutions of the antibiotic on agar plates having a lawn of bacteria.

LIMITING DILUTION: A test in which the potency of an antibiotic can be determined by observing zones of inhibition of bacterial growth to graded amounts of an antibiotic standard.

SELECTIVE MEDIA AUGMENTATION: A procedure for determining the minimum inhibitory concentration (MIC) and the concentration enabling growth (CEG) of antibiotics by adding antibiotics to culture media containing the bacteria of interest.

and cost effective. Furthermore, state and federal surveillance networks are in place for tracking ARB in clinical and agricultural settings using these techniques (Harrison and Lederberg, 1998; Tollefson et al., 1999).

Variations of all three methods have been applied to different waters; however, at present, standardized protocols have not been validated for water-based matrices (Rizzo et al., 2013). In addition, some reports have used culture-based techniques to evaluate horizontal gene transfer between bacteria (Kowalchuk, 2004; Sørensen et al., 2005). Culture-based analyses are confined to detecting antibiotic resistance only in bacteria that can grow on the particular media used and will not detect antibiotic resistance determinants from other sources, such as viable but non-culturable bacteria, injured organisms, and extracellular DNA. It is estimated that over 90 percent of environmental bacteria cannot currently be cultured; thus, culture-based assays may lead to an underrepresentation of antibiotic resistance (Wade, 2002).

Methods Terminology

qPCR: Also known as real-time polymerase chain reaction, where measurements are made during DNA amplification, allowing the amount of DNA to be determined.

INTEGRON INTEGRASE: An enzyme that enables a gene to be integrated into the DNA of the infected cell.

METAGENOMICS: The study of the structure and function of nucleotide sequences, especially of a microbial community, isolated directly from environmental samples.

7.4.2 Molecular Methods

Molecular analyses generally refer to methods that involve the detection and/or quantification of specific DNA/RNA sequences (i.e., genes and mRNA) and/or proteins. At present, quantitative **POLYMERASE CHAIN REACTION (qPCR)** is the most prevalent molecular methodology for the assessment of ARG in the environment. Assay targets can include the resistance genes themselves, as well as sequences related to horizontal gene transfer mechanisms, such as **INTEGRON INTEGRASES** (Gillings et al., 2008).

Molecular detection is rapid, identifies antibiotic resistance in organisms that cannot be cultured, and can quantify low prevalence targets. Currently, standardized molecular protocols for ARG are not available. Molecular analyses using PCR also require a pre-defined target. Consequently, antibiotic resistance from non-targeted bacteria and genes are not detected; however, functional and sequence-based **METAGENOMIC** approaches have recently been used to provide a more comprehensive picture of antibiotic resistance in environmental matrices without the requirement of strictly pre-defined targets (Gomez-Alvarez et al., 2012; Li et al., 2015; Munck et al., 2015; Nesme et al., 2014; Yang et al., 2013; Ma et al., 2016).

Metagenomic analysis (Schmieder and Edwards, 2012) currently is not applicable to routine testing due to the requirement for specialized analysts and equipment, along with the high costs associated with this technology (but costs are rapidly declining). A more complete list of advantages and disadvantages for each methodology is outlined in **Table 7-4**.

Table 7-4: A Comparison of Culture-Based and Molecular Approaches for Monitoring Antibiotic Resistance

Methodology	Advantages	Disadvantages
Culture-based analyses	Measures antibiotic resistance or horizontal gene transfer in live organisms	Can only detect antibiotic resistance in bacteria that can be cultured; greater than 90 percent of bacteria have not been cultured; injured organisms or bacteria in viable, but non-culturable states may not be detected
	Measure any antibiotic resistance mechanism being expressed by the organism; no pre-defined knowledge of ARG needed	Methods are not rapid; data are obtained in greater than 1 day
	Measures mechanisms that actively result in antibiotic resistance (i.e. expressed antibiotic resistance)	Will not detect extracellular DNA or ARG in non-targeted species
	Can be performed by most water treatment laboratories	Antibiotic resistance expression may be affected by growth conditions
	Relatively inexpensive	Isolation of minority species may be difficult due to overgrowth of non-target bacteria
	Detection of horizontal gene transfer processes possible	
	Standardized methods already have been developed in clinical laboratories	
	Compatible with data from clinical laboratories	
	Over 40 years of clinical data available on ARB	
Molecular analyses[a]	Detection and quantification of DNA targets from all sources in a sample	No standardized methods
	Highly specific and sensitive; can be designed to quantify specific ARG	Detects DNA from non-viable organisms; difficult to differentiate DNA from live versus dead bacteria
	Detection of minority targets; can detect underrepresented ARG among high concentrations of background DNA	Susceptible to inhibition from environmental matrices
	Relatively rapid; data possible in less than 1 day	Higher costs
	Detection and quantification of horizontal gene transfer mechanisms	Sample preparation can be time consuming
	Can obtain taxonomic and antibiotic resistance data	Quantitative polymerase chain reaction (qPCR) susceptible to large inter-laboratory variations
		Requires specialized equipment and knowledge not common in water treatment laboratories
		Must have a predesignated target; will miss antibiotic resistance related to non-targeted genes

[a] Excludes high-throughput DNA sequencing technologies, which are not viable monitoring options at present.
ARG = Antibiotic resistance genes. ARB = Antibiotic resistant bacteria.

The examination of analytical targets that include both antibiotic resistance determinants, as well as MGE, could provide additional benefits with respect to public health. Functional metagenomic studies have shown that different environments (e.g., activated sludge, soil, and the human gut) each contain a wide array of genes that can convey antibiotic resistance, although a majority of these genes have not been described in human pathogens (Schmieder and Edwards, 2012; Martínez, 2011; Munck et al., 2015). In WWTPs specifically, pathogen-associated ARG were found to be present, but comprised only a minority of the total ARG in activated sludge (Munck et al., 2015). In particular, a metagenomic study from Denmark (Munck et al., 2015) identified *tetA* (tetracycline resistance), $bla_{tem\text{-}1}$, $bla_{oxa\text{-}1}$ (β-lactam resistance), and *ermB* (macrolide resistance) as the most prevalent clinically relevant ARG in activated sludge; however, these genes accounted for less than 600 of the more than 9,000 sequence reads that mapped to functional ARG. Munck et al. (2015) also observed that the dissemination of wastewater ARG into other environments was limited and that MGE were associated with some ARG that had disseminated into other environments. For example, an *aadA* ARG associated with a class I integron from Danish activated sludge samples also was identified in a pig manure sample and within an *E. coli* isolate from a bloodstream infection (Munck et al., 2015). Mobilization, therefore, is an important factor contributing to the dissemination of ARG (Munck et al., 2015). For that reason, ARG that have already mobilized into human pathogens represent a more direct risk to public health compared to other environmental ARG. Consequently, analyses focusing on clinically relevant mobilized ARG would likely provide more pertinent data with regards to human health than ARG alone.

7.5 Occurrence and Removal of Antibiotic Resistant Bacteria and Antibiotic Resistance Genes Through Water and Wastewater Treatment

7.5.1 Occurrence and Removal in Wastewater Treatment Plants

Wastewater treatment practices in use today are designed to reduce the health risk associated with known and unknown chemical, organic and microbial hazards. Conventional wastewater treatment plants (WWTPs) use a series of distinct stages to treat incoming wastewater. Primary treatment consists of physical separation of oil and grease, as well as sedimentation of large particles and suspended solids. Secondary treatment consists of biological degradation to reduce dissolved organic matter. Tertiary-stage treatment refers to any process after secondary treatment that is employed to further improve water quality. Examples of tertiary treatments include additional biological processes to remove specific chemical constituents (such as nitrogen-containing compounds) or physical barriers that improve the microbiological and physical characteristics of the water. Finally, one or more disinfection steps are commonly applied to treated effluents, resulting in additional microbial inactivation prior to discharge to receiving streams or water reuse applications.

The ability of WWTP processes to significantly diminish indicator bacteria concentrations in wastewater has been well documented. Indicator bacteria generally are decreased between 2- and 3-$\log_{10}$ units by primary and secondary treatment steps, while subsequent disinfection can result in an additional 1- to 4-$\log_{10}$ reduction (Rose et al., 2004). Moreover, advanced tertiary treatment can produce further decreases in microbial concentrations related to the specific process employed. The effects of treatment processes on the removal of ARG and ARB, however, are less well characterized and differ for different ARG (Christgen et al., 2015; Jury et al., 2011).

Regulatory levels related to microbial constituents have been established for the protection of public health, but not for antibiotic resistance. Currently, there are no regulations pertaining to antibiotic resistance in wastewater or potable water due to several factors, including the lack of data on the dose-

response relationship between antibiotic resistance determinants in water and adverse human health effects (Ashbolt et al., 2013); however, information on the occurrence and concentration of some antibiotic resistance elements in water and wastewater is available. Until risk assessment tools are established, evaluating the quantity of ARG and/or ARB through water treatment processes offers the best approach for limiting any potential risk related to antibiotic resistance. For instance, antibiotic resistance detection methods can be used to identify treatment practices that efficiently remove antibiotic resistance determinants or as part of a framework verifying the operational effectiveness of particular treatments.

Resistance to all classes of antibiotics have been described in raw wastewater (Rizzo et al., 2013), although the presence and quantity of specific ARG and ARB can differ according to geographic location, type of treatment employed, and operational parameters (Yang et al., 2012; Munir et al., 2011; Bouki et al., 2013). In general, resistance to β-lactams, tetracycline, macrolides, and sulfonamides have been the most prevalent types of clinically relevant antibiotic resistance described in wastewater (Rizzo et al., 2013; Li et al., 2015; Yang et al., 2013; Munck et al., 2015). Specifically, tetracycline, sulfonamide, and β-lactam resistance genes have been reported at concentrations ranging from 10^7 to 10^{11} copies/100 milliliters (mL) in raw wastewater (**Table 7-5**), whereas culture-based resistance in indicator bacteria ranged from 10^5 to 10^8 colony forming unit (CFU) or most probable number (MPN)/100 mL (**Table 7-6**).

Additionally, pathogen-associated antibiotic resistance has been examined in wastewater. In particular, vancomycin-resistant enterococci (Rosenberg Goldstein et al., 2014) and *mecA* (methicillin resistance gene) (Borjesson et al., 2009; Bockelmann et al., 2009), as well as various clinically relevant resistance phenotypes within fecal indicator bacteria (an indicator for enteric bacterial pathogens, see **Table 7-5**), have been characterized in wastewater matrices (see **Tables 7-5** and **7-6** for additional information).

While the presence of ARG and ARB in wastewater present a potential risk to humans, water treatment processes have shown the ability to remove or inactivate these constituents. A summary of ARB and ARG removal by different wastewater treatment practices is shown in **Tables 7-5** and **7-6**. Briefly, primary treatment of wastewater results in little reduction (less than 1-$\log_{10}$ unit) of individual ARG, whereas secondary treatment produces a more substantial removal with ARG typically reduced by 1-to 3-$\log_{10}$ units (see **Table 7-5**) and ARB between less than 1- and 5-$\log_{10}$ units (see **Table 7-6**). Tertiary-level treatment reductions vary, but can provide up to an additional 4-$\log_{10}$ reduction of ARG and ARB by filtration and disinfection processes. For example, media filtration coupled with disinfection resulted in the removal of *tet* and *sul* genes that ranged from less than 1- to 2-$\log_{10}$ (Fahrenfeld et al., 2013). Furthermore, in one study examining ARB and ARG removal through WWTPs, activated sludge treatment along with media filtration and disinfection (ultraviolet [UV] disinfection or chlorine), were shown to reduce tetracycline and sulfonamide ARB and ARG to 3- to 4-$\log_{10}$ from the concentrations found in raw wastewater (Munir et. al., 2011). Additionally, membrane bioreactor technology incorporating UV disinfection has demonstrated greater reductions of ARG compared to conventional WWTPs using tertiary sand filtration and disinfection (Munir et al., 2011); however, the removal of antibiotic resistance determinants was not consistent, as some ARG and ARB were found in higher numbers than others after particular treatments (see **Tables 7-5** and **7-6**). The reason for the prevalence of some antibiotic resistance determinants in certain treatment matrices has not yet been determined.

Table 7-5: Reported Antibiotic Resistance Genes (ARG) Removal by Wastewater Treatment Processes

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
	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 7-6: Reported Antibiotic Resistant Bacteria (ARB) Removal by Wastewater Treatment Processes

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, 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.

7.5.2 Antibiotic Resistance Occurrence and Removal by Drinking Water Treatment Processes

Potable water matrices (both source and treated waters) have been shown to contain ARB and ARG (Armstrong et al., 1982; Xi et al., 2009). In general, the most prevalent types of antibiotic resistance found within WWTPs (β-lactams, tetracycline, macrolides, and sulfonamides) were also the predominant resistance determinants described in drinking water matrices (Armstrong et al., 1982; Armstrong et al., 1981; Gomez-Alvarez et al., 2012; Narciso-da-Rocha et al., 2013; Shi et al., 2013; Vaz-Moreira et al., 2011; Vaz-Moreira et al., 2012; Jia et al., 2015). The concentrations of ARG reported in source waters varied depending on location, water type, and analysis target (**Table 7-7**), but were typically less than those found in WWTP effluents (Iwane et al., 2001; LaPara et al., 2011; LaPara et al., 2015; Marti et al., 2013).

More specifically, resistance to β-lactams, tetracycline, macrolides, and sulfonamides in source water ranged from not-detected to 10^6 copies/100 mL (ARG) and from not-detected to 10^6 CFU/100 mL in culturable bacteria (see **Table 7-7**). Furthermore, the quantities detected in groundwater were lower than those in surface water by approximately 1- to 3-$\log_{10}$ units (see **Table 7-7**). The occurrence and concentration of antibiotic resistance determinants in surface water also has been shown to differ depending on the sources impacting the water (Pruden et al., 2012).

Typical drinking water treatment schemes involve coagulation/flocculation, filtration, and disinfection steps. Advanced water treatments, such as biological filtration, activated carbon, and advanced oxidation processes, also may be employed. To date, only a few studies have quantitatively described the removal of antibiotic determinants in drinking water treatment processes; therefore, additional research is needed to provide a more robust analysis of removal rates. A summary of current work on the occurrence and removal of ARG and ARB is provided in **Table 7-7**. In general, total ARB reductions between raw source water and final treated water (without advanced water treatment) were within the range 4- and 6-$\log_{10}$ units (see **Table 7-7**). Furthermore, treatment processes decreased the amount of ARG between less than 1- to nearly 2-$\log_{10}$ units, resulting in concentrations on the order of 10^3 copies/100 mL or less in finished water (see **Table 7-7**). The quantity of ARB and ARG also was observed to increase through drinking water supply distribution systems, presumably the result of biofilm formation and sloughing (Xi et al., 2009), which are greatly enhanced within premise plumbing (Ashbolt, 2015). The effects of these increases on public health and the environment are largely unknown.

7.5.3 Disinfection and Advanced Oxidation Processes

Disinfection procedures commonly are employed in both potable treatment plants and WWTPs as a means to further reduce harmful microbes. Chlorination is the most prevalent disinfection technique used in the United States, followed by UV irradiation and ozone (Dodd, 2012). Under typical disinfection parameters for wastewater, chlorine disinfection (10 to 450 mg x min./L), UV irradiation (dose of 10 to 200 mJ/cm^2), and ozone (approximately 30 to 60 mg x min./L) can achieve 4-$\log_{10}$ or more of indicator bacteria inactivation (Huang et al., 2011; Macauley et al., 2006; Yuan et al., 2015; Oppenheimer et al., 1997; Oh et al., 2014); however, the ability of these disinfection methods to reduce DNA concentrations differ. Moreover, bacteriophages are a vector for the transfer of ARG (via transduction); therefore, reductions in bacteriophage concentrations will need to be effective to limit the spread of ARGs.

Table 7-7: Reported Concentrations and Log_{10} Reductions of Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARG) in Drinking Water Treatment Matrices

Water Type or Treatment Process[a]	ARG/ARB[b]	ARG/ARB Concentration[c]	Log_{10} Reduction[d]	References
Surface water	Tetracyclines	HP: 10^4-10^5 ARG: ND-10^6 MGR: 0.71	NA	Xi et al., 2009; Jia et al., 2015; Bockelmann et al., 2009; Armstrong et al., 1982
	β-lactams	HP: 10^4-10^5 ARG: ND-10^4 MGR: 1.4	NA	Xi et al., 2009; Jia et al., 2015; Bockelmann et al., 2009; Armstrong et al., 1982
	Macrolides	ARG: ND-10^6 MGR: NR	NA	Jia et al., 2015; Bockelmann et al., 2009
	Chloramphenicol	HP: 10^3-10^4 ARG:10^2-10^3 MGR: 0.41	NA	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982
	Quinolones	HP: 10^5 MGR: 0.05	NA	Xi et al., 2009; Jia et al., 2015
	Aminoglycosides	HP: 10^3-10^6 MGR: 2.94	NA	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982
	Sulfonamides	HP: 10^4-10^6 ARG:10^3-10^4 MGR:4.82	NA	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982
Groundwater	Tetracyclines	ARG: ND-10^{3e}	NA	Bockelmann et al., 2009
	β-lactams	ARG: ND[e]	NA	Bockelmann et al., 2009
	Macrolides	ARG: ND[e]	NA	Bockelmann et al., 2009
Primary stage	Tetracyclines	HP: 10^1 ARG: ND MGR: 0.41	HP: 2-3 ARG:NA	Jia et al., 2015; Armstrong et al., 1982
	β-lactams	HP: 10^1 ARG: ND MGR: 1.16	HP: 2-3 ARG:NA	Jia et al., 2015; Armstrong et al., 1982
	Chloramphenicol	HP: 10^1 MGR: 0.38	HP: 2-3	Jia et al., 2015; Armstrong et al., 1982
	Quinolones	MGR: NR		Jia et al., 2015
	Aminoglycosides	HP: 10^1-10^2 MGR: 3.42	HP: 2-3	Jia et al., 2015; Armstrong et al., 1982
	Sulfonamides	HP: 10^2 MGR: 7.33	HP: 2-3	Jia et al., 2015; Armstrong et al., 1982

Water Type or Treatment Process[a]	ARG/ARB[b]	ARG/ARB Concentration[c]	Log_{10} Reduction[d]	References
Finished water	Tetracyclines	HP: 10^{-2}-10^{0} ARG: ND MGR: 0.47	HP: 5-6 ARG:NA	Bockelmann et al., 2009; Jia et al., 2015; Xi et al., 2009; Armstrong et al., 1982
	β-lactams	HP: 10^{-2}-10^{1} ARG: ND-10^{2} MGR: 1.43	HP: 4-5 ARG: <1-2	Bockelmann et al., 2009; Jia et al., 2015; Xi et al., 2009; Armstrong et al., 1982
	Macrolides	ARG: ND	ARG: NA	Bockelmann et al., 2009
	Chloramphenicol	HP: 10^{-2}-10^{1} ARG: 10^{1}-10^{2} MGR: 0.59	HP: 4-5 ARG:1-2	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982
	Quinolones	HP: 10^{0} MGR: 0.05	HP: 5	Xi et al., 2009; Jia et al., 2015
	Aminoglycosides	HP: 10^{-1}-10^{1} MGR: 1.04	HP: 4-5	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982
	Sulfonamides	HP: 10^{-1}-10^{0} ARG: 10^{1}-10^{3} MGR: 2.63	HP: 5-6 ARG: 1-2	Xi et al., 2009; Jia et al., 2015; Armstrong et al., 1982

[a] Primary treatment signifies drinking water processes of pretreatment, flocculation, and sedimentation. Finished water refers to samples taken after filtration and disinfection processes.

[b] Indicates significant antibiotic classes studied for resistance in water matrices. Each category includes data for all ARGs or ARB variants described in the accompanying references.

[c] The values represent the ARB or ARG concentration ranges coalesced from the accompanying published reports rounded to the nearest power of 10. The ARB data refer to indicator organisms that typically do not contain extensive numbers of pathogens. HP = Heterotrophic bacteria per 100 milliliters. ARG: Antibiotic resistance gene by quantitative polymerase chain reaction (qPCR) (copies per 100 milliliters). MGR = Metagenomic reads are given as antibiotic resistance reads per million total reads. ND = Not detected. NR = Not reported.

[d] The values represent the log_{10} reduction range between the source water 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.

[e] The values represent only data from the Torreele, Belgium groundwater recharge site described in the accompanying reference as it was the only recharged groundwater further treated for potable use.

Reductions in microbe and ARG concentrations in water are a function of the disinfectant concentration applied and the length of treatment, which results in a collective term referred to as CT (i.e., residual disinfectant concentration, C, mg/L, multiplied by the contact time, T, minutes). In reference to the removal of ARB in water, chlorine CTs less than 100 mg x min./L decrease ARB by less than 1- to 4-$\log_{10}$, with chlorine concentration having a greater effect than reaction time (Huang et al., 2011; Murray et al., 1984; Oh et al., 2014; Yuan et al., 2015; Zhuang et al., 2015). The removal of ARG by chlorination processes in drinking water and treated wastewater has been reported between 0.1- to 1.49-$\log_{10}$ (Oh et al., 2014; Xi et al., 2009; Yuan et al., 2014; Zhuang et al., 2015). Current Title 22 recycled water criteria for the State of California mandate a chlorine CT of 450 mg x min/L to be considered a tertiary treated disinfected water.

Elevating the CT to 450 produced substantially more removal of ARB and ARG than other less stringent chlorination parameters (Macauley et al., 2006; Zhang et al., 2015), but was less effective than ultrafiltration (UF) processes (Breazeal et al., 2013; **Table 7-8**). Chloramine also is used as a disinfectant in water matrices and was found to be less effective at reducing ARG compared to chlorine in laboratory experiments (Zhang et al., 2015). Notably, the amount of ARG reduction in wastewater effluent treated with chlorine has also been shown to plateau as a function of treatment concentration and time (Yuan et al., 2015; Zhuang et al., 2015). Additional research is needed to determine the optimal disinfection parameters for the removal of ARB and ARG in drinking water and treated wastewater.

Recent metagenomic work has demonstrated that ARG diversity decreased after drinking water chlorination, although the relative abundance of some ARG increased (Jia et al., 2015). Certain antibiotic resistance phenotypes and ARG have been associated with bacterial survival at low to moderate chlorine doses (Shi et al., 2013; Yuan et al., 2015). In particular, certain genes associated with β-lactam, tetracycline, macrolide, and chloramphenicol resistance were shown to increase in percentage after treatment, with CTs of less than 70 mg x min./L chlorine (Huang et al., 2011; Shi et al., 2013; Yuan et al., 2015). Despite the increase in relative abundance of some ARG following chlorination, the specific ARG found to be present were also found in species that did not survive disinfection, suggesting that the relationship between ARG and disinfection processes is not completely understood. A metagenomic study characterizing ARG and bacterial communities before and after drinking water chlorination demonstrated that the abundance (i.e., number of ARG sequences as a proportion of the total number of reads) of some ARG increased after chlorination, while others decreased (Jia et al., 2015). Additionally, bacterial community shift was found to contribute more than MGE to the observed changes in the resistome after chlorination (Jia et al., 2015). Thus, the effects of antibiotic resistance with regards to chlorine susceptibility have not been completely determined. Interestingly, sulfonamide ARG (which are prevalent in wastewater and potable water prior to disinfection) were successfully reduced with chlorine (Jia et al., 2015; Shi et al., 2013; Xi et al., 2009; Zhuang et al., 2015).

Ozone and UV irradiation are alternative disinfection processes that have been applied in the treatment of wastewater and potable waters. UV disinfection is effective at reducing ARB populations, but shows very little activity against ARG (Auerbach, 2007; McKinney and Pruden, 2012; Munir et al., 2011; Zhang et al., 2015). Typically, UV treatment has resulted in poor removal of ARG (less than 1-$\log_{10}$) at dosages commonly applied to water treatment. In particular, dosages greater than 200 mJ/cm^2 were necessary to achieve a 3-$\log_{10}$ reduction of ARG compared to 20 mJ/cm^2 to achieve similar reductions in ARB (McKinney and Pruden, 2012).

Table 7-8: Log_{10} Reduction of Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARG) in Water by Disinfection and Barrier Processes

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 log	NR	EPA, 1999; Dodd, 2012; Armstrong et al., 1982[c]
	WWTP disinfection (typical)	30-300 mg × min./L	3-5 log	<1	Huang et al., 2011; Yuan et al., 2015
	WWTP disinfection (CA Title 22)	450 mg × min./L	2->4 log	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 log	<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 log	1-3	Dodd, 2012; Lüddeke et al., 2015; Oh et al., 2014; Zhuang et al., 2015
Ultrafiltration[d]	Filtration	NA	NR	4->5.9	Breazeal et al., 2013
Reverse osmosis	Filtration	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 = Millijoule per centimeters squared.

Data on the effects of ozone on ARB and ARG in water are sparse, but it appears to be a promising technology for eliminating antibiotic resistance, exhibiting better reactivity with DNA than chlorine (Dodd, 2012). More than 2-$\log_{10}$ of ARB and ARG can be removed from synthetic wastewater using ozone at a CT of 31 mg x min./L (Oh, 2014); however, a recently published report demonstrated that the relative abundance (qPCR copies normalized to DNA concentration) of some clinically relevant ARG increased after ozone treatment of secondary effluent while others decreased, suggesting that further characterization is needed to fully understand the impacts of ozone treatment on the removal of ARB and ARG (Alexander et al., 2016).

Data on the relationship of antibiotic resistance determinants and advanced water treatments associated with DPR, in particular, UF and reverse osmosis (RO), is lacking. UF is an effective means of reducing bacteria and viruses in treated wastewater, removing indicator bacteria to below detectable limits and MS2 by up to 7-$\log_{10}$ (Gómez et al., 2006; Madaeni et al., 1995; Madaeni, 1999). Moreover, when coupled with RO, the combined process provides an additional barrier, preventing microbial contamination in the final treated water. Consequently, the probability of bacteria (including ARB) occurring in the final permeate at concentrations greater than those found in typical drinking water sources is small; however, contamination during maintenance processes and/or biofilm formation within the drinking water supply distribution system present potential sources of bacteria that could affect the quality of the finished water.

Along with intact bacteria, extracellular (free) DNA containing ARG is a potential source of antibiotic resistance, and the effects of UF and RO on removals of free DNA have not been studied extensively. Limited published data has shown that UF is an effective barrier to ARG. In particular, using a laboratory-scale, stirred cell filtration apparatus, membranes with an average pore size of 10,000 Daltons (Da) were able to remove 4.9-$\log_{10}$ units of plasmid from treated wastewater (Breazeal et al., 2013). Decreasing the pore size to 1,000 Da retained all of the plasmid within the filter, resulting in a decrease of greater than 5.9-$\log_{10}$ (Breazeal et al., 2013). Unfortunately, no published data regarding the removal of ARG through pilot-scale and full-scale membrane processes were found. Issues associated with the differences between the laboratory and full-scale processes (such as o-ring integrity or membrane imperfections) could affect the removal of ARG. No published data were found that specifically detailed the removal of ARG by RO processes (i.e., molecular weight cutoff of 100 Da or less). Considering the UF data provided by Breazeal et al. (2013), along with the physical parameters of RO, it is reasonable to conclude that the probability of finished water (UF and RO treated) containing significant concentrations of ARG would be unlikely, although additional research is needed. Furthermore, Böckelmann et al. (2009) analyzed secondary treated wastewater that underwent UF, RO, and groundwater infiltration prior to entering a drinking water treatment facility. The UF process removed greater than 4-$\log_{10}$ units of ARG from the secondary effluent, resulting in undetectable levels (by qPCR) in all samples; however, one sample collected after the UF and RO treatments exhibited a small, but detectable, concentration for one ARG, which implies bacterial regrowth or biofilm shedding may be a source of antibiotic resistance in these waters. In general, the UF/RO processes described by Böckelmann et al. (2009) demonstrated ARG concentrations that were below those observed in other drinking water sources, such as groundwater and surface waters, suggesting the human health risk associated with UF- and RO-treated wastewaters would be less than or equal to that found in traditional source waters. Moreover, the drinking water produced from the treatment system described above was negative for all the ARG tested, indicating that any ARG in the UF/RO source water were successfully eliminated by conventional potable water treatment processes (Böckelmann et al., 2009).

7.6 Findings of the Exert Panel

In terms of ARB and ARG, the Expert Panel finds that:

- **Finding #7-1:** Antibiotic resistance is a valid and serious worldwide public health concern.

- **Finding #7-2:** Risk levels associated with ARB/ARG in water have not been determined; however, concentrations of ARB/ARG in waters subjected to DPR treatment processes would likely be lower than that from current water sources entering drinking water treatment facilities, suggesting that risk levels would be comparable to, or less than, those associated with current source waters.

- **Finding #7-3:** ARB and ARG are found in wastewater and in other environments, such as soils and other source waters (not necessarily impacted by wastewater).

- **Finding #7-4:** There are currently no standardized tests for ARB/ARG/ mobile genetic elements (MGE) in environmental samples.

- **Finding #7-5:** The determination of ARB/ARG concentrations in water can be helpful in assessing treatment process efficiencies to remove antibiotic resistance determinants.

- **Finding #7-6:** Current wastewater treatment technologies (e.g., activated sludge, tertiary filtration, and chlorine disinfection) reduce ARB and ARG concentrations.

- **Finding #7-7:** The current knowledge base regarding urgent and serious potentially waterborne drug-resistant bacteria is limited for known antibiotic resistance determinants and their fate during treatment.

- **Finding #7-8:** Information about the performance of advanced water treatment processes related to ARG removal is limited. Disinfection and oxidation (e.g., chlorine, ozone, UV) differ in their effectiveness of removing ARG in treated wastewater. There is limited information regarding the efficiency of membrane processes (e.g., UF, RO) at pilot-scale and full-scale on the removal of ARG.

- **Finding #7-9:** Considering all the available information, a combination of secondary wastewater treatment and advanced water treatment processes (i.e., a sequence of treatment train processes such as microfiltration/UF, RO, and ultraviolet disinfection/advanced oxidation processes) leading to a finished potable water is likely to reduce ARB and ARG concentrations in recycled water to levels well below those found in conventional treated drinking water.

- **Finding #7-10:** Ongoing research in the U.S., Europe, and Asia is examining wastewater and other sources (e.g., hospitals, agriculture) for ARG and ARB and their removal by different treatment processes. Examples of this research are included in **Appendices 7A** to **7F**.

7.7 Recommendations of the Expert Panel

Based on the above findings (see **Section 7.6**), it is unclear whether water is a significant disseminator of ARB and ARG relative to other sources; therefore, the Expert Panel believes it is important to continue to characterize the role of potable reuse in disseminating antibiotic resistance and identified the following three research recommendations. The following recommendations (which are not listed by priority) should be addressed at a broader national level.

- **Recommendation #7-1:** Additional research is needed to determine the risk to humans associated with ARB and ARG in water relative to other sources of exposure. In particular, research is needed on defining dose-response relationships between ARB and ARG concentrations in water and their ability to be acquired by human pathogens and transferred to environmental microbiota and the gut microbiome.

- **Recommendation #7-2:** Standardized tests to determine ARB and/or ARG concentrations in potable water and wastewater should be developed. These tests should be financially and technologically accessible to a majority of water and wastewater treatment agencies. Ideally, the tests would quantify ARB and ARG that are relevant to humans. Methodology should be developed that also provides an assessment of ARG transferability within water matrices (including biofilms).

- **Recommendation #7-3:** Characterize and evaluate ARB and ARG removal using advanced water treatment processes. Projects practicing DPR should quantitatively determine the removal of ARB and/or ARG and identify the most promising and robust technologies within their treatment trains to reduce antibiotic resistance determinants for potable reuse.

7.8 References

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PART III: PERFORMANCE OF DIRECT POTABLE REUSE SYSTEMS

CHAPTER 8: PERFORMANCE OF DIRECT POTABLE REUSE SYSTEMS

- Potential treatment trains for direct potable reuse.
- Assumptions for quantifying the reliability of treatment facilities.
- Performance criteria for microbial and chemical contaminants.
- Identifying hazards and hazardous events.
- Quantifying robustness, resilience, and redundancy.
- The roles of operations and maintenance and source control.

8.1 Introduction

Drinking water augmentation with recycled water has been practiced in the form of IPR for more than 50 years in California (Crook, 2010; Drewes and Khan, 2015; Drewes and Horstmeyer, 2016). Thus, a long track record exists in California and worldwide, demonstrating that planned IPR can be practiced with no apparent detrimental effect on public health (NRC, 2012; Khan, 2013). A key element of an IPR system is its reliance on an environmental buffer (e.g., groundwater aquifer or reservoir). While some environmental buffers might offer opportunities for the additional removal of contaminants (Drewes and Khan, 2011), the major technical reason for requiring environmental buffers in IPR projects is to provide sufficient residence time within the environmental buffer to allow detection and preventive action before harmful contaminants can reach the public drinking water supply. Environmental buffers also provide, in all cases, the equalization or leveling out of the concentration of hazardous constituents to further reduce the potential of subjecting consumers to unreasonably high concentration levels.

The main difference between direct potable reuse (DPR) and indirect potable reuse (IPR) is that with DPR, environmental buffers are eliminated or substantially reduced in size from that required for IPR. The major question that needs to be addressed in developing DPR regulations is ***how the significant benefits of residence time and equalization offered by environmental buffers associated with IPR practices can be incorporated into a DPR system to provide at least an equivalent degree of reliability and redundancy***. Two major options have been proposed to fulfill the core functions of the environmental buffer in DPR systems, either by providing additional treatment redundancy or/and by adding engineered storage with a defined holding time prior to release into the drinking water supply distribution system.

The lack of an environmental buffer means that DPR is a more closely coupled system, in which there is less time to monitor process water quality and respond to out-of-specification quality water issues. This factor is especially important for water quality concerns related to acute risks, such as those presented by pathogens and selected chemicals like nitrate. Providing additional and redundant treatment barriers can reduce acute risks from water quality deviations in the finished water as long as the additional treatment process is capable of accommodating the failure of another process (i.e., representing an additional safety factor).

8.2 Examples of Potential Treatment Trains for Direct Potable Reuse

The design of DPR treatment trains in California likely will build upon the long track record of IPR schemes for subsurface spreading augmented by additional treatment barriers; therefore, the Expert Panel decided to contrast the IPR scheme for subsurface spreading to five potential DPR alternatives (**Figure 8.1**). Four of these alternatives contain integrated membrane systems (i.e., microfiltration and reverse osmosis), as well as an advanced oxidation process (AOP). Example 1 represents a treatment scheme that involves an environmental buffer with a shorter reservoir retention time than required in the proposed criteria for IPR using surface water augmentation (SWA). Examples 2 to 4 consider different combinations of treatment processes. Example 5 represents a treatment train that does not rely on reverse osmosis.

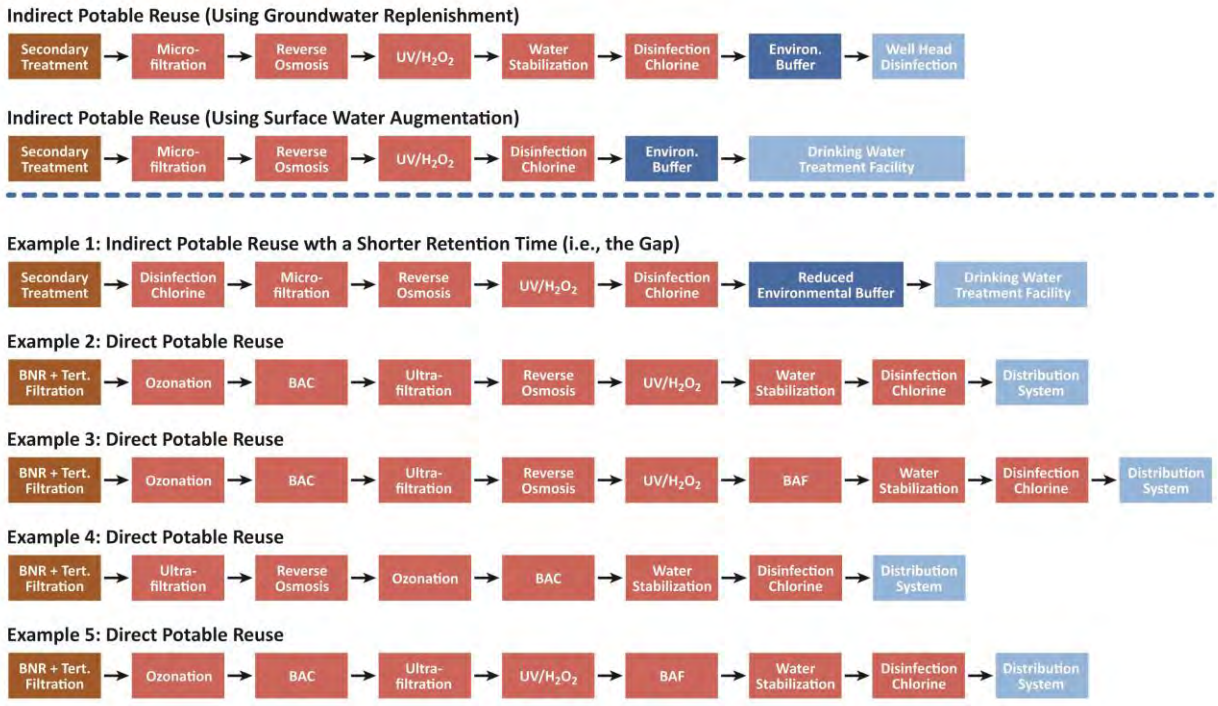

Figure 8-1: Examples of potential direct potable reuse treatment trains in comparison to examples (above the dashed line where California regulations exist or proposed regulations are under development) for indirect potable reuse via subsurface spreading and surface water augmentation. UV = Ultraviolet disinfection. H_2O_2 = Hydrogen peroxide. Tert. = Tertiary. BNR = Biological nutrient removal. BAC = Biological activated carbon. BAF = Biologically active filtration.

These examples do not represent an endorsement of an ultimate treatment train design for DPR by this Expert Panel, but are intended to serve as a basis for illustrating reliability concepts and relative risk assessments of different IPR and DPR schemes. It should be noted that in many of these treatment trains, water stabilization is a critical unit operation. The purpose of stabilization is to minimize corrosion in the subsequent drinking water distribution system, service lines, and building (premise) plumbing of consumers. Typically, stabilization is achieved by adding chemicals like calcium hydroxide,

sodium bicarbonate (or carbonate), various phosphates, or sodium hydroxide. Given the greater attention to corrosion control following the Flint Water Crisis in 2014, new DPR systems should undertake careful studies of corrosion control prior to distributing water, and any major changes in operations should trigger the reassessment of corrosion control.

8.3 Underlying Assumptions for Quantifying Treatment Facility Reliability

The potential of exposure to a given contaminant in the finished water from a potable reuse scheme may be estimated from its concentration in the untreated wastewater together with its variable degree of removal by each of the multiple set of treatment processes used in the wastewater treatment and potable reuse schemes (Eisenberg et al., 2001; Haas and Trussell, 1998; Tanaka et al., 1998). Wastewater naturally is variable with respect to the concentrations of hazards, such as the occurrence of pathogens and trace chemicals, which may vary over hourly, daily, and longer periods. The design and operation of a potable reuse system must cope with such variability to produce a consistently acceptable product.

Furthermore, the performance efficiency of advanced water treatment processes, such as reverse osmosis and AOP, varies depending upon a range of factors, including flow rate, water composition, and temperature. As a result, the final concentrations of microbial and chemical contaminants in water (and, hence, exposure to them) will vary. For DPR projects, the final water quality is expected to have greater temporal variability as compared to the final water quality of IPR projects because the equalizing effect of an environmental buffer has been eliminated. In addition, a shorter time period to detect and respond to failures and process upsets, as well as the potential for higher water quality variability, places a higher demand on the ability of the DPR system to prevent and rapidly respond to such failures and excursions (Drewes and Khan, 2011; Pecson et al., 2015b). The main goal of any potable reuse scheme is to provide a high degree of consistent public health protection (i.e., reliability).

In the context of this report, the following definitions as related to reliability include:

- ***Reliability*** is the ability of a potable reuse system to verifiably deliver a water quality that consistently exceeds the public health protection expected of conventional drinking water supplies.

- ***Redundancy*** is the addition of measures beyond minimum requirements to ensure treatment goals are met reliably and performance targets are achieved consistently, if not exceeded. Redundancy is accomplished through the use of independent parallel operations of one or more similar treatment trains, permitting continuous operation even if one entire treatment train may become inoperative for maintenance or other reasons.

- ***Robustness*** is the ability of a potable reuse system to address a broad variety of contaminants and resist catastrophic failures. In particular, considering the broad variety of chemical contaminants and pathogens, robustness is the use of a diverse group of barriers to control a variety of contaminants (NRC, 2012).

- ***Resilience*** is the capacity of a potable reuse system to successfully adapt and/or respond to a failure.

Redundancy, robustness, and resilience in potable reuse schemes are provided through the use of multiple technical, operational, and management barriers, which together provide opportunities for risk prevention, risk management, monitoring and compliance, and individual action (NRC, 2012; Tchobanoglous et al., 2015). Here, multiple barriers are obtained through the use of several different treatment processes operated in series to provide redundancy and robustness in the removal of both pathogens and unwanted chemicals and to ensure the failure of a single process does not render the system vulnerable to penetration by microbial or chemical contaminants that pose a significant risk to public health. Among the technical barriers are engineered physical, chemical, and biological processes, each of which removes multiple classes of contaminants, such as the diverse set of chemicals and pathogens of concern.

The degree of the reliability of a potable reuse system can be evaluated by two approaches. First is the use of the ***"multiple barrier"*** concept, which is a core design principle in potable reuse, as well as in conventional drinking water supply, where redundancy, robustness, and resilience can be demonstrated. There is the expectation that a multi-barrier system can maintain treatment goals even if a single unit treatment process fails. The multiple barrier concept in potable reuse is established at multiple levels: (1) source control program; (2) conventional wastewater treatment; (3) advanced water treatment; (4) management of the environmental (engineered) buffer; and (5) drinking water treatment, including management of the drinking water distribution system (Drewes and Khan, 2011); however, in the absence of comprehensive water quality data and a well-defined methodology for assessment, many established potable reuse projects have followed rather conservative criteria in selecting individual treatment barriers and configuring these to overall potable reuse treatment trains (Drewes and Khan, 2011).

A second approach for the assessment of reliability is the use of ***probability distribution functions*** **(PDFs)**, which provide insight on each treatment process by describing how frequently a barrier achieves different levels of performance (Haas and Trussell, 1998; Olivieri et al., 1999). By combining multiple independent treatment barriers, it is possible to generate an overall PDF of a potable reuse treatment train to quantitatively determine the probability of the system in satisfying treatment goals (e.g., required LRVs) (Khan, 2009; Drewes et al., 2010).

8.4 Performance Criteria for Microbial Contaminants

For microbial contaminants, the requirements under existing IPR regulations in California specify 12-$\log_{10}$ reduction of viruses, 10-$\log_{10}$ reduction of *Giardia,* and 10-$\log_{10}$ reduction of *Cryptosporidium* (referred to as "12/10/10"). The basis for these requirements are described in **Chapter 2** and will not be discussed further in this chapter.

8.5 Performance Criteria for Regulated and Unregulated Chemical Contaminants

For a proposed potable reuse scheme, performance goals for chemical contaminants should include contaminants of recognized health concern that have published guideline values or standards for regulated and unregulated contaminants (i.e., California Notification Levels and USEPA Health Advisories), as well as surrogate measures for bulk water characteristics that can provide assurance of the proper removal of chemical contaminants in potable reuse schemes **(Figure 8-2).**

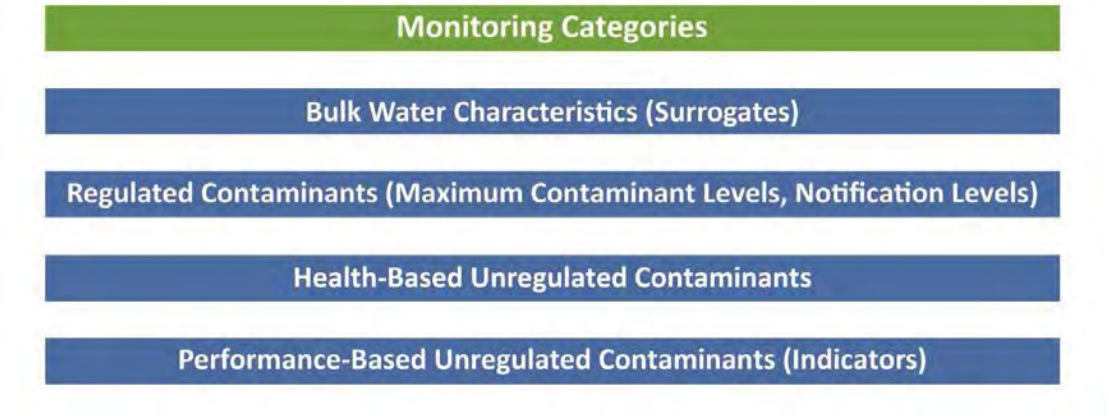

Figure 8-2: Monitoring categories for the proper control of chemical contaminants during potable reuse.

8.5.1 Health-Based Contaminants

Regulated contaminants include chemicals with acceptable health risks specified, for example, as primary or secondary maximum contaminant levels (MCLs), notification levels commonly used in California, public health goals published by the Office of Environmental Health Hazard Assessment (OEHHA), or maximum contaminant level goals by the USEPA. Chemical contaminants that are noteworthy with respect to monitoring plans for DPR projects are listed in **Table 8-1**. For a more detailed discussion, please refer to **Chapter 3**.

For ***unregulated contaminants*** with available toxicological information, a *de minimis* risk approach can be used. To specify *de minimis* levels for these health-based contaminants, any of the following can be adopted and usually modified by a relative source contribution and used as a point of departure (POD) for estimating risks for carcinogens and non-carcinogens by applying appropriate uncertainty factors (Schwab et al., 2005; Snyder et al., 2008; Bull et al., 2011; Khan, 2013):

- Reference dose (RfD) which is derived from a NOAEL or LOAEL and applying several uncertainty factors depending upon the nature of the toxicological data.
- Benchmark dose (usually a model-predicted lower confidence level of a 10-percent effect level [designated by as a $BMDL_{10}$], this procedure is increasingly being used as the RfD because of the imprecision of NOAEL and LOAELs) by application of appropriate uncertainty factors.
- Acceptable daily dose (ADD).
- Predicted no-effect concentration (PNEC) that expresses the toxicological potency of health-based contaminants.

For example, *de minimus* levels can be considered a risk-based action level (RBAL) by applying attributable source contribution of 0.2 (WHO, 2010, NRC, 2008).

Table 8-1: Health-Based Contaminants Proposed for Monitoring Programs for Potable Reuse Projects

Parameter	Chemical (Maximum Concentration)	Notes
N-Nitrosodimethylamine (NDMA)	10 ng/L	Notification Level, California
N-Nitrosodiethylamine (NDEA)	10 ng/L	Notification Level, California
1,4-Dioxane	1 µg/L	Notification Level, California
Perfluorooctanoic acid (PFOA)	70 ng/L[a]	Health advisory (USEPA, 2016)
Perfluorooctane-sulfonic acid (PFOS)	70 ng/L[a]	Health advisory (USEPA, 2016)
Bromate	10 µg/L	USEPA Primary Maximum Contaminant Level

[a] PFOA and PFOS (individually or combined) should not exceed the 70 ng/L health advisory.

ng/L = Nanogram per liter. µg/L = Microgram per liter.

When neither existing guideline values nor relevant toxicological data are available to develop benchmark values, an empirical approach based on structural features can be used for deriving thresholds of toxicological concern (TTCs) (Khan, 2013). The TTC approach is based upon the statistical evaluation of a large group of chemicals with similar structure and functional groups. It allows identifying a 95-percent lower confidence level for a chronic no adverse effect level for that particular group of compounds (called Cramer Classifications), and then applies uncertainty factors similar to that used in non-cancer risk assessments. The use of TTCs is well established internationally and has been used by the U.S. Food and Drug Administration and the World Health Organization for determining whether toxicological data is needed for minor contaminants of a product; ***however, these data should not be treated as the equivalent of MCLGs, PHGs, or MCLs***. The method has been developed for deciding whether it is necessary to develop toxicological data for minor contaminants of a product. Essentially, it is a triage approach that says, based upon the activity of other chemicals in the similar-structure group, the measured concentrations are sufficiently below the 95-percent lower confidence level that the chemicals need not be of concern. If one looks at the levels arrived at using known compounds, the TTC value typically is far below MCLs for chemicals (e.g., brominated haloacetic acids [HAAs] versus the currently regulated five HAAs by the USEPA [i.e., HAA5]).

Because of the large number of contaminants, deviations in published RfD or PNEC values for individual contaminants, and differences in expert opinion regarding the appropriate uncertainty factors (UncFactor) for carcinogenic contaminants, a uniform list of contaminants that should be monitored in potable reuse schemes does not yet exist. Nevertheless, several scientific groups and panels have proposed contaminants with human health relevance that should be used periodically for potential inclusion in monitoring programs for potable reuse projects (see NWRI, 2013; Drewes et al., 2013; SWRCB, 2013).

8.5.2 Performance-Based Contaminants and Surrogate Measures

Performance validation and verification of established and alternative treatment trains can be obtained through direct measurements of certain performance-based indicator contaminants that correlate with core removal mechanisms (i.e., biotransformation, adsorption, size exclusion, chemical oxidation) of individual treatment processes (Drewes et al., 2008; Dickenson et al., 2009, 2011). The following factors must be considered for the selection of performance-based indicator contaminants to assess the treatment efficacy of potable reuse schemes (Drewes and Horstmeyer, 2016):

- Target contaminants chosen to assess treatment performance must occur frequently enough and at concentrations significantly above their analytical method detection limit (preferably, the ratio between the measured environmental concentration and the method detection limit should exceed 10).

- Appropriate and commercially available analytical methods must exist to quantify the target contaminants in recycled water.

- Performance-based indicator contaminants used for monitoring should broadly represent the range of physicochemical and biological properties affecting their removals by the various treatment processes within a potable reuse treatment train.

In addition, specific performance-based bulk measurements that can be monitored continuously (e.g., electrical conductivity, UV absorbance) can serve as surrogate parameters for contaminants of interest whose removal is similar. Such surrogate parameters also can indicate out-of-specification performance or treatment process failure (Drewes et al., 2008; Wert et al., 2009; Drewes et al., 2010). These approaches have the advantage in that they can be established as real-time monitoring strategies where high-time resolution system performance control is desired. A summary is provided in **Table 8-2** of some proposed surrogate and performance-based indicator contaminants and their expected removal requirements.

To illustrate the usefulness of measuring surrogate parameters in real time for the assessment of treatment process efficacy, a spiking experiment was conducted at a DPR demonstration facility in San Diego, California (**Figure 8-3**). To evaluate the efficiency of ozonation followed by biological active carbon (BAC) filtration and an integrated membrane system (UF/RO) in treating a secondary-treated wastewater effluent, acetone was spiked at a high microgram-per-liter range. The spike was administered twice for short time periods, initially prior to the RO process and subsequently prior to the ozone/BAC and UF/RO treatment. During both spiking events, total organic carbon (TOC) concentrations in the RO permeate increased, confirming the breakthrough of acetone, which is difficult to remove by the processes employed. This experiment demonstrated the value of TOC as a surrogate parameter for detecting a spike in acetone concentrations; however, it is possible that some highly volatile organic chemicals will not be captured by conventionally configured monitors of TOC. Other materials that might break through during spikes could be low molecular weight inorganics, such as tritium and bromide (which will be a function of the nature of the incoming wastewater).

Table 8-2: Performance-Based Surrogate and Indicator Contaminants Proposed for Monitoring Programs for Potable Reuse Projects

Parameter	Surrogate/Indicator (maximum concentration or minimum percent removal)	Notes and References
Total organic carbon	0.5 mg//L	Performance trigger level for reverse osmosis membranes (SWRCB, 2013)
1,4-Dioxane	>69%	Performance trigger level for advanced oxidation (CCR, 2015)
Caffeine	>90%	Performance trigger level for advanced oxidation (SWRCB, 2013)
DEET	>90%	Performance trigger level for advanced oxidation (SWRCB, 2013)

[a] Failure to achieve these minimum removals or percentages does not imply an adverse health effect.

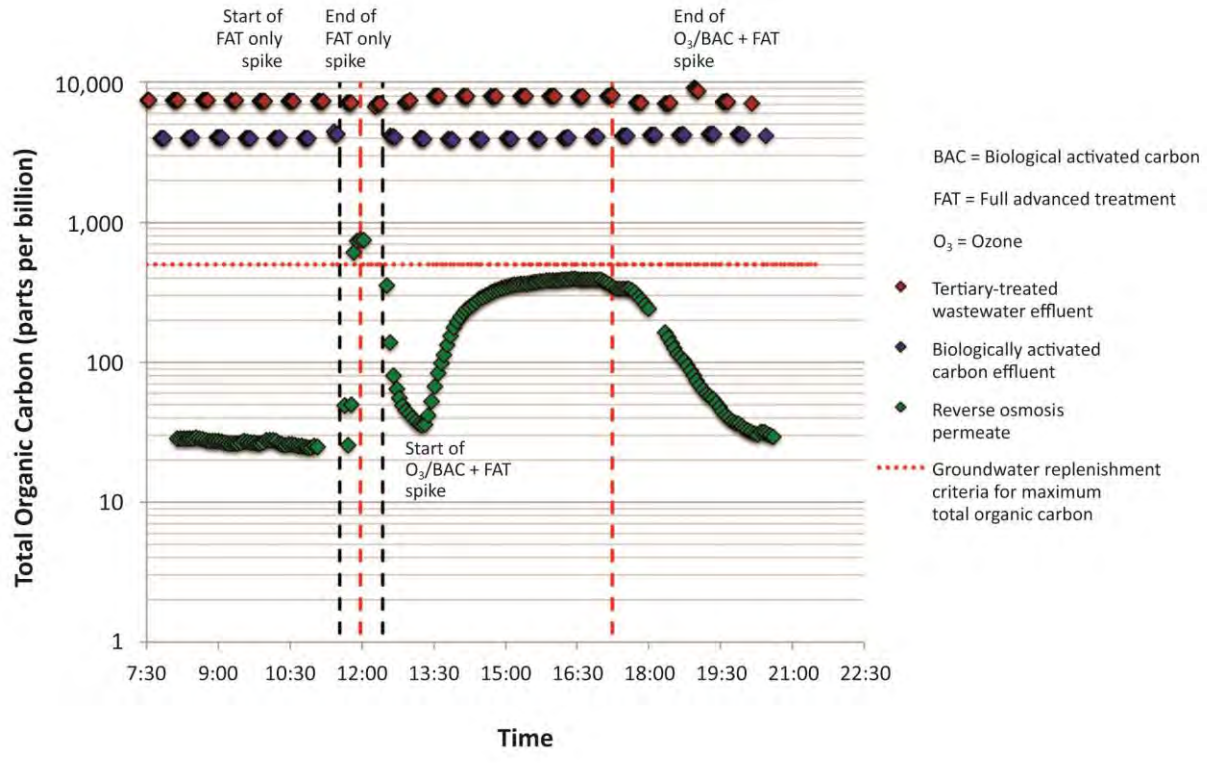

Figure 8-3: Spiking of acetone at a demonstration-scale facility comprised of ozone (O_3), biological activated carbon (BAC), ultrafiltration (UF), reverse osmosis (RO), and advanced oxidation processes (AOPs) (Tackaert et al., 2016).

8.6 Identification of Hazards and Hazardous Events

Multiple hazards could impact the ability of a DPR system to provide water of acceptable quality, including:

- Introduction of "slugs" of chemical or microbial contaminants into the system that may overwhelm the treatment processes (see **Figure 8-3**).
- Mechanical failure of equipment or process control systems.
- Human error (e.g., operator, laboratory).
- Power failure (especially on a regional scale, such that multiple sources of power are disrupted simultaneously).
- Natural disasters (e.g., earthquake, fire, storms) that mechanically disrupt components needed for adequate functioning.
- Intentional malicious activity.

The design of a DPR system to achieve an adequate level of reliability requires the use of principles to compensate for as many of the hazards that can occur as feasible. The specification of the necessary level of reliability is a regulatory and management decision that must be made, recognizing there will be a tradeoff between reliability and cost.

8.7 Barriers in Indirect Potable Reuse Schemes Providing Reliability

8.7.1 Role of Engineered Storage Barriers and Environmental Buffers in Existing Indirect Potable Reuse Schemes

In California, one regulation has been adopted for IPR using groundwater replenishment, as codified in Title 22 (CCR, 2015), specifying the practice of groundwater replenishment via surface application or via subsurface application of recycled municipal wastewater. Currently, a second regulation is under development (as of June 2016) for SWA using a surface water reservoir as the environmental buffer.

With IPR using surface application (i.e., surface spreading), the environmental buffer includes both the soil and vadose zone through which treated recycled municipal wastewater first passes prior to reaching the groundwater aquifer, where the recycled water moves to a recovery well, possibly after mixing with native water in the aquifer, where it is then extracted and further treated, if necessary, prior to entering the drinking water distribution system. With subsurface application (i.e., direct injection), the aquifer serves as the sole environmental buffer into which treated recycled water is directly injected and remains in the aquifer until recovery through an extraction well. With SWA, the environmental buffer is provided by the reservoir receiving the advanced treated water.

Brief summaries are provided in **Tables 8-3** and **8-4** of the pathogen removal requirements by a treatment train and by the environmental buffer used for each of the three IPR options described above (i.e., surface application, subsurface application, and SWA).

Table 8-3: Treatment Train Requirements for Virus, *Cryptosporidium,* and *Giardia*

System	Treatment	Pathogen Reduction (Log_{10})		
		Enteric Virus	*Cryptosporidium*	*Giardia*
Surface Application[a]	Filtration and disinfection, soil aquifer treatment	≥12	≥10	≥10
Subsurface Application[a]	Reverse osmosis and oxidation	≥12	≥10	≥10
Surface Water Augmentation A[b]	Reverse osmosis and oxidation	≥8	≥7	≥8
Surface Water Augmentation B[c]	Reverse osmosis and oxidation	≥9	≥8	≥9

[a] Pathogen reductions for surface and subsurface applications represent the sum of removals from the wastewater and recycled water treatment train processes, subsurface environmental buffer, and water treatment processes applied to extracted groundwater.

[b] Pathogen reductions for surface water augmentation must be achieved by the wastewater treatment plants and advanced water treatment facilities prior to release of source water to the reservoir. The drinking water treatment facilities using the surface water must achieve additional log pathogen reductions of 4/3/2 to result in an overall log reduction of 12/10/10.

[c] Pathogen reductions for surface water augmentation must be achieved by the wastewater treatment plants and advanced water treatment facilities prior to the release of source water to the reservoir. An additional log_{10} reduction is required to replace the reduced level of dilution in the reservoir (i.e., 100:1 reduced to 10:1). The drinking water treatment facilities using the surface water must achieve additional log_{10} pathogen reductions of 4/3/2 to result in an overall log_{10} reduction of 12/10/10.

Table 8-4: Environmental Buffer Requirements and Pathogen Reduction Allowances

System	Theoretical Retention Time, and Required Dilution	Pathogen Reduction Allowances (Log_{10})		
		Enteric Virus	*Cryptosporidium*	*Giardia*
Surface Application	≥2 months	(1 log_{10} per month underground)	*	*
Subsurface Application	≥2 months	(1 log_{10} per month underground up to a maximum of 6 log_{10})	**	**
Surface Water Augmentation A	≥4 to 6 months, ≤1% per day	No reduction credit	No reduction credit	No reduction credit
Surface Water Augmentation B	≥4 to 6 months, ≤10% per day	No reduction credit	No reduction credit	No reduction credit

* Surface Application: For retention times ≥6 months a total of 10-log_{10} reduction credit will be given for the entire system (treatment facility prior to application and subsurface system). For less than 6 months retention time, no log_{10} reduction credit is given for the subsurface system.

** Subsurface Application: Regardless of retention time, no log_{10} reduction credit will be given for the subsurface system.

In general, the State Water Board assumes that less than the required 10^{-4} risk from pathogens in drinking water would be achieved if the overall pathogen removal from concentrations in raw wastewater to drinking water supplied to consumers is a 12-$\log_{10}$ reduction for virus and 10-$\log_{10}$ reduction each for *Cryptosporidium* and *Giardia*. Of these, the proportions of the pathogen reductions that can be allotted to the different environmental buffers are somewhat dependent upon the reductions obtained through wastewater treatment processes.

The minimum required subsurface Retention time for both surface and subsurface groundwater replenishment applications is 2 months with $\log_{10}$ reduction credits as described in Table 8-4. In any event, the overall $\log_{10}$ reduction requirement for pathogens is the same for both applications (i.e., 12/10/10 for virus, *Giardia*, and *Cryptosporidium*, respectively.).

As suggested in **Table 8-3,** less overall pathogen $\log_{10}$ reductions are required in the two scenarios of the proposed surface water augmentation IPR regulations than for IPR via groundwater replenishment; however, surface water supplies that are used for drinking water, in general, must be treated according to California regulations under Title 22, Article 2, to achieve $\log_{10}$ reductions of pathogens equivalent to a 4/3/2 level for virus, *Giardia,* and *Cryptosporidium,* respectively. This requirement, plus the pathogen reductions outlined in **Table 8-3** for wastewater treatment under the surface water augmentation A scenario, provides the same overall 12/10/10 $\log_{10}$ reductions as for IPR via groundwater replenishment. No specific pathogen reduction is assumed for the reservoir that serves here as the environmental buffer; however, an additional requirement for surface water augmentation A scenario is no more than 1 percent of the surface water withdrawn at any time can come from recycled water discharged into the reservoir on any previous single day. That means if there were a failure in the treatment train on a given day, no more than 1 percent of that water would reach consumers. In effect, there would be a 100:1 dilution of the water failing to meet regulations; however, with the surface water augmentation B scenario, where a 1-$\log_{10}$ greater reduction of pathogens is required for wastewater treatment, the dilution requirement could be as low as 10 percent rather than 1 percent. Implied is an assumption that a 1-day failure would result in no more than a 1-$\log_{10}$ increase in pathogen concentrations at the DWTF intake, a value that could result from a 1-day tenfold increase in pathogen concentrations in the recycled wastewater supplied to the reservoir.

Chemical contaminant regulations for inorganic and organic chemicals, disinfection byproducts, and radioactivity are similar for all three IPR scenarios (i.e., surface application, subsurface application, and SWA). Maximum concentrations generally are specified for the treated municipal wastewater being used for recycling, with no benefit allotted for reductions that may occur within the environmental buffer or in the treatment schemes that may be used on water extracted from a given environmental buffer. As a result, chemical contaminant regulations are treated somewhat differently than biological contaminants. Most past concerns with chemicals have been from long-term exposure rather than short-term exposure; therefore, in general, a short response time to an excessive concentration was not considered as essential with chemicals as with pathogen contamination. Even so, a sudden chemical spill (such as from an industrial accident) potentially could cause concentrations that affect health from short-term exposure, so this possibility cannot be ignored.

If measures can be developed for DPR that substitute the benefits provided by environmental buffers (i.e., time to respond and equalization), then potential acute health problems resulting from hazardous chemicals and pathogens possibly could be overcome.

8.7.2 Benefits Provided by Environmental Buffers in Indirect Potable Reuse Schemes

For IPR pathogen log_{10} reductions to be comparable for DPR, LRVs would need to be 12/10/10. In the proposed criteria for IPR using SWA, the environmental buffer is assumed to provide no credit for pathogen or hazardous chemical reduction other than providing dilution and residence time; actual reduction must be accomplished by the combination of engineered treatment processes used to treat the wastewater before discharge to the reservoir and those subsequently used to treat the extracted reservoir water. It is conceivable that the same total series of engineered treatment processes might be used in a DPR scheme. If such process combinations are sufficient to reduce biological and chemical contaminants for IPR schemes, and if these combinations all perform as expected (perhaps quantified by the use of probability distribution functions), they should – when applied to a DPR scheme – reduce the contaminants to the same degree; however, spiking studies mimicking hazardous events at a DPR demonstration-scale facility illustrated that even additional treatment barriers, like ozonation and BAC followed by UF/RO processes and UV-AOP, were not capable of completely reducing chemicals like acetone or formaldehyde, which also resulted in an exceedance of TOC concentrations beyond 0.5 mg/L (**Figure 8-4**).

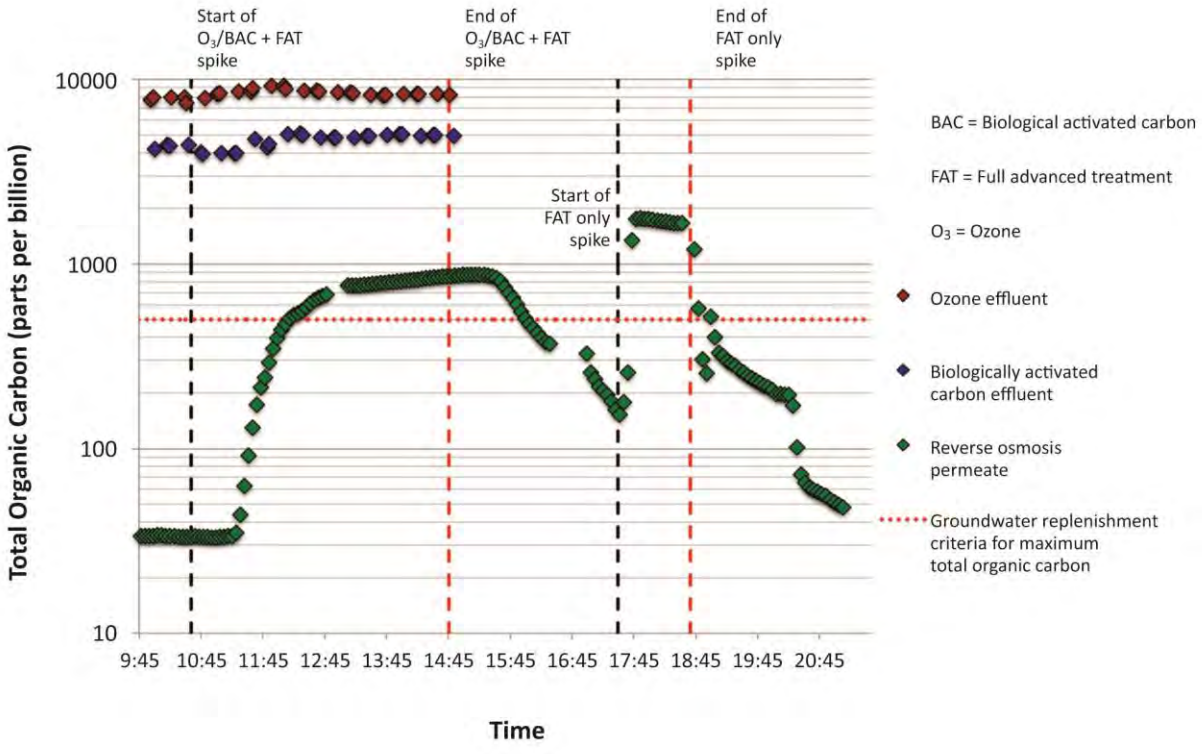

Figure 8-4: Spiking of acetone and formaldehyde at a demonstration-scale facility comprised of ozone (O_3), biological activated carbon (BAC), ultrafiltration (UF), reverse osmosis (RO), and advanced oxidation processes (AOPs) (Trussell et al., 2016).

With engineered processes alone, how quickly can a failure be detected in DPR, and how can consumers be protected from receiving unacceptably contaminated water? These questions, perhaps, are among the biggest challenges for DPR and need to be addressed. In the scenario for Surface Water Augmentation B from **Tables 8-3** and **8-4**, the greater risk of allowing 10 percent rather than 1 percent of any day's recharged water to reach consumers was reduced by requiring a 1-$\log_{10}$ increase in pathogen reduction by any of the treatment systems. This approach is one of several that might be applied in DPR. For example, the requirement for an overall $\log_{10}$ reduction of pathogens might be increased over 12/10/10 to counteract the risk resulting from the failure of a process in the overall treatment train. Other possibilities need examination.

Another significant advantage of environmental buffers is the equalization or leveling out of wastewater concentrations, which vary greatly over time. Thus, a 12/10/10 $\log_{10}$ reduction in pathogens for the less variable wastewater concentrations provided by the environmental buffer would tend to be more risk-free than for the same $\log_{10}$ reductions applied when peak pathogen concentrations in wastewater occur. The hazard analysis and critical control point (HACCP) approach uses performance-based standards for each step in a process sequence to ensure that the overall performance of the treatment train yields acceptable quality. It can be coupled with quantitative risk assessment, and has been proposed for the control of drinking water quality (Havelaar et al., 1994; Notermans et al., 1995; Walker et al. (in press, a,b). The use of HACCP coupled with a quantitative risk assessment could illustrate the loss of redundancy during various hazardous events or treatment process failures. This knowledgebase might provide justification to propose alternative treatment barriers for a DPR scheme that would provide the same level of safety as an IPR scheme using an environmental buffer.

8.7.3 Typical Removals of Pathogens in Treatment Processes

California requirements do not specify completely the processes to be used to meet pathogen reduction requirements for IPR, nor do they specify in general the pathogen reductions that can be obtained by each treatment process. The task of obtaining this information is required of the utility requesting permission to build and operate an IPR facility. Literature values have been provided by Tchobanoglous et al. (2015) and Pecson et al. (2015b) for treatment efficiencies obtainable for pathogen reductions by some treatment processes, and approved $\log_{10}$ reduction values for groundwater replenishment projects in California are summarized in **Table 2-2**. A summary is included in **Chapter 2** of the $\log_{10}$ reduction credits approved by the State Water Board for particular treatment processes. There may be several different manners in which treatment processes may be joined together to obtain the overall 12/10/10 $\log_{10}$ pathogen reduction requirement. What then becomes important are the best engineered barriers to use and the best ways to combine them with other technical, operational, and management barriers to achieve the reliability, redundancy, and resilience necessary for producing a safe water supply for the public.

The aim of quantifying reliability is to assess whether a treatment train is capable of delivering acceptable quality water for a high percentage of time; therefore, reliability can be used as an iterative tool in design, as well as a check on operational performance. Several methods and tools can be used to assess the reliability of a DPR system. In the multi-barrier approach, the removal of any single contaminant does not depend solely on one step, so that if one step in the treatment chain fails entirely, reduction requirements in principle can still be achieved. This concept is illustrated in **Table 8-5** using a hypothetical treatment train and pathogen as examples.

Table 8-5: Hypothetical Example of Calculating Log_{10} Reduction Requirements for the Multi-Barrier Approach

Influent Concentration	10,000 per Liter (L)	Log_{10} Reduction
Stage A Log_{10} Reduction		2
Stage B Log_{10} Reduction		3
Stage C Log_{10} Reduction		2
Maximum Acceptable Concentration	1/L	
Log_{10} Reduction Required		4

In this hypothetical example, a treatment system has three stages (Stages A, B, and C), which achieve 2-, 3-, and 2-log_{10} reductions, respectively. The influent to the treatment system contains 10,000 per liter of a pathogen (which might be some estimate of reasonable maximum concentration, as in the genesis of the IPR removal requirements). In addition, it has been determined (e.g., by risk assessment) that a final maximum concentration of 1 organism per liter is acceptable; therefore, 4-log_{10} reduction is required. Based on **Table 8-5**, if any one of Stage A, B, or C is disabled, the ability to remove 4 log_{10} or more still exists; therefore, reliability with respect to this pathogen would be deemed adequate.

Although useful for the development of IPR processes, the multiple independent barrier approach has several limitations, including:

- If total failures were rare, but frequent periods of poor performance of multiple processes were typical, unacceptable performance could still occur.
- Correlations may exist between poor performances of different processes. The processes may not be truly independent, for example:
    - Inadequate particle conditioning affects performance in coagulation/flocculation and filtration.
    - Elevated TOC (and total organic nitrogen) interferes with adsorption and disinfection.
    - Common underlying variables (e.g., temperature, alkalinity) affect performance.
    - Common failure mechanisms occur for multiple membrane systems.

A second, more detailed (and more data-intensive) method to assess reliability is to use a Monte Carlo approach, as described by Haas and Trussell (1998). For this method, as depicted in **Figure 8-5**, the influent concentration (N_0) is described by a probability distribution, $f_0\left(N_0\right)$. Each stage-wise removal is described by a conditional probability distribution; for example, $f_A\left(N_A|N_0\right)$. In the (very commonly used) case of first-order removal, these latter distributions can be described by an unconditional distribution of the quantity ($1-\varepsilon$), where ε is the fractional removal efficiency.

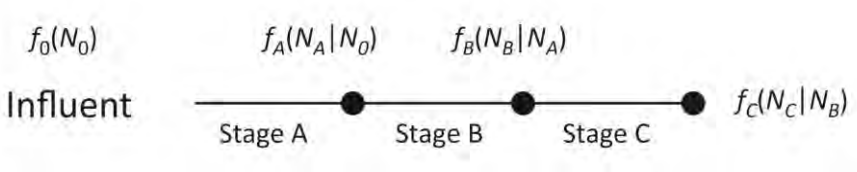

Figure 8-5: Schematic diagram of a probabilistic analysis of a treatment train.

The advantage of this method is that it allows for the calculation of exact reliability probabilities. A key disadvantage is the need to determine the stage-wise transformation distributions. The calculations can be done using a Monte Carlo approach where a random draw from each of the relevant distributions is made and the product water concentration is computed. By repeating this calculation a large number of times, the product water distribution of concentration can be computed.

In **Table 8-6,** a simple example is used to illustrate this approach. Depicted here are the distributions for a system using MF, RO, AOP, UV disinfection, and chlorination. Note that these are hypothetical distributions and are not informed by real datasets. The results of the simulation, which included 10,000 trials, are provided in **Figure 8-6**. As shown, for example, the overall treatment system achieves more than 99.9-percent reliability for producing water with less than 10^{-4} organisms per liter.

Table 8-6: Distributions for an Example Problem

Influent	Distribution gamma, scale=5, shape=2
Microfiltration $\log_{10}$ removal	Normal (5,1)
Reverse osmosis $\log_{10}$ removal	Normal (5,1)
Advanced oxidization removal	−log [beta (0.9, 29.1)]
Ultraviolet disinfection removal	−log [beta (3, 27)]
Chlorine removal	−log [beta (7, 3)]

Notably, this approach carries a number of assumptions that must be validated by additional data. First, the removal achieved through one stage is only a function of what organisms have come through the prior stage and, in particular, is not a function of the time history of prior treatment performance (which may be important if flows and quality are highly variable). Second, it is assumed that removal efficiencies are first order.

The work being conducted at the DPR Demonstration Project in San Diego, California, by Trussell Technologies is supportive of these assumptions, and shows that reductions during sequential processes appear to be statistically independent, which greatly simplifies the computational task (Pecson et al., 2016); however, with increasing data being collected on potential DPR configurations at the pilot scale, it is expected that the use of this technique will become more practical.

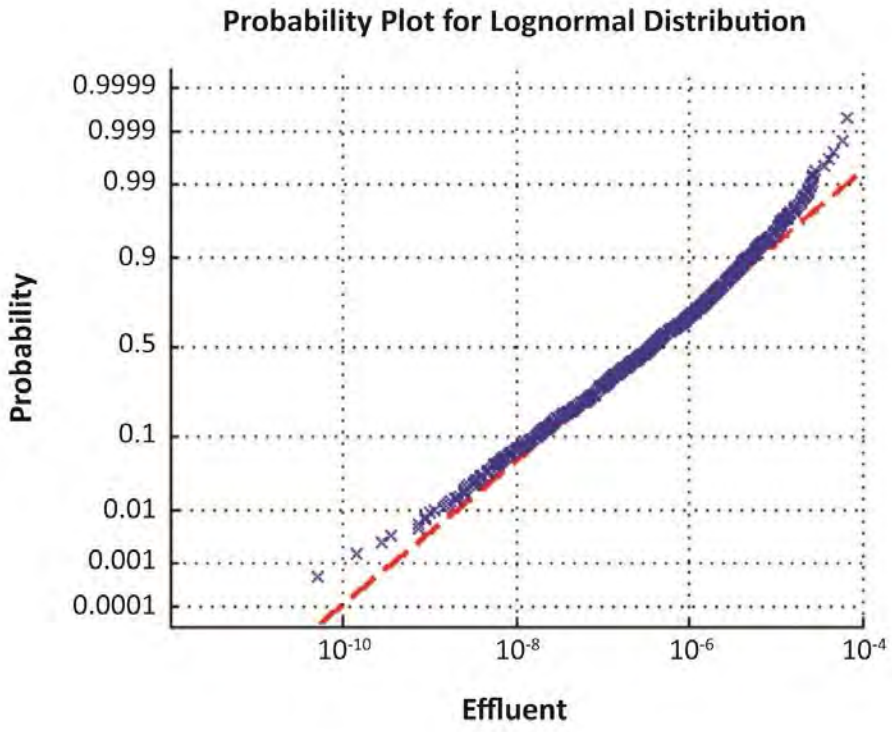

Figure 8-6: Results from Monte Carlo: Probability that effluent will be less than or equal to indicated concentration, using lognormal coordinates.

8.7.4 Robustness, Resilience, and Reliability

To be feasible, DPR systems must meet or exceed the attributes of robustness, resilience, and reliability (which were defined in **Section 8.3**). A resilient system is one that can recover rapidly from a disturbance. To some degree in IPR, resilience is provided by a storage barrier prior to final release for consumption. Robustness is defined as the presence of different ***types*** of treatment processes acting via different mechanisms such that a yet-unknown pollutant likely will be removed by multiple stages. The concept of robustness is illustrated in **Figure 8-7**.

With DPR systems, the resilience provided by the environmental buffer in IPR comes from a combination of multiple parallel treatment trains, high frequency monitoring of surrogate constituents, the presence of an adequate inventory of replacement equipment, and a detailed response action plan when target levels of surrogates are exceeded at any step. An example feasibility analysis is provided in **Chapter 9**.

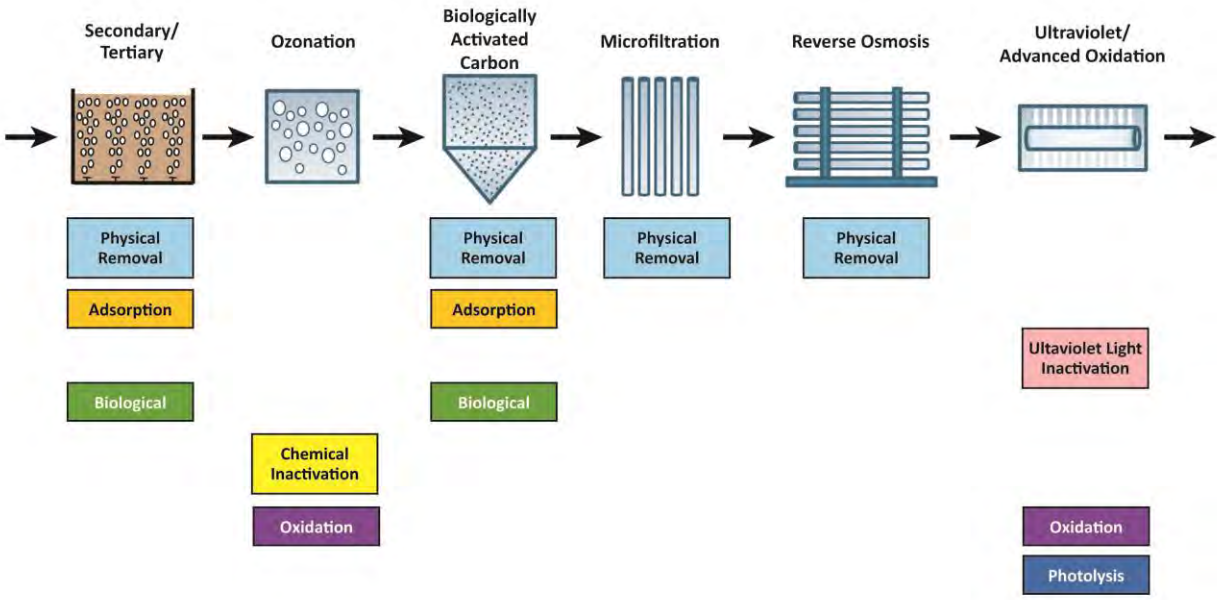

Figure 8-7: Schematic of the concept of robustness. Adapted from Pecson et al. (2016).

8.8 Operation and Maintenance

The production of advanced treated water involves the use of a number of advanced treatment processes. Aggressive operation and maintenance (O&M) is necessary to ensure that advanced treated water meets all public health objectives and the DPR system operates consistently and reliably. O&M activities begin with the design and construction of the DPR system and continue throughout its lifetime (Tchobanoglous et al., 2015).

One essential element is the O&M plan, which provides an approach to demonstrate the various treatment processes of the DPR system are performing as designed. The O&M plan also must include regulatory compliance sampling and monitoring, as well as performance monitoring (NWRI, 2016). Components of an O&M plan are listed in **Appendix 8A**.

Another important element is operator training. AWTFs are complex systems and must be operated and maintained by well-trained, highly skilled operations staff. These operators must be able to effectively respond to any issues or challenges that arise at the AWTF, as well as receive ongoing training or certification as new processes and techniques become available. Efforts are underway in the State of California to determine what is needed for DPR operator training and certification (NWRI, 2016). For example, the State Water Board collaborated with the California Urban Water Agencies and four other organizations to develop a framework for potable reuse operator training and certification in California (CUWA, 2016).

Overall, in regards to O&M, the Expert Panel recommends the following (NWRI, 2016):

- Utilities with DPR systems should develop plans for initial startup, annual startup, shutdown, asset management, and O&M. The O&M plan must include regulatory compliance sampling and monitoring, as required by the State Water Board.

- Start-up monitoring should be reported to the State Water Board for approval of adequacy.

- Performance and compliance monitoring should be reported in the monthly operating report consistent with the State's drinking water program reporting requirements.

- The State Water Board will need to determine (1) the number and types of sampling required with online monitoring; (2) the type and frequency of monitoring used to demonstrate compliance; and (3) the frequency and types of monitoring used to demonstrate the protection of public health.

- The presence and availability of highly trained staff and access to expert support/assistance are critical to the safe, successful functioning of DPR systems.

  - O&M requirements for a DPR system exceed the demands of a wastewater or drinking water supply, requiring special operator skills and experience. It is recommended that DPR system operators have a high level of certification in either or both water and wastewater and be trained specifically for operating the DPR system.

  - Certified water/wastewater operators will be needed to run a DPR system. Operators should be available 24 hours a day, 7 days per week.

8.9 Pretreatment and Source Control for Direct Potable Reuse

The primary sources of wastewater from a community include discharges from residences and commercial, institutional, and public facilities. Other significant sources include industrial discharges and stormwater infiltration/inflow. Because of the diversity of these sources, the organic and inorganic constituents contained in wastewater can vary. When planning a DPR project, communities should strive to look for the best available wastewater stream with the lowest fraction of non-domestic flow.

The National Pretreatment Program for commercial and industrial dischargers has reduced the discharge of many constituents that are difficult to manage from a treatment and environmental standpoint, but has not completely eliminated the discharge of such constituents (pretreatment is one component of a comprehensive source control program). Source control programs are designed to further control, limit, or eliminate the discharge of constituents into wastewater that can be difficult to treat or impair the final quality of treated wastewater intended for human consumption (Tchobanoglous et al., 2015; APAI, 2015).

8.9.1 Background on Pretreatment and Source Control

8.9.1.1 National Pretreatment Program

The National Pretreatment Program is a component of the National Pollutant Discharge Elimination System (NPDES) and was established as part of the Clean Water Act. The program is designed to protect the infrastructure of publicly owned treatment works (POTWs) and reduce the level of contaminants discharged by industries and other non-domestic wastewater sources into municipal wastewater collection systems, thereby reducing the amount of contaminants released into the environment through wastewater (USEPA, 2014). The objectives of the program include:

- Prevent the introduction of pollutants into a POTW that will interfere with its operation, including interference with its use or disposal of municipal sludge.
- Prevent the introduction of pollutants into a POTW that will pass through the treatment works or otherwise be incompatible with it.
- Improve opportunities to recycle and reclaim municipal and industrial wastewater and sludge (USEPA, 2011).

Under this federal program, industrial and commercial dischargers, referred to as industrial users, are required to obtain permits or other control mechanisms to discharge wastewater to POTWs, which collect and transport wastewater to treatment facilities. The General Pretreatment Regulations (40 CFR Part 403) of the National Pretreatment Program require all large POTWs (i.e., those designed to treat flows of more than 5 mgd and smaller POTWs that accept wastewater from industrial users that could affect the treatment plant or its discharges) to establish local pretreatment programs (USEPA, 2011).

For wastewater agencies not subject to the National Pretreatment Program, the local, state, or federal permitting authority may not, in some cases, require the implementation of an approved pretreatment program or a program that meets all federal requirements; ***however, an agency that intends to operate a DPR project should develop a source control program as the first barrier to protect the quality of advanced treated water, even if it is not a permit requirement*** (APAI, 2015). The key elements of the National Pretreatment Program per 40 CFR 403.8(f) are listed in **Table 8-7** (Tchobanoglous et al., 2015)**.**

8.9.1.2 Federal Pretreatment Standards

POTWs must enforce both general and specific prohibitions in the General Pretreatment Regulations. The regulations do not allow an industrial user to discharge constituents that pass through or cause interference with the treatment process. Discharge prohibitions include requirements for infrastructure protection (including the POTW collection system) and worker safety (Tchobanoglous et al., 2015).

Categorical pretreatment standards include technology-based numeric limits or best management practices (BMPs) developed in accordance with Section 307 of the Clean Water Act to limit pollutant discharges to POTWs from specific process wastewaters. These national technology-based standards apply to an industrial user regardless of whether the POTW has an approved pretreatment program or the industrial user has been issued a control mechanism or permit. The standards are established based on the list of priority pollutants (APAI, 2015). Additional standards and requirements may be added by state and local regulatory agencies, as needed, to protect the POTW (i.e., in 1989, the USEPA delegated authority to the State and Regional Water Boards to administer pretreatment and source control programs in California). After approval in accordance with 40 CFR 403.5(c), these local limits – also called Pretreatment Standards –are enforceable for the purposes of the Clean Water Act (Tchobanoglous et al., 2015).

8.9.2 Existing Industrial Pretreatment and Pollutant Source Control Requirements for Indirect Potable Reuse

The State of California recognizes the importance of pretreatment and source control in potable reuse systems and already has regulations in place to address the treatment and control of contaminants from industrial, commercial, and other sources into municipal wastewater systems that practice IPR.

Table 8-7: Key Elements of the National Pretreatment Program

Element	Description
Legal authority	The POTW must have the legal authority to apply and enforce any pretreatment standards and requirements.
Procedures	The POTW must develop and implement procedures to ensure compliance with pretreatment standards and requirements, including procedures for: (1) receiving and analyzing self-monitoring reports and other notices submitted by industrial users; (2) random sampling and analysis of effluent from industrial users; and (3) conducting surveillance activities to identify compliance or noncompliance independently from information supplied by industrial users.
Funding	The POTW (and multijurisdictional entities) must have sufficient resources and qualified personnel to carry out the authorities and procedures specified in its approved pretreatment program.
Local limits	The POTW must develop technically based local limits to regulate the discharge of pollutants of concern from IUs and address the specific needs and concerns of a POTW.
Enforcement response plan	The POTW must develop and implement an enforcement response plan that contains detailed procedures indicating how the POTW will investigate and respond to instances of industrial noncompliance.
List of Industrial Users	The POTW must maintain a list of all industrial users.

POTW = Publicly owned treatment work.

Sources: USEPA (2011); APAI (2015); Tchobanoglous et al. (2015)

For example, the existing regulations for IPR using groundwater replenishment and the proposed criteria for IPR using SWA require that the wastewater management agency: (a) administers an industrial pretreatment and pollutant source control program; and (b) implements and maintains a source control program that includes, at a minimum, the following:

- An assessment of the fate of State Water Board-specified and Regional Water Quality Control Board-specified chemicals and contaminants through the wastewater and recycled municipal wastewater treatment systems.
- Chemical and contaminant source investigations and monitoring that focuses on State Water Board-specified and Regional Water Quality Control Board-specified chemicals and contaminants.

- An outreach program to industrial, commercial, and residential communities within the portions of the municipal wastewater collection agency's service area that flows into the water recycling plant subsequently supplying the IPR project, for the purpose of managing and minimizing the discharge of chemicals and contaminants at the source.

- A current inventory of chemicals and contaminants identified and evaluated, including new chemicals and contaminants resulting from new sources or changes to existing sources, that may be discharged into the wastewater collection system (CCR, 2015; DDW, 2016).

The details of how a project sponsor will administer an industrial pretreatment and pollutant source control program and implement and maintain a source control program is included in the project's Engineering Report that must be submitted to – and approved by – the State Water Board prior to implementing an IPR project.

The Expert Panel believes that the comprehensive source control program required by the State Water Board may be effective in minimizing the frequency with which pulses of hazardous chemicals are likely to enter AWTFs used for DPR projects, but are unlikely to eliminate them. Hence, the Expert Panel suggests that research on online continuous monitoring of selected constituents and/or parameters in the wastewater collection system is needed to identify the presence of hazardous constituents in wastewater.

8.9.3 Pretreatment Program for Direct Potable Reuse

Although not all POTWs are required to implement pretreatment programs, any community or utility pursuing a DPR project in California, regardless of size, would be required to determine the impacts of industrial and commercial contributions on the wastewater supply and implement an aggressive local pretreatment program (Tchobanoglous et al., 2015). The following activities should be included in pretreatment programs for DPR projects (NWRI, 2016):

- **Understanding the Sewershed.** Investigate what chemicals are used and disposed of by homeowners and/or commercial establishments (e.g., pesticides and cleaning products). Also, identify the potential for spills and other sources of chemicals (e.g., dry cleaners) that may enter the wastewater collection system episodically. Action response plans are needed for spills.

- **Survey.** Conduct (1) an initial survey of discharges into the system to determine what industrial contaminants already exist, and (2) sample the raw wastewater and secondary effluent of the current system for drinking water constituents and CECs. If done routinely, this sampling will provide important information about pollutants in the raw wastewater and the ability of the primary and secondary wastewater treatment processes to reduce these pollutants. The information then can be used to determine what advanced treatment processes and monitoring are necessary to protect public health.

- **Classification of businesses.** Compile a list of current commercial and industrial entities that discharge into the wastewater system. Use the Standard Industrial Code (SIC) approach to inventory businesses that discharge into the collection system. Source control criteria will need to be established for new industries or businesses (e.g., medical care facilities, dental clinics, photo processors, and silver jewelry manufacturers) that move into the area.

- **Residential programs.** Education and outreach programs can be used to inform the public about the proper disposal of pharmaceuticals and household products containing chemicals that may be difficult to treat (NWRI, 2016).

8.9.4 Overview of Source Control for Direct Potable Reuse

While beneficial, pretreatment programs generally do not completely eliminate pollutant loadings from industrial sources. Hence, an important preventative approach when pursuing and planning for DPR is the implementation of a source control program in conjunction with a pretreatment program to eliminate or control the discharge of constituents of concern that might impact the production of advanced treated water from an AWTF (Tchobanoglous et al., 2015; NWRI, 2016).

Source control is a critical element in safely implementing DPR and includes more than simply focusing on wastewater compliance. It should be enhanced to control for constituents of concern from the perspective of drinking water. These enhancements should go beyond requirements in the Clean Water Act and pretreatment regulations defined in the Code of Federal Regulations (40 CFR Part 403) to address constituents of concern that pose a risk to drinking water quality in areas where potable reuse occurs or is planned (NWRI, 2016).

Tchobanoglous et al. (2015) identified the main goals of an effective source control program for DPR to be:

- Minimize the discharge of potentially harmful or difficult-to-treat chemical constituents to the wastewater collection system from industries, health care facilities, commercial businesses, and homes.

- Improve the quality of wastewater effluent quality and the performance of advanced water treatment.

- Provide the public with confidence that the wastewater collection system is being managed with potable reuse in mind.

It should be recognized that source control cannot eliminate all constituents of concern from the wastewater stream; however, it is important to identify contaminants that may be present in the sewershed, mechanisms by which they may be introduced into the wastewater collection system, and actions that can be taken to minimize their introduction into the wastewater collection system (NWRI, 2016). Understanding is needed of the sources and concentrations of hazardous constituents entering the sewershed from readily-managed point sources. In some cases, to minimize the impact from large industrial users, it may be appropriate to consider diverting their wastewater discharges to alternative treatment facilities.

The principal elements of an effective source control program for DPR are provided in **Appendix 8B** (from Tchobanoglous et al., 2015)**.**

8.10 Findings of the Expert Panel

The central focus of the Expert Panel has been the evaluation of the feasibility of developing uniform water recycling criteria for DPR systems that are adequately protective of public health. To provide the same levels of protection afforded by projects practicing IPR either using groundwater recharge or SWA, all of which include an environmental buffer, the key functions provided by the environmental buffer must be achieved by other means (e.g., the reliability of mechanical systems and treatment plant performance), thereby ensuring the delivery of a water quality that is protective of human health. To do this, regulations specifying DPR practices need to provide the following reliability and/or performance features in addition to requirements already specified in IPR regulations within the State of California:

- **Finding #8-1:** The DPR system must be reliable. Reliability is achieved by (1) providing multiple, independent treatment barriers, (2) incorporating the frequent monitoring of surrogate parameters at each step to ensure treatment processes are performing properly, and (3) developing and implementing rigorous response protocols (such as a formal HACCP system). Other key attributes that promote reliability include:

    a) Using a treatment train (see **Section 9.3.3** in **Chapter 9**) with multiple, independent treatment barriers (i.e., redundancy) that meet performance criteria greater than the public health threshold LRV goals established for microorganisms.

    b) Ensuring the independent treatment barriers represent a diverse set of processes (i.e., robustness) in the treatment train that are capable of removing particular types of contaminants by different mechanisms. This diversity provides better assurance that if a currently unrecognized chemical or microbial contaminant is identified in the future, there is a greater degree of likelihood it will be removed effectively by the treatment train.

    c) Using parallel independent treatment trains (i.e., resilience and redundancy) and providing sufficient replacement parts, along with trained personnel, to rapidly carryout the most frequently needed repairs.

    d) Encouraging the use of a probabilistic analysis of treatment train performance at the design stage. The analysis should be based on data from other pilot-scale or full-scale facilities. After the full-scale facility is commissioned, it should be updated periodically using actual performance data and operational experience.

    e) Providing the ability to divert advanced treated water that does not meet specifications (i.e., water that is “off spec”).

    f) Implementing a rigorous source control program designed to control the discharge of toxic chemicals and other contaminants to the wastewater collection system that serves the DPR project. The source control program must include stringent sewer ordinances and ongoing surveillance.

    g) Providing certified operational personnel who are able to conduct rigorous O&M at AWTFs and DWTFs.

h) Unauthorized short-term peak discharges of chemicals into the wastewater collection system serving a DPR system have the potential to compromise final product water quality; therefore, incorporating a final treatment process (to be specified) after the advanced water treatment train may result in some "averaging" of these potential chemical peaks.

i) Ensuring that the operation and performance of each unit treatment process in the DPR treatment train achieves the proposed and/or anticipated LRVs for pathogen.

j) Ensuring the chemical and microbial stability of water in the drinking water distribution system will be maintained after introducing advanced treated water, in particular for a DPR system in which final treated water will directly enter the drinking water distribution system.

- **Finding #8-2:** The State Water Board should not codify a specific set of treatment processes as part of developing uniform statewide water recycling criteria for DPR, as it could stifle technological innovation in this growing area of need. The criteria should allow for alternatives to any treatment processes specified in the regulations if it is demonstrated to the State Water Board that the alternatives provide at least an equivalent level of public health protection.

- **Finding #8-3:** An approach to stage the introduction of recycled water from a DPR system into a community's drinking water supply should be considered by the State Water Board as part of the review and approval of a project. This approach is consistent with California's regulatory practices for groundwater replenishment using recycled water.

8.11 Recommendations of the Expert Panel

In terms of general recommendations regarding the performance of DPR systems, the Expert Panel recommends the following:

- **Recommendation #8-1:** A formal process should be established by the State that includes an internal process to administer the periodic review of the performance of permitted potable reuse projects by an external expert panel on a 5-year cycle. Based on this review process, the State should incorporate new knowledge into potable reuse regulatory permits.

8.12 Research Recommendations of the Expert Panel

The Expert Panel identified several areas of research (not listed in priority order) to be conducted to further ensure the protectiveness of DPR – some of which would best be directly supported by the State, and others that are matters of broader national need. Note these research activities can be undertaken either before and/or concurrently with the development of criteria for DPR.

- **Research Recommendation #8-1:** The State Water Board should adopt the use of probabilistic QMRA to confirm the necessary LRVs of viruses, *Cryptosporidium*, and *Giardia* needed to maintain a risk of infection equal to or less than 10^{-4} per person per year. The State should provide oversight, direction, and funding for implementing probabilistic QMRA. The purpose of using probabilistic QMRA is to provide a better assessment of the performance of DPR treatment trains and to provide an opportunity to identify additional effective DPR treatment

trains. Input values for pathogen concentrations should be based on descriptive pathogen statistics resulting from additional review of the literature (as well as information collected from **Research Recommendation #8-3**). Also, as full-scale DPR systems are built, owners and regulators need to take advantage of these systems to sample and assess actual as-built performance and reliability characteristics.

- **Research Recommendation #8-2:** To better inform decisions associated with updating LRVs, as well as conducting probabilistic-based QMRA modeling, the State Water Board should include monitoring requirements in regulatory permits to measure pathogens (i.e., *Giardia* cysts, *Cryptosporidium* oocysts, and several human viruses) in raw (untreated) wastewater feeding a DPR system to provide more complete information on concentrations and variabilities. Improved methods should be used that will allow for the better characterization and improved precision of concentrations of pathogens. Note this recommendation also is listed in **Chapter 2** (see **Research Recommendation #2-1**).

- **Research Recommendation #8-3:** The State Water Board should encourage short-term research be conducted to identify suitable treatment options for final treatment processes that can provide some "averaging" with respect to potential chemical peaks (in particular, for chemicals that have the potential to persist through advanced water treatment). These options might involve: (1) the use of a buffer tank (clear well) of a sufficient size, potentially blended with an alternative water source prior to release into the drinking water distribution system, or using two tanks feeding into the drinking water distribution system; (2) removal of volatile contaminants during a degassing step (decarbonization) similar to the approach that is commonly employed after reverse osmosis treatment in established AWTFs for potable reuse; (3) use of a biologically active filter after reverse osmosis/advanced oxidation, to provide an additional opportunity for microorganisms (if microorganisms will be able to survive in that environment) to degrade contaminants that may otherwise pass through the filter; or (4) other options.

8.13 References

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CHAPTER 9: POTABLE REUSE REGULATORY FEASIBILITY ANALYSIS

- Comparison of alterative water supply options.
- Approach and assumptions for evaluating the regulatory feasibility.
- Example analysis of regulatory feasibility.

The purpose of this chapter is to describe the approach used by the Expert Panel to evaluate the feasibility of developing uniform water recycling criteria for direct potable reuse (DPR). The potable reuse regulatory feasibility analysis was performed for microbial pathogens using *Cryptosporidium* as the reference pathogen. The use of *Cryptosporidium* does not suggest that it is the only pathogen of concern;[23] however, conducting this analysis with other pathogens is not expected to change the Expert Panel's opinion about the feasibility of DPR. Altogether, the feasibility analysis was comprised of three steps: (1) define alternative scenarios for drinking water supply; (2) define the approach and assumptions used for the analysis; and (3) conduct a feasibility analysis for the reference pathogen.

9.1 Define Alternative Scenarios for Drinking Water Supply

Three alternative water supply scenarios were used to conduct the potable reuse regulatory feasibility analysis and to allow for a relative comparison between DPR and two approved water supplies in California. These scenarios include:

1. A current source of drinking water source that is partially impacted by upstream wastewater effluent discharge (therefore, it also is characterized as *de facto* or unplanned potable reuse).
2. A potential IPR alternative that involves an environmental buffer with a shorter retention time than required by proposed regulations in California.
3. A DPR project in which recycled water is used to augment a community's drinking water supply.

A brief description of each scenario is provided in **Sections 9.1.1** to **9.1.3**.

[23] Other pathogens of interest include adenovirus and norovirus, yet for the reasons noted below, these pathogens were not used as examples for the feasibility analysis.

- Data for adenovirus were not analyzed by the Expert Panel because the existing dose-response data and mathematical relationship (Couch et al., 1966; Crabtree et al., 1997) apply to inhalation, which is not applicable to the exposure routes considered herein.
- Norovirus was not analyzed for several reasons: (1) there remains significant uncertainty associated with the selection of a dose-response model (Van Abel et al., 2016); (2) no dose response model has been accepted from a regulatory perspective; (3) norovirus has not been cultivated successfully using conventional tissue culture methods (consequently, no work is available to establish the ratio between genome density and infectious unit density in the water environment); and (4) while genome-based methods are more sensitive at detecting the presence of copies of the genome of a virus, these methods do not provide information on viral infectivity (NRC, 2012).

9.1.1 Scenario 1: Current Source of Drinking Water (Also Representative of *De Facto* Potable Reuse)

The Sacramento-San Joaquin River system (referred to as the "Delta") was selected as a scenario for this analysis to represent a raw drinking water supply that is partially impacted by upstream wastewater effluent discharge (**Figure 9-1**). This system also can be characterized as *de facto* potable reuse. As described in **Chapter 1**, *de facto* potable reuse occurs when downstream surface waters subject to a significant contribution[24] from upstream wastewater discharges are used as sources of drinking water.

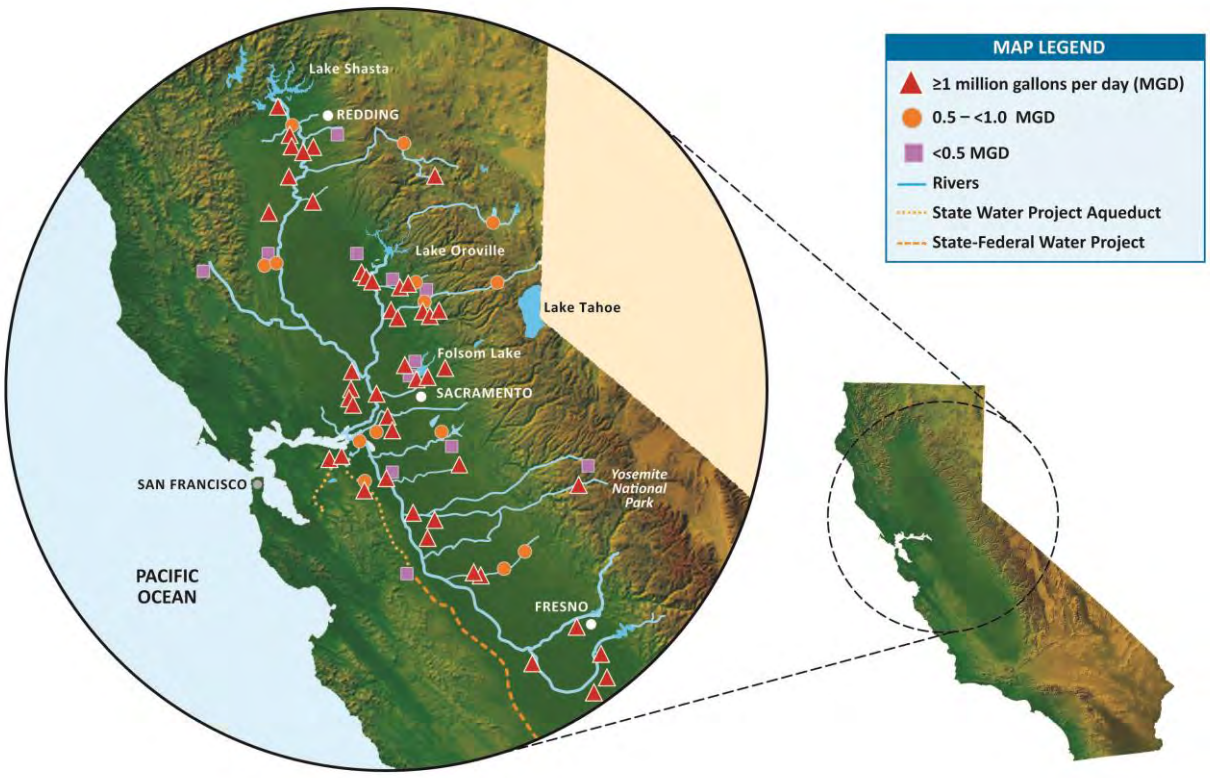

Figure 9-1: Map showing the Sacramento-San Joaquin River system (referred to as the "Delta"). Note that National Pollutant Discharge Elimination System (NPDES) permits for wastewater discharges in the region have been identified.

[24] A significant contribution would constitute greater than 3 percent of wastewater effluent in a surface water source (SWPCA, 2011).

As noted in **Section 1.1.3** in **Chapter 1**, about two-thirds of the population of California receives drinking water from the State Water Project (SWP), which is fed by water from the Delta. Twelve wastewater treatment plants (WWTPs) discharge to the Delta, and many others discharge to its tributaries. Currently, the average volume of dry weather wastewater discharge reaching the Delta is around 350 mgd; it is expected to increase in the future (SWPCA, 2011). Notably, efforts are underway to improve the quality of wastewater reaching the Delta, including upgrading WWTPs to include granular media filtration (SWPCA, 2011).

All California SWP contractors completed monitoring required by the Long Term 2 Enhanced Surface Water Treatment Rule, the results of which are documented in SWPCA (2011). The results have been classified in Bin 1, representing low *Cryptosporidium* levels (i.e., a running annual average of less than 0.075 oocysts per liter); therefore, no additional action related to reducing *Cryptosporidium* concentration is required at this time.

9.1.2 Scenario 2: Indirect Potable Reuse with an Environmental Buffer with Reduced Retention Time (the "Gap")

As discussed in **Section 1.1.2** in **Chapter 1**, all current and proposed regulations for indirect potable reuse (IPR) in the State of California include the use of a regulatory-defined environmental buffer; however there are likely to be potential potable reuse projects where an environmental buffer is available, but does not meet the proposed operational and performance criteria for an IPR project using surface water augmentation (SWA). This scenario addresses such a situation.

The proposed criteria for IPR using SWA include (1) an operational criterion of a monthly-average theoretical hydraulic residence time of at least 4 to 6 months and (2) a performance criterion requiring the dilution of a 1-day pulse of "off-spec" water of at least 1:100 or of 1:10 when an additional 1-$\log_{10}$ reduction of each pathogen (i.e., virus, *Cryptosporidium* oocysts, and *Giardia* cysts) is provided by the AWTF (NWRI, 2015a,b). These criteria ensure that a substantial environmental buffer is in place to provide the following three benefits:

- Storage of advanced treated water for subsequent potable reuse.
- Attenuation (e.g., by dilution and die-off) of any contaminants that may evade sufficient treatment.
- Time to respond to treatment plant upsets during production.

Notably, the proposed criteria for IPR projects using SWA do not include an alternatives clause (NWRI, 2015b) like that in the regulations for IPR using groundwater replenishment (CCR, 2015), where a project may be allowed to use an alternative to ***any*** requirement if it "assures at least the same level of protection to public health." Consequently, an IPR project for SWA using an environmental buffer that does not meet regulatory criteria would be defined as DPR. This situation creates a regulatory "Gap" between IPR projects with smaller environmental buffers and DPR projects with no environmental buffers. Based on the previous analysis of an environmental buffer conducted by the Expert Panel as part of its review of proposed criteria for IPR using SWA (NWRI, 2015a,b), the Expert Panel considers IPR projects with a theoretical hydraulic retention time of <2 months to be a DPR project (i.e., the Gap between IPR using SWA and DPR covers projects with theoretical hydraulic retention times of ≥2 months and <4 months). See **Section 1.1.2** of **Chapter 1** for more information.

As part of developing proposed IPR criteria for SWA, the State Water Board recognized that the requirement for a minimum criterion of 1:100 dilution of a 1-day pulse of off-spec water could place restraints on some projects; therefore, the proposed criteria also allowed for a minimum dilution of 1:10 if 1-$\log_{10}$ additional reduction for each pathogen class is provided. As a result, the proposed criteria award equivalent credit between 1-$\log_{10}$ less dilution and 1-$\log_{10}$ more treatment. This equivalence is important because it allows dilution and treatment to each pathogen class to receive $\log_{10}$ reduction credit. It is recognized that, in general, attaining a minimum of 1:10 dilution within the reservoir and providing 1-$\log_{10}$ of additional treatment may often be easily achieved. Further, a potential Gap project might incorporate an allowance for a 1:10 dilution, but with a parallel requirement for additional $\log_{10}$ reductions through treatment (i.e., 2 or more $\log_{10}$ reductions for each reference pathogen).

It was earlier demonstrated (NWRI, 2015a,b) that if a reservoir was represented as a continuous-flow stirred tank reactor (CFSTR), it could achieve a 1:10 dilution with a theoretical hydraulic residence of as little as 10 days (Anderson, 2015); however, a reservoir with such a short theoretical hydraulic residence time would fail to serve as a significant environmental buffer as it would provide only minimal time to respond to a treatment plant upset, as well as provide limited dilution (**Table 9-1**).

Table 9-1: Properties of a Surface Water Reservoir When Represented as a Continuous-Flow Stirred-Tank reactor with Increasing Theoretical Hydraulic Residence Times (t_r) Subject to a 1-Day Pulse of Off-Spec Advanced Treated Water[a,b]

t_r (Months)	Dilution Factor[c]	t_2 (Days)[d]	% *In Situ* Removal[e]
0.33	10	0.2	43
1	30	0.6	69
2	60	1.3	81
3	90	2.0	87
4	120	2.7	90
5	150	3.3	92
6	180	4.0	93

[a] Adapted from Anderson (2015) and NWRI (2015a).

[b] Actual dilutions, travel times, and *in situ* removals in a reservoir will depend upon complex hydrodynamics and other factors, which can deviate substantially from these values.

[c] Assumes the flow of advanced treated water constitutes total flow through the system.

[d] Time for 2 percent of a conservative tracer or unreactive contaminant to exit the reservoir.

[e] Assuming a first-order decay rate of k=0.077 per day.

Under an idealized CFSTR representation, a reservoir with a 10-day hydraulic residence time (or 0.33 month assuming a 30-day month) could provide no more than a 1:10 dilution and would export to a downstream drinking water treatment facility (DWTF) 2 percent of a universally distributed conservative contaminant mass within about 0.2 days (or 5 hours) or approximately 10 percent after 24 hours. With short-circuiting in the reservoir, even less time could be available. A reservoir achieving only 1:10 dilution, thus, would provide very little time to implement corrective action (e.g., increasing disinfection at the DWTF or switching to an alternative source supply). Moreover, such a short residence would also provide little opportunity for *in situ* removal. By contrast, increased hydraulic residence times provide greater dilution, increased time to respond, and (although not credited in the proposed criteria for IPR using SWA) increased removal of pathogens (and some chemical contaminants) prior to the delivery of water to the downstream DWTF (see **Appendix 9A**, Anderson, 2016).

9.1.3 Scenario 3: Direct Potable Reuse

This scenario (as described in Example 2 of **Figure 8-1** in **Chapter 8**) represents a DPR project that produces recycled water suitable to be fed directly into a community's drinking water distribution system. A treatment train employed for this scenario might use a secondary-treated wastewater effluent after biological nutrient removal, followed by ozonation and biologically-active carbon filtration (BAC), an integrated membrane system (microfiltration [MF]/reverse osmosis [RO]), an advanced oxidation process (AOP), water stabilization, and final disinfection.

9.2 Define the Approach and Assumptions of the Potable Reuse Regulatory Feasibility Analysis

For the regulatory feasibility analysis, several topics and assumptions are addressed, including discussions of the following:

- Tolerable level of public health risk (i.e., adequate public health protection) from human exposure to finished drinking water.
- Reference pathogen and risk-based reduction targets.
- Anticipated treatment plant performance necessary to attain and maintain the adequate protection of public health.

9.2.1 Tolerable Level of Public Health Risk

Specifying the tolerable level of public health risk was addressed in **Chapter 1** and is briefly summarized here. The Office of Drinking Water of the USEPA employed a goal of 10^{-4} risk of infection per person per year for the evaluation of risks associated with microbial pathogens, where 10^{-4} serves as the tolerable risk goal (Regli et al., 1991). Performance goals for potable reuse projects in California also are based on the Safe Drinking Water Act's tolerable risk level of 10^{-4} annual risk of infection and occurrence data of pathogens in raw wastewater (CCR, 2015; NWRI, 2013).

Under the Safe Drinking Water Act, the USEPA sets national health-based standards to protect against both naturally occurring and manmade constituents that may be found in drinking water. Drinking water regulations for the State of California are consistent with national primary standards. In addition, relative to chemical contaminants, the State Water Board issued a Recycled Water Policy in 2009 (and updated in 2013) (Anderson et al., 2010; Drewes et al., 2013; SWRCB, 2013) that, among other efforts,

sought to incorporate the most current scientific knowledge on chemicals of emerging concern (CECs) into regulatory policies for use by state agencies. The State's primary drinking water standards, IPR regulations, and recycled water policy have established a substantial foundation to define the adequate protection of public health from which to consider the feasibility of developing uniform water recycling criteria for DPR. Note that these tolerable risk levels refer to the final drinking water quality and apply independent of whether potable reuse is practiced indirectly or directly.

9.2.2 Reference Pathogen and Risk-Based Reduction Targets

The reference pathogen selected for this regulatory feasibility analysis was *Cryptosporidium*. Discussed below are the risk-based target reduction levels presented in a probabilistic framework. The Expert Panel used the framework to evaluate the regulatory feasibility of DPR, as well as to compare DPR against unplanned and planned potable reuse options.

The Expert Panel's primary focus was on the acute risk of infection associated with microbial pathogens and, as a result, quantitatively evaluating pathogen reduction against a tolerable risk level. Currently, California has microbial $\log_{10}$ reduction values (LRVs) for IPR projects using groundwater replenishment defined as part of State Water Recycling Criteria (CCR, 2015) and as part of proposed criteria for IPR projects using SWA (NWRI, 2015a,b). These LRVs are based on a tolerable risk of one infection per year among 10,000 people (10^{-4} per person per year), consistent with the Surface Water Treatment Rule. A more thorough presentation and review of the supporting basis for the LRVs, as well as what levels have been currently credited to groundwater replenishment projects, is provided in **Chapter 2**. The approved LRVs are summarized in **Chapter 2** (see **Table 2.1**) and are referred to as the "12/10/10" LRVs for virus, *Giardia*, and *Cryptosporidium*, respectively.

For the purposes of this feasibility analysis, the Expert Panel developed a cumulative density function (CDF)[25] plot of risk-based $\log_{10}$ reduction targets for *Cryptosporidium* (the reference pathogen), as shown in **Figure 9-2**. The assumptions used to develop the CDF plot are described in **Section 2.1.1.2** of **Chapter 2**.

The CDF plot represents the LRVs necessary to attain the tolerable risk level of 10^{-4} per person per year, as previously described, and is used as an acceptable risk-based baseline from which to evaluate the feasibility of the example DPR treatment trains discussed below. As illustrated by the plot in **Figure 9-2**, the general requirement for a 10-$\log_{10}$ reduction in *Cryptosporidium* is quite conservative.

9.2.3 Anticipated and/or Projected Treatment Plant Performance for Reference Pathogen Reductions

To carry out a comprehensive and useful evaluation of the reliability of a water treatment system, a methodology was developed that relies on a range of assumptions and observations to characterize treatment facility reliability with respect to the following conditions:

- Variability of treatment process effectiveness under normal operation.
- Probability of observed mechanical failures resulting in off-spec water production.
- Impacts of projected mechanical failures (e.g., a 24-hour influent slug concentration).

[25] Cumulative density is the probability that a particular quantity has a value less than or equal to a particular level.

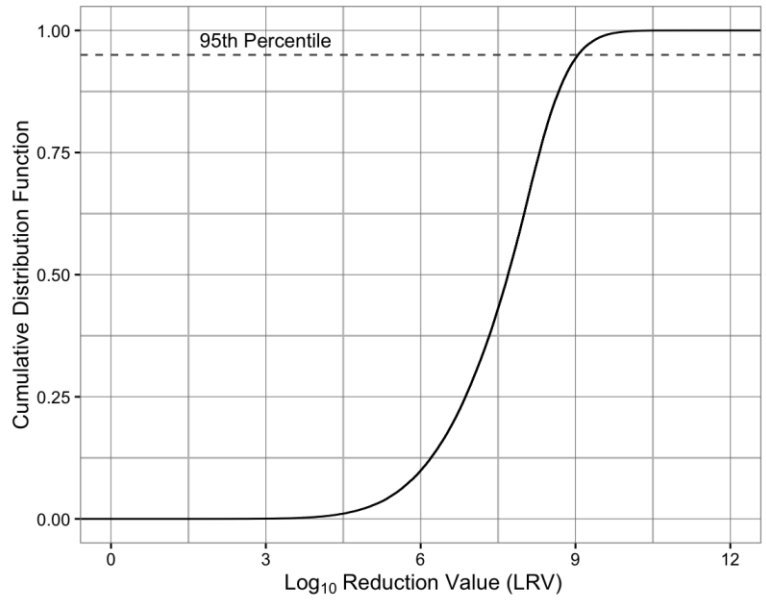

Figure 9-2: Cumulative distribution function (CDF) plot of estimated log_{10} reduction values (LRVs) for *Cryptosporidium* spp. (based on the dose response in USEPA, 2006) to meet an annual tolerable risk of infection of 10^{-4} per person per year (same as plotted in Figure 2-1).

The methodology incorporates the use of individual treatment process performance data from a DPR pilot-plant demonstration project in San Diego (Pecson et al., 2016) and the review and compilation of mechanical reliability data from a number of sources, including the aforementioned DPR Demonstration Project, San Diego Aqua II and III Health Effects Studies (Olivieri et al., 1998), and Water Factory 21 at the Orange County Water District (McCarty et al., 1982).

What follows is a discussion of the various assumptions used to quantitatively describe treatment process performance (i.e., inherent reliability) and mechanical reliability as a means to evaluate overall treatment plant reliability under the three conditions noted above.

9.2.3.1 Unit Process Performance (Inherent Reliability)

The DPR Demonstration Project in San Diego (Pecson et al., 2016) has been focused primarily on documenting the inherent reliability of key treatment operations used for a contemporary potable reuse project. Summaries of the treatment system design criteria and water quality conditions at the demonstration facility are provided in **Appendix 9B**.

Illustrated in **Figures 9-3a-d** are individual CDFs for the LRVs of *Cryptosporidium* associated with four key treatment processes (i.e., ozonation, microfiltration, reverse osmosis, and UV disinfection) and performance data collected over 1 year of operation at the demonstration facility. Note the CDF plots only provide data on treatment process performance and not information on mechanical reliability.

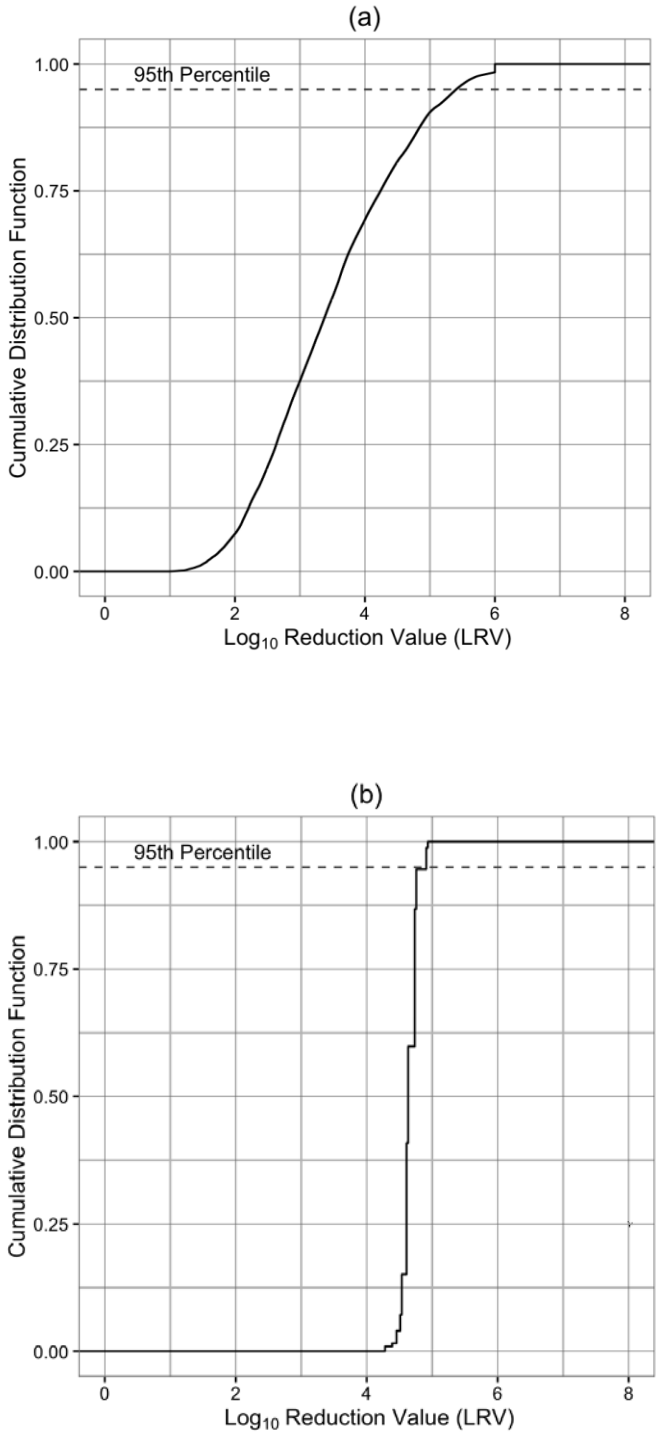

Figure 9-3a,b: **Empirical cumulative distribution function plot estimates for treatment process performance log_{10} reduction values (LRVs) for *Cryptosporidium.* The treatment processes represented include: (a) ozonation and (b) microfiltration. Adapted from data provided by the DPR Demonstration Project in San Diego, California (Pecson et al., 2016).**

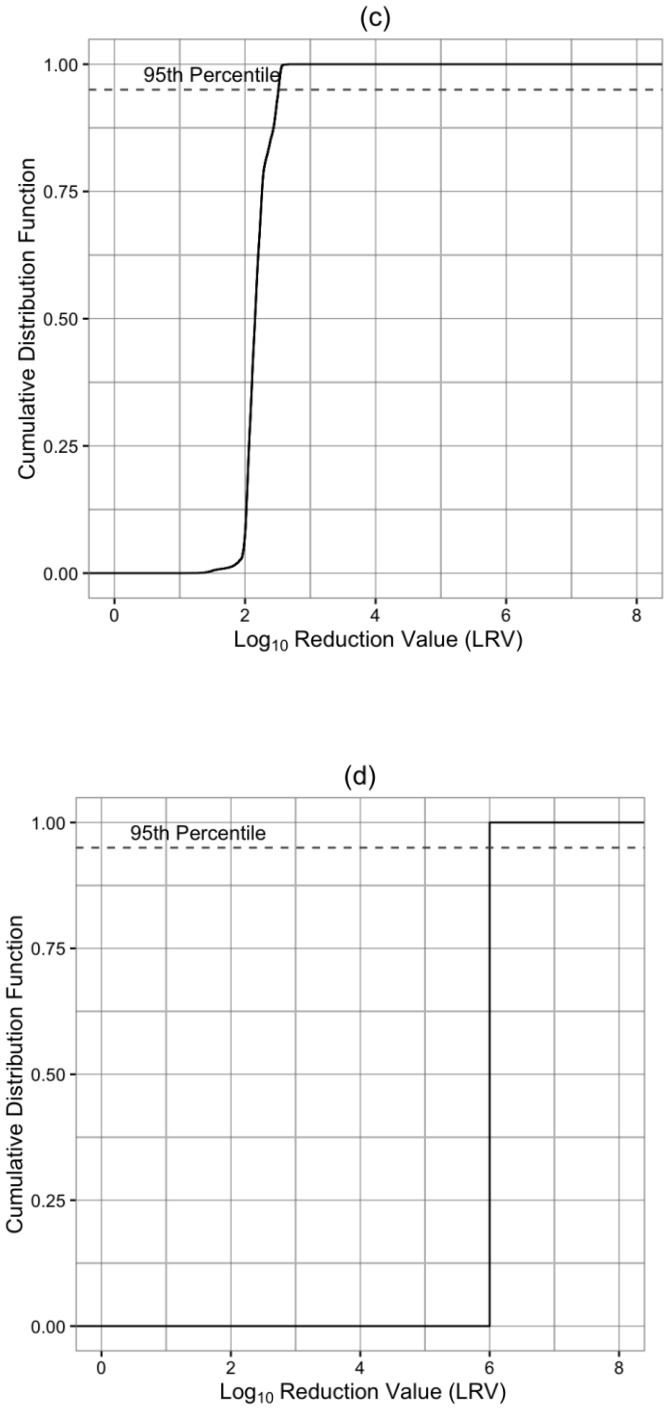

Figure 9-3c,d: **Empirical cumulative distribution function plot estimates for treatment process performance log_{10} reduction values (LRVs) for *Cryptosporidium.* The treatment processes represented include: (c) reverse osmosis (based on total organic carbon) and (d) ultraviolet disinfection. Adapted from data provided by the DPR Demonstration Project in San Diego, California (Pecson et al., 2016).**

Based on previous investigations (Haas and Trussell, 1998; Olivieri et al., 1999), it was assumed that the treatment processes were independent, allowing for an easier estimation of the performance of the full treatment train. This assumption was investigated using data and analyses provided as part of the DPR Demonstration Project (Pecson et al., 2016). As illustrated in **Figure 9-4**, the estimated CDF performance of the full treatment train, based on CDFs of empirical treatment processes, overlaps the actual (measured) data collected for each treatment process; therefore, for the purposes of this feasibility analysis, the assumption that individual treatment processes are independent has been established as valid.

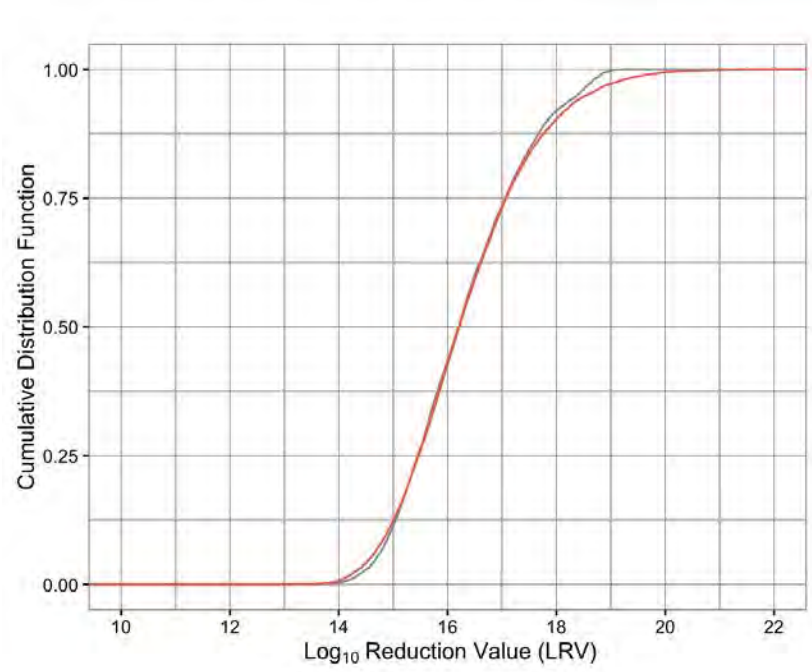

Figure 9-4: Comparison of Empirical (gray) and Monte-Carlo Analysis (red) cumulative distribution function (CDF) plots for an advanced treatment train log_{10} reduction value for *Cryptosporidium,* illustrating the independence of treatment processes. The advanced treatment train consists of ozone, biological activated carbon, microfiltration, reverse osmosis, and ultraviolet/advanced oxidation.

9.2.3.2 Mechanical Reliability

The fundamental method of assessing the mechanical reliability of a treatment system requires (1) first determining the key equipment in the treatment plant in which failures may affect effluent quality, and (2) then determining the probability that the facility will function according to design specifications when that equipment fails to operate properly. This component of a reliability analysis may be used to quantify the dependability of a treatment plant in terms of operation, and to identify potential deficiencies of the treatment processes. The reliability of the treatment system may be improved by identifying and correcting these deficiencies.

A review of the literature indicates a number of approaches are available for analyzing the mechanical reliability of a treatment plant. Some more common approaches include a fault tree analysis, event tree

analysis, failure modes and effects analysis, and critical component analysis. All four approaches have been used by a variety of industries to assess the mechanical reliability of various types of facilities.

The "critical component analysis" approach – originally developed by the USEPA to determine the in-service reliability, maintainability, and operational availability of selected critical wastewater treatment components (USEPA, 1982) – is the main approach used in all examples of this feasibility analysis. Although the main objective of the critical component analysis is to determine which mechanical components in the treatment plant would have the most immediate impact upon effluent quality should failure occur, for the purpose of this feasibility analysis, the fraction of time that a unit or component was operating (either including or excluding preventative maintenance) was collected from the four example demonstration plants, as discussed later in this section.

Note that all the demonstration plants are not permanent facilities and do not include many of the provisions established in a full-scale system, such as standby pumps, standby chemical feeders, and standby process units. In performing this analysis, the Expert Panel was mindful of what the impact of such failures would be on the reliability of a full-scale system. Also note these failures generally are prevented through design guidelines and regulatory requirements for standby facilities to ensure reliable operation. Requirements for standby facilities are enforced regularly during the review process for drinking water permits.

- **DPR Demonstration Project in San Diego (2016).** As noted in **Section 9.2.3.1**, the focus of this project is on documenting the reliability of key treatment processes likely to be used in DPR treatment train. Because full-scale mechanical and electrical treatment systems were used at this demonstration facility, the data collected also can be used to examine mechanical reliability. Throughout the demonstration, event logs were maintained to keep track of plant issues, shutdowns, and downtime (Pecson et al., 2016). Many of the events recorded were direct artifacts of the design of the demonstration facility. For example, the UV/AOP system experienced significant downtime due to online communications issues. This type of mechanical reliability event can and should be minimized at a full-scale facility where a PLC system would undergo extensive testing and verification during start-up and commissioning. The demonstration facility did not undergo the same degree of commissioning as a full-scale facility, so many of the mechanical reliability events are artifacts of scale. Notably, only excursions that might realistically occur at a full-scale facility were included in this analysis.

  Data collected from the event logs (Pecson et al., 2016) also were used to count the total number of failures over the 12-month testing period for each treatment process. An assumption was made that any failure would result in a 15-minute period during which the treated water system would experience a complete failure (i.e., the LRV was reduced to zero). Using this methodology, both the total number of failure events and total number of hours per year that a given unit treatment process experienced failure were estimated (see **Table 9-2**).

  Again, because the facility was designed for short-term operation (i.e., 1 year at demonstration-scale), standby capacity was not included; consequently, the frequency of mechanical reliability events at the demonstration facility was higher than expected at a full-scale facility.

Table 9-2: Summary of Estimated Failure Events per Year and Off-Spec Production Times per Year at the Direct Potable Reuse Demonstration Project in San Diego, California[a]

Process	Ozonation	Microfiltration	Ultrafiltration	Reverse Osmosis	Ultraviolet Disinfection/ Advanced Oxidation Process
Number of off-spec/year	20	24	20	6	18
Estimate of off-spec production time/year (hours)	5	6	5	1.5	4.5
Estimated operational availability (%)	>99.94	>99.93	>99.94	>99.98	>99.95

[a] Adapted from Pecson et al. (2016).

To gain a more meaningful understanding of mechanical reliability, it would be best to analyze a full-scale DWTF. DWTFs are designed to provide a high degree of availability (i.e., up-time) while ensuring that treated water is continuously meeting specifications (i.e., high reliability). These facilities also face many of the same challenges that a future DPR facility would in that DPR facilities may have little to no capacity downstream of the treatment plant to divert or store water prior to distribution to consumers; therefore, they must be designed to maximize up-time and process performance. For example, operational flexibility through standby and redundant treatment capacity could be used to maximize plant up-time. In addition, high-frequency monitoring and associated alarms should capture many (if not most) off-spec situations, with offline storage (i.e., reservoir and/or engineered storage) used to continue providing water during system downtime. In the same sense, AWTFs for potable reuse should be designed with similar requirements.

- **Potable Reuse at the Aqua II and III Projects in San Diego.** Mechanical reliability data were collected at two separate AWTFs over a total of 4.5 years (3 years at Aqua II and 1.5 years at Aqua III). While a direct comparison of the unit treatment processes is neither possible nor practical, the combined operational availability for both facility datasets indicates that the RO units were available >99.9 percent of the time and the UV units were operational >99.99 percent of the time (Olivieri et al., 1998).

- **Potable Reuse at Water Factory 21.** Water Factory 21 (WF21) was a 15-mgd AWTF constructed by the Orange County Water District in 1976 to improve the quality of secondary-treated municipal wastewater from the Orange County Sanitary District for injection into the groundwater basin to prevent seawater intrusion. Water Factory 21 included advanced water treatment processes (i.e., high-lime treatment, air stripping, chlorination, filtration, activated-carbon adsorption, RO, and final chlorination). At the time, it was the most advanced treatment

train ever built and the first to include RO treatment for the reuse of municipal wastewater. The performance of WF21 was characterized during a 3-year period from March 1978 to March 1981 (McCarty et al., 1982). The effluent from this treatment system met the health criteria for all priority pollutants at that time.

The ability of WF21 to operate without failure also was evaluated; however, because there was no need to treat or inject water at all times, redundancy in equipment use was not of high concern. Equipment failures at times required the shutdown of some or all processes (though other failures could be handled by reducing flow to some processes, while others required no reduction in flow though the plant). A summary is provided in **Table 9-3** of the causes of downtime at WF21 during an evaluation period of 1,127 days. Furthermore, for many occasions, plant shutdowns were planned; therefore, a direct comparison of the data to more recent investigations might not be appropriate. Notably, a review of the data indicates that unplanned shutdowns of the entire treatment facility are best represented by the category "other equipment failures" and constitute <5 percent of operational time.

The advanced water treatment portion of WF21 was operated 73 percent of the time, while the RO system was operated about 90 percent of the time. There were two major events that reduced the operation of advanced water treatment, both occurring during the first 1.5 years of study.

- Flow to the chlorination basin initially was stopped during the first 3 months of study to remove activated carbon, lost from the GAC column when they were operated in an upflow manner. It was then discovered that the lining on the chlorination basin was damaged, requiring 3 months for repair. The activated carbon columns were modified for downflow operation to prevent future spills.
- In 1979, the Geology Department evaluated flow velocities in the groundwater zone impacted by injection; as part of this effort, water subjected to RO treatment only was injected into the subsurface to provide mineral-free water that could serve as a tracer for movement through the ground.

Both these events are unusual and do not reflect the normal operations of the AWTF. If these events are removed from consideration in the analysis, then the AWTF was available about 88 percent of the time, which is nearly the same availability as the RO plant. Of this amount, about one-half of the shutdown time was for planned routine maintenance and the other half was the result of equipment failures. Days required for equipment failures included thermo disc cooler repair (9 days), pump failure (7 days), convey repair (31 days), electric repair (4 days), acid leaks (45 days), RO sump control repair (2 days), chlorine system repair (12 days), line leaks (3 days), and general repairs (26 days).

A review of the above advanced water treatment mechanical reliability data provided by the three pilot facilities and one full-scale facility were grouped into three example alternative ranges of operational reliability (i.e., the percentage of time the AWTF operates as expected), shown in **Table 9-4** as Options 1 to 3. As will be discussed in **Section 9.3**, the operational reliability grouping was used together with AWTF performance data to investigate (1) the impact of reducing operational reliability on treatment plant performance and (2) how overall performance is impacted positively by redundancy.

Table 9-3: Percentage of Shutdown Time at Water Factory 21 Due to Various Causes[a]

Cause of Down Time	Advanced Water Treatment			Reverse Osmosis		
	Days[c]	Down Time Distribution (%)	Total Time (%)	Days[c]	Down Time Distribution (%)	Total Time (%)
Geological Department study	83	27	7.4	--	--	--
Chlorine basin repair	88	28	7.8	--	--	--
Activated carbon column modification	12	4	1.1	--	--	--
General maintenance	57	19	5.1	60	34	5.3
External causes[b]	18	6	1.6	5	3	0.4
Other equipment failure	49	16	4.4	111	63	5.6
Total	307	100	27.4	176	100	11.3

[a] Source: McCarty et al. (1982).

[b] Represents power shutoff, no influent water available, and/or lack of chemicals.

[c] During 1,127 days of evaluation.

Table 9-4: Example Ranges of Operational Reliability (i.e., the Percentage of Time the Advanced Water Treatment Facility Operates as Expected)

Option	Operational Reliability	Description
1	Ideal Operation	>99.999-percent operational availability (upper bound).
2	Best Operation	>99-percent operational availability or ≤1-percent off-spec.
3	Worst Operation	>95-percent operational availability or ≤5-percent off-spec (lower bound).

9.3 Conduct an Example Potable Reuse Regulatory Feasibility Analysis

An example regulatory feasibility analysis was used to provide a comparison of the three drinking water scenarios described in **Section 9.1**, relative to the tolerable risk level for the selected reference pathogen, *Cryptosporidium*.

- Sacramento-San Joaquin River (Delta) system, which is impacted partially by upstream wastewater effluent discharge (representing *de facto* potable reuse).
- Potential IPR alternative with an environmental buffer representing a shorter retention time than required by proposed regulations in California (representing the "Gap").
- DPR project in which a community's drinking water supply is augmented with recycled water.

9.3.1 Scenario 1: Delta Drinking Water Supply (*De Facto* Potable Reuse)

For this analysis, the Delta was used as a representative of both drinking water supply and *de facto* potable reuse. In addition to WWTP flows, the Delta receives urban runoff from Sacramento, Stockton, eastern Contra Costa County, and a number of small communities. Other communities discharge urban runoff to tributaries of the Delta. It also has been estimated that over 12-million visitors recreate annually in the Delta, including about 500,000 boaters. Recreation includes picnicking, hiking, camping, hunting, boating, fishing, water-skiing, and other leisure activities along the Delta's 57,000 acres of navigable waterways. All these activities have the potential to impact water quality in the Delta, and all are projected to increase in the future (SWPCA, 2011).

Delta source waters most likely contain pathogenic bacteria, viruses, and protozoa, along with non-pathogenic naturally occurring microorganisms due to the various inputs discussed above. Routine monitoring for all possible pathogens is impractical, so the focus of most source water monitoring is on indicator bacteria and pathogenic protozoa, *Giardia,* and *Cryptosporidium*. Monitoring results based on requirements of the Long Term 2 Enhanced Surface Water Treatment Rule are contained in SWPCA (2011). As discussed in **Chapter 2**, results indicate:

- The overall Delta raw water source monitoring results have been classified in Bin 1, meaning the concentration of *Cryptosporidium* is low (i.e., the running annual average is less than 0.075 oocysts/L), and no additional action related to *Cryptosporidium* is required.
- An evaluation of the total coliform, fecal coliform, and *E. coli* data indicates that 2-$\log_{10}$ *Cryptosporidium*, 3-$\log_{10}$ *Giardia*, and 4-$\log_{10}$ virus removal and inactivation are the appropriate level of treatment (SWPCA, 2011).
- Modeling of wastewater discharges into the Delta indicates that the volume of wastewater in the Delta surface water ranges on average from zero to about 3 percent (SWPCA, 2011).
- While drinking water regulations in California are currently being met for unplanned potable reuse, relying on a *de facto* approach may not be acceptable for managing and protecting potable water sources from the pressures associated with future population growth and climate change and, therefore, should be evaluated by the State.

9.3.2 Scenario 2: Indirect Potable Reuse with a "Reduced Environmental Buffer" (The Gap)

Based upon the proposed IPR criteria for SWA under consideration and as described in **Section 9.1.2**, a Gap exists between IPR using SWA with an environmental buffer that does not meet regulatory criteria and a DPR project that has no environmental buffer. In recognition of this Gap, the Expert Panel supports the following approach by the State Water Board:

- Incorporate an alternatives clause that covers Gap projects into the proposed criteria for IPR using SWA.
- Require that agencies proposing potable reuse projects failing to meet the criteria for IPR using SWA demonstrate – through hydrodynamic and public health risk modelling – public health protection equivalent to that achieved by full compliance with criteria.
- Establish a consistent framework as part of the established regulatory process for the preparation of project-related engineering reports and subsequently reviewing and permitting Gap projects.
- Conduct a peer review of several Gap project proposals and engineering reports to assist in the establishment of a consistent technical basis for Gap projects.
- Consider the potential benefits of environmental buffers, irrespective of size, as a means to take advantage of temperature equalization, storage, and peak attenuation.

9.3.3 Scenario 3: Water Supply for Direct Potable Reuse

Unit performance data and mechanical reliability assumptions from the DPR Demonstration Project in San Diego were used to investigate the overall question of treatment plant performance relative to attaining the Safe Drinking Water Act's tolerable risk goal for *Cryptosporidium*. To investigate these assumptions, the data collected on inherent unit treatment process performance and mechanical reliability from the DPR Demonstration Project (Pecson et al., 2016) were used to represent unit treatment process performance as the processes used at this facility are more current than the processes used at Water Factory 21 (McCarty et al., 1982) and the Aqua II and III Projects in San Diego (Olivieri et al., 1998).

As described in **Chapter 8** (see **Figure 8.1**), the DPR treatment train includes the following treatment processes: O_3, BAC, MF, RO, UV-AOP, and Cl_2. Influent to the treatment train is filtered secondary-treated wastewater effluent that has undergone nitrification/denitrification. No LRVs are applied to secondary-filtered wastewater effluent,[26] and no LRV credit is assumed for BAC and final chlorination prior to entry into the drinking water distribution system. A series of curves are contained in **Figure 9-5** that illustrate the following concepts:

- **LRV-CDF for Tolerable Risk Level**: The first curve, shown in black, illustrates a range of LRVs that define the necessary treatment to achieve the tolerable risk for the USEPA dose response function (USEPA, 2006). For a complete description of how these curves were developed, refer

[26] Some evidence exists to support applying LRVs to secondary-filtered wastewater (Rose et al., 2004; Olivieri et al., 2007; Sanitation Districts of Los Angeles County, 1977). Work currently is underway to investigate this topic (e.g., WRRF #14-02 on "Establishing Additional Log Reduction Credits for Wastewater Treatment Plants," being undertaken by Dr. Zia Bukhari of American Water).

to **Chapter 2** (see **Section 2.1.1.3**). Further, refer to **Section 2.1.1** for a discussion and review of the basis for California's LRVs for IPR using groundwater recharge and the basis for supporting the 10 LRV for *Cryptosporidium.* Note that the California approach for estimating LRVs is based on a single observed maximum concentration assumed to occur in raw wastewater all the time.

- **Single Treatment Train Performance**: The second set of curves (green) represent a single advanced water treatment train that combines the LRVs associated with the CDFs for the unit processes noted above with the mechanical reliability assumptions discussed in **Section 8.8.2.3**. Combining the LRVs of the treatment unit process with the mechanical reliability assumptions involved 100,000 Monte Carlo simulations, with each simulation representing a 15-minute time increment for a total of roughly 2.8 years of operation. Specifically, 100,000 simulations were conducted for each unit treatment process based on a binomial assumption that the unit treatment processes were either working (in which case an LRV was randomly selected from the appropriate CDF) or were not working (in which case an LRV of zero was assigned). The simulations were bound by the stated mechanical reliability assumptions noted above regarding operational availability. In addition, off-spec water production was defined as a 15-minute interval that was ≤10 LRV. The results indicate that under the worst-case mechanical reliability assumption, the CDF for LRV performance crosses the 10 LRV tolerable risk threshold (i.e., the existing LRV requirement for IPR) less than 5 percent of the time. This result does not mean there is an unacceptable public health risk (i.e., annual infection rate of $>10^{-4}$ per person per year) considering the analysis did not define the level of exposure (i.e., all off-spec occurrences do not necessarily occur at the same time, resulting in relevant exposures; the subject of the frequency of multiple groupings of the occurrence of off-spec water is discussed later in this section). Finally, under best-case mechanical reliability assumptions, the CDF for LRV performance crosses the tolerable risk threshold (i.e., 10 LRV) <1 percent of the time.

- **Redundancy (Standby Treatment Train Capacity) Performance**: For an analysis of redundancy, an approach was used to develop blended water produced from the above CDFs for the performance of the two single treatment trains. The performance CDF of the blended treatment train was estimated by sampling the performance CDFs of the two single treatment trains to create a blended CDF. Where off-spec water is produced (i.e., ≤10 LRV) in a single treatment train, the water from that train is diverted off-stream and the supplied water LRV is that of the redundant train. When both single train performances were below the assumed off-spec threshold, the water was assumed to be diverted off-stream. As a result, based on the assumptions used to conduct the regulatory feasibility analysis, the two red CDFs shown in **Figure 9-5** illustrate that under best- and worst-case assumptions, the variability of lower LRVs is damped out during the redundant operation of two treatment trains, and the potential off-spec water produced theoretically does not enter a hypothetical drinking water distribution system.

To consider the frequency of off-spec water produced, as well as the variability of single train and redundant (or blended train) LRVs, a time series was developed for a sample of simulations from among the 100,000 simulations. A review of the times series information shown in **Figure 9-6** illustrates the frequency of occurrences of LRVs (up and down spikes), as well as the grouping of occurrences for single and redundant treatment trains for the best- and worst-case mechanical reliability assumptions. The red line represents the LRV performance for the blended (or redundant) treatment plant and illustrates the damping out of off-spec water occurrences through the operation of redundant treatment trains.

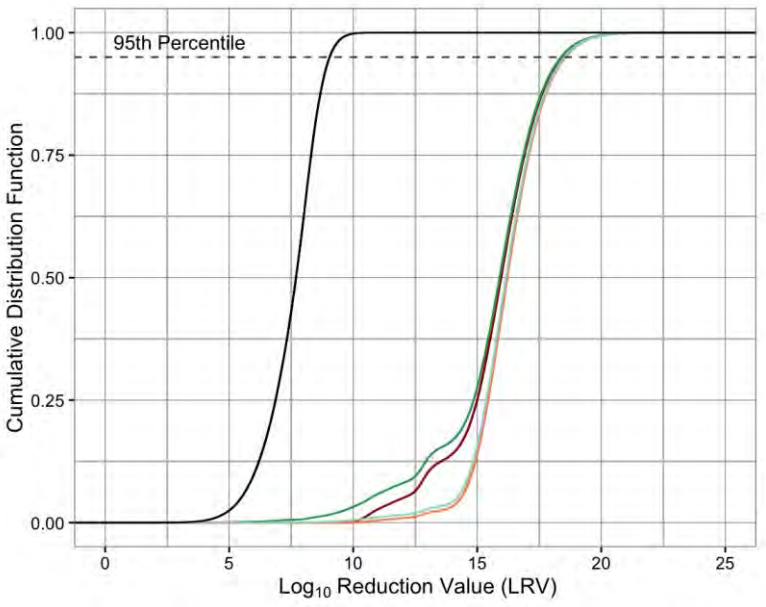

Figure 9-5: Comparison of log_{10} reduction value (LRV) cumulative distribution functions (CDFs) for meeting a tolerable risk of *Cryptosporidium* CDF (as shown in Figures 2-1 and 9-2) versus a single (green) and redundant (blended, shown as red) direct potable reuse treatment train performance CDFs for best- and worst-case mechanical reliability examples.

The following observations can be made based on a review of the operational simulation results for a period of 2.8 years:

- **Ideal Case (<1 Mechanical Failure)**. While not included in **Figure 9-6**, the ideal case simulation analysis indicates that the performances of single and redundant trains were not observed to fall below the 10-log_{10} reduction threshold for *Cryptosporidium*.

- **Best Case (≤1 Percent Mechanical Failure)**. Estimated log_{10} reductions during off-spec observations for Single Train A and a separate (but identical) Single Train B below a 10-log_{10} reduction threshold ranged between a minimum LRV of 3.7-log_{10} to a maximum of 9.9-log_{10}, with a median value of 9.3 log_{10}. More importantly, these simulations indicate that during the operation of redundant trains, one occasion occurred over the 2.8-year simulation period where log_{10} performance fell below the 10-log_{10} reduction threshold, but not below 9.6 log_{10}.

- **Worst Case (≤5 Mechanical Failure)**. Estimated log reductions during off-spec observations for Single Train A and Single Train B that fell below the 10-log_{10} reduction threshold ranged between a minimum LRV of 0 log_{10} to a maximum of 9.9 log_{10}, with a median value of 9.0 log_{10}. During redundant train operations, 95 incidences of off-spec production occurred over the 2.8-year simulation period (or roughly 30 hours of off-spec production over 2.8 years of simulated operation), with estimated log_{10} reductions ranging from 6.0 to 9.9 log_{10} (median of 9.5 log_{10}). More importantly, these simulations indicate that during the operation of redundant trains, five 15-minute incidences off-spec production occurred (or roughly 75 minutes over 2.8 years of simulated operation), with a log_{10} reduction of less than 9.

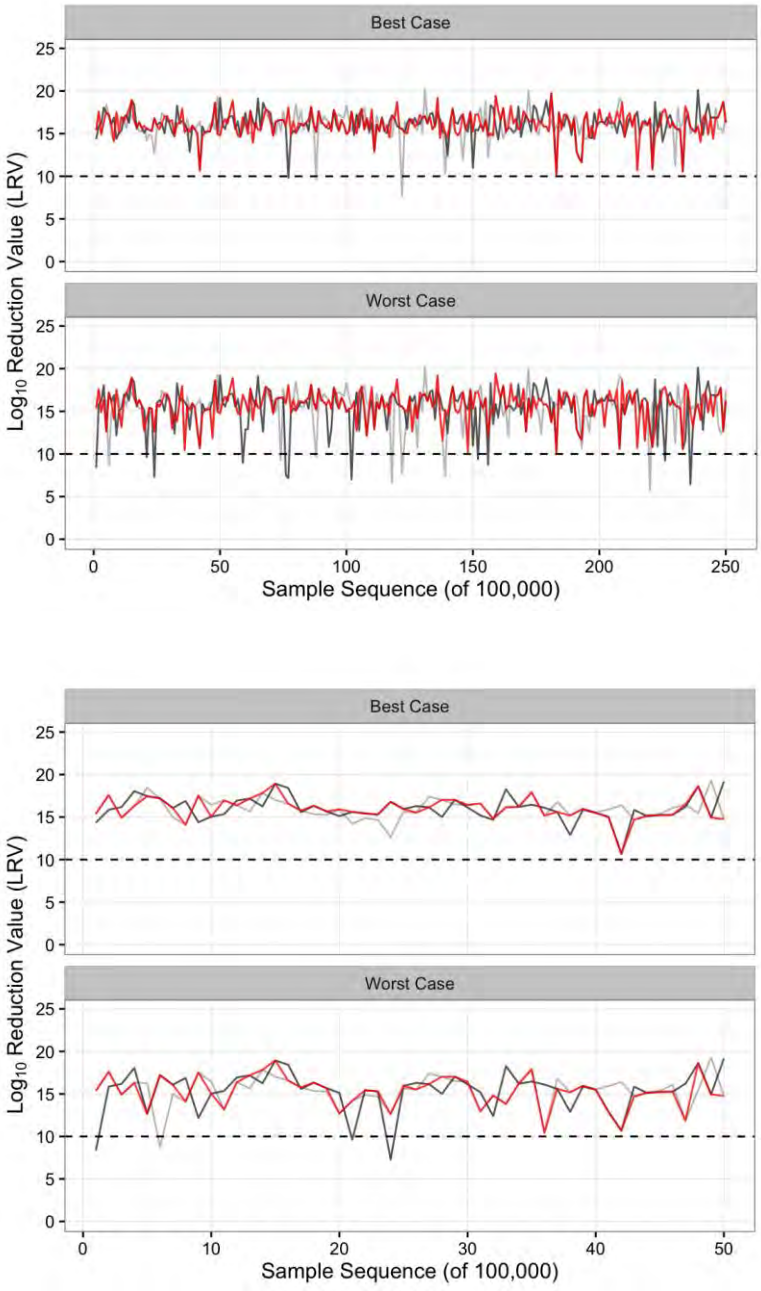

Figure 9-6: Illustration of the portions of simulations for the two single (gray lines) treatment trains and the redundant (red line) direct potable reuse treatment train operations for best- and worst-case assumptions. The full simulation represents a 2.8-year period.

Overall, under best-case operational assumptions, the production of off-spec water is infrequent, while under worst-case operational assumptions, the frequency increases; however, proper operational flexibility together with high-frequency monitoring, associated alarms, and predetermined actions most likely could mitigate the production of off-spec water.

9.4 Findings of the Expert Panel

Based on the results of the potable reuse regulatory feasibility analysis, the Expert Panel concludes the following:

- **Finding #9-1:** Conducting the probability analysis for the example treatment train (i.e., O_3/BAC/UF/RO/UV-AOP) resulted in quantifying the associated risk caused by the target pathogen *Cryptosporidium,* which demonstrated the usefulness of this approach for potable reuse projects.

- **Finding #9-2:** The probability analysis allowed for quantifying the reliability (i.e., resiliency, redundancy, and robustness) of multiple barriers in the design of treatment trains for potable reuse.

- **Finding #9-3:** The example treatment train (i.e., O_3/BAC/UF/RO/UV-AOP) demonstrated ample additional protection over the broadly accepted risk-based treatment performance goal for *Cryptosporidium* in a conventional drinking water supply. It also is above the accepted risk-based performance criteria (i.e., 12/10/10) for current IPR projects in California. Note the example treatment train was selected for the purpose of conducting this regulatory feasibility analysis; a similar analysis could be done for other treatment train options (e.g., credits given for secondary wastewater treatment and final disinfection, as well as for variations of advanced water treatment processes; see the examples in **Figure 8-1** of **Chapter 8**).

9.5 Recommendations of the Expert Panel

The Expert Panel recommends the following research studies be considered in the future. These recommendations are not listed in priority order.

- **Recommendation #9-1**: A comparison is needed of different waterborne viruses to determine virus risk, including the use of rotavirus (i.e., concentration and dose-response based on culturability) and other viruses like Norovirus GII (i.e., concentration and dose-response based on gene copies determined by qPCR).

- **Recommendation #9-2:** Conduct dose-response studies with *Cryptosporidium* at low doses (<5 oocysts) and re-evaluate dose-response models for low doses.

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PART IV: OTHER TOPICS

CHAPTER 10: MANAGEMENT CONTROLS

- Operator training and certification.
- Technical, managerial, and financial capacity.
- Institutional barriers such as interagency jurisdiction, the changing role of utilities involved with direct potable reuse, and others.

10.1 Introduction

The sponsors ofdirect potable reuse (DPR) projects would benefit from implementing management controls that recognize these facilities will require different or additional procedures and assumptions from existing water and wastewater facilities. In this chapter, the Expert Panel describes several management controls that should be considered as part of implementing a DPR project.

10.2 Managerial Considerations for Implementing Direct Potable Reuse

10.2.1 Operator Training and Certification

DPR systems should be operated by experienced and well-trained staff to ensure treatment processes function properly, regulatory requirements are met consistently, and the water produced is safe for public consumption. A training and certification program, such as those developed for water and wastewater operations, would provide a means to train operators for employment at advanced water treatment facilities (AWTFs). Also, public acceptance of DPR can be enhanced if the public understands the levels of training and certification that operators must achieve to be qualified to operate these facilities.

Certification could take different forms, such as an add-on license available to water and wastewater operators who are already certified at a specified level and would include both work experience and course work in addition to a certification exam. The certification program should emphasize topics related to the operation of advanced water treatment technologies, such as public health components, emergency response procedures, and regulations related to drinking water.

If certification for potable reuse operators is not part of the California Water Code, requirements should be included as a permit condition issued for these facilities. The development of a DPR certification program would benefit from the experience of certified operators at existing AWTFs for indirect potable reuse (IPR). The certification program could be administered by the State Water Resources Control Board (State Water Board) or by water industry trade associations experienced in implementing operator certification and training programs; however, the State Water Board should be involved in the development of any industry certification process. Options for a certification program were provided in a recent white paper titled “Potable Reuse Operator Training and Certification Framework,” prepared by the California Urban Water Agencies (CUWA, 2016).

10.2.2 Assessing the Technical, Managerial, and Financial Capacity of Direct Potable Reuse Systems

Technical, managerial, and financial (TMF) capacity is the ability of a water utility to provide safe and dependable water to its customers. The Safe Drinking Water Act requires states to incorporate TMF capacity into public water system operations. This requirement helps ensure that public water systems – including small drinking water systems – have long-term sustainability and are able to maintain compliance with all applicable drinking water laws and regulations. In general, TMF can be described as follows:

- **Technical:** Addresses the performance and operation of an AWTF.
- **Managerial:** Addresses governance (e.g., the responsibilities of overseeing the AWTF; employees and contractors).
- **Financial:** Addresses the financial ability to operate and maintain existing infrastructure and financial planning for future needs (including grants and loans). It is assessed through budget statements, asset management, and financial audits.

As DPR projects are considered for implementation by small and large communities all throughout California, it is clear that the complexity of the treatment processes will require technical support for operation and maintenance (O&M) that is much more sophisticated than is available currently or has been provided in the past for most water and wastewater treatment plants. For example, this support could be in the form of publicly funded circuit riders or possibly requiring that the utility establish a relationship with a commercial O&M provider for technical assistance and troubleshooting.

The State of California already has an existing TMF capacity development program for public drinking water systems, per requirements in the Safe Drinking Water Act of 1996, to assess the TMF capacities of water systems and assist those in need of developing or improving TMF capacity. The State's existing TMF capacity development program for public drinking water systems may need to be modified or expanded upon to specifically address AWTFs and DPR (NWRI, 2016). The goal of a TMF capacity assessment for AWTFs should be to help utility staff identify any potential or existing weaknesses and to improve the AWTF's ability to provide safe and reliable advanced treated water.

A resource for TMF capacity for the State of California can be found on the State Water Board's website at www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/TMF.shtml.

10.2.3 Institutional Barriers to Implementing Direct Potable Reuse

During the planning and implementation of a DPR project, a number of institutional barriers can arise that could delay the project or provide enough uncertainty to prevent its consideration. These barriers will need to be addressed by the sponsors of DPR projects in collaboration with regulators, decision makers, and the public. With appropriate planning, resources, and time, these barriers can be managed successfully. Possible areas of institutional barriers include:

- **Interagency Jurisdiction.** DPR projects require strong interagency cooperation and responsiveness between the agencies operating the wastewater treatment plant (WWTP) and AWTF/drinking water treatment plant (DWTF). For DPR projects where the agency that administers the WWTP is not the agency that operates the AWTF/DWTF, a memorandum of understanding or other contractual agreement should be considered so that appropriate issues

are addressed and actions can be taken, when necessary. The project sponsor of a DPR system will need to ensure that agreements with the WWTP agency are in place on topics such as the availability of treated wastewater effluent, water quality parameters, source control, and the need for wastewater discharge requirements.

- **Role of Wastewater Agencies.** The principal focus of wastewater treatment in the past has been to produce a wastewater effluent suitable for discharge into the environment. As potable reuse becomes more common, WWTPs can be designed to produce an effluent optimized for further processing by AWTFs. Until then, several improvements may need to be made to existing WWTPs to improve the quality of effluent for subsequent advanced treatment. In addition, the water quality produced by WWTPs for use in DPR systems will differ from the water quality for wastewater discharge (Tchobanoglous et al., 2015).

  Modifying existing WWTPs for DPR may involve upgrades to the wastewater management infrastructure, along with related O&M activities. In such case, WWTPs agencies will need to optimize overall performance, enhance reliability, and produce a wastewater effluent quality that is suitable for DPR. Measures that should be considered to improve performance and enhance the reliability of WWTPs include:

  - Source control.
  - Influent flow equalization.
  - Eliminating untreated return flows.
  - Modifying the operation of biological treatment processes to provide nutrient removal.
  - Effluent filtration.
  - Process performance monitoring.

  As a result, wastewater agencies will need to be responsive to a range of changes, including treatment processes, water quality parameters, and source control programs.

- **Technical, Operational, and Management Barriers**. The implementation of a DPR project involves an understanding of several key components necessary for a DPR program: (1) regulatory considerations; (2) technical issues; and (3) public outreach. The success of any DPR project will depend on the attention given to these components, which serve as barriers to safeguard the performance and production of advanced treated water (Tchobanoglous et al., 2015). DPR project sponsors should identify and implement the technical, operational, and management barriers needed to ensure reliable treatment and the protection of public health (personnel, etc.). The use of multiple independent barriers results in an overall high level of reliability by reducing the risk associated with a single barrier so that overall system resilience is enhanced. Notably, the management of a DPR system is more demanding and different than operating a WWTP or DWTF.

  For additional information, refer to the report on *Direct Potable Reuse Framework* (WRRF 14-20) (Tchobanoglous, 2015) at https://watereuse.org/watereuse-research/framework-for-direct-potable-reuse/.

- **Project Acceptability.** Water supply project options, including DPR projects, will need a robust analysis of acceptability. Agencies may benefit from using assessment tools, such as a triple bottom line (TBL) analysis for various factors (including economic, social, and environmental), as part of determining the acceptability of implementing a DPR project. Sustainability is a consideration for any new water supply project. For DPR projects, these analyses should take the following into account: economics by measuring the cost of a particular project, compared to other available water supply alternatives; social impacts, including the goal of providing the water needs of the community; and environmental impacts and benefits of the water supply alternatives, such as providing water for ecosystems.

  As a resource, refer to the white paper on *The Opportunities and Economics of Direct Potable Reuse*" (WRRF 14-08) at https://watereuse.org/watereuse-research/the-opportunities-and-economics-of-direct-potable-reuse/.

- **Water Rights.** An exploration of any water rights issues associated with the wastewater effluent will need to be conducted. For any DPR project, water rights issues must be addressed.

- **Communication, Outreach, and Public Acceptance (Stakeholder Involvement).** Public understanding and acceptance is critical for communities considering DPR; therefore, communicating effectively about the DPR project with the public is essential. Agencies will need to develop a proactive and comprehensive educational outreach program early in the development of a DPR project. The project sponsor will need the support of customers, including acceptance that the DPR project will produce water that adequately protects public health. An agency involved with a DPR project should organize a public outreach program that begins early in the process and is maintained after the project is implemented (Millan et al., 2015; Tchobanoglous et al., 2015).

  A useful resource is the report on *Model Communication Plans for Increasing Awareness and Fostering Acceptance of Direct Potable Reuse* (WRRF 13-02) at https://watereuse.org/watereuse-research/13-02-model-communication-plans-for-increasing-awareness-and-fostering-acceptance-of-direct-potable-reuse/.

- **Pilot-Scale and Demonstration Plants.** Pilot-scale and demonstration plants, particularly in communities with no experience with potable reuse, allow stakeholders and the public to view firsthand the high level of treatment, monitoring, and water quality provided at the AWTF.

10.3 Findings of the Expert Panel

In regards to management controls, the Expert Panel concludes the following:

- **Findings #10-1:** The project sponsor needs to demonstrate TMF capacity to reliably implement a DPR project**.**

10.4 References

CUWA (2016). *Draft White Paper: Potable Reuse Operator Training and Certification Framework*, California Urban Water Agencies, Walnut Creek, CA. http://www.cuwa.org/index.html (last accessed 1/20/2016).

Millan, M., P.A. Tennyson, and S. Snyder (2015). *Model Communication Plans for Increasing Awareness and Fostering Acceptance of Potable Reuse*. Project Number WRRF-13-02, WateReuse Research Foundation, Alexandria, VA.

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Tchobanoglous, G., J. Cotruvo, J. Crook, E. McDonald, A. Olivieri, A. Salveson, and R.S. Trussell (2015). *Framework for Direct Potable Reuse*, WateReuse Association, Alexandria, VA. http://www.twdb.texas.gov/publications/reports/contracted_reports/doc/1248321508_Vol2.pdf (accessed 9/3/2015).

PART V: FINDINGS AND RECOMMENDATIONS OF THE EXPERT PANEL

CHAPTER 11: KEY FINDINGS AND RESEARCH RECOMMENDATIONS OF THE EXPERT PANEL REGARDING THE FEASIBLITY TO DEVELOP UNIFORM WATER RECYCLING CRITERIA FOR DIRECT POTABLE REUSE

The purpose of this report is to address the legislative mandate contained in Section 13565(a)(1) of the California Water Code (see **Appendix P1**) that, in summary, requires the Expert Panel to:

- Advise the State Water Board on public health issues and scientific and technical matters regarding the feasibility of developing uniform statewide water recycling criteria for direct potable reuse (DPR).
- Assess what, if any, additional areas of research are needed to be able to establish uniform statewide regulatory criteria for DPR, and recommend an approach for accomplishing the additional needed research in a timely manner.

The Expert Panel selected the following main topics as part of addressing the legislative mandate:

- Public health surveillance tools and methods to quantify and mitigate risks (**Chapter 3**).
- Analytical approaches for measuring chemical water quality (**Chapter 4**).
- Application of bioanalytical tools (i.e., bioassays) to water analyses (**Chapter 5**).
- Traditional and molecular methods for assessing microbial water quality (**Chapter 6**).
- Antibiotic resistant bacteria and antibiotic resistance genes (**Chapter 7**).
- Performance of DPR systems (**Chapter 8**).
- Potable reuse regulatory feasibility analysis comparing an example DPR system against an existing potable water supply in California that is protective of public health (**Chapter 9**).
- Management controls (**Chapter 10**)

What follows is a summary of the overall results of the Expert Panel's key findings and recommendations (including key research recommendations) relative to addressing the legislative mandate in the California Water Code. Note that further details, including additional Expert Panel findings on the main topics listed above, as well as suggested national research recommendations, are provided in specific chapters of this report (as designated). Furthermore, although DPR is the subject of this report, many of the key aspects presented and discussed herein can be applied to indirect potable reuse (IPR); accordingly, relevant aspects of IPR also are discussed.

11.1 Overall Expert Panel Findings Relative to the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse

The Expert Panel finds it is feasible to develop uniform water recycling criteria for DPR that would incorporate a level of public health protection as good as or better than what is currently provided in

California by conventional drinking water supplies, IPR systems using groundwater replenishment, and proposed IPR projects using surface water augmentation.

For DPR to provide the levels of protection afforded by IPR projects using either groundwater replenishment or surface water augmentation (both of which include the use of an environmental buffer), the functionality provided by the environmental buffer for IPR projects (i.e., storage, attenuation, and response time) must be addressed by other means for DPR projects (e.g., reliability of mechanical systems and plant performance), thereby ensuring the delivery of a water quality that is protective of human health. To do so, regulations specifying DPR practices need to provide the following features in addition to requirements already specified in IPR regulations for California:

- The DPR system must be reliable. Reliability is achieved by (1) providing multiple, independent treatment barriers, (2) incorporating frequent monitoring of surrogate parameters at each step to ensure treatment processes are performing properly, and (3) developing and implementing rigorous response protocols (such as a formal Hazard Analysis and Critical Control Point [HACCP] system). See **Chapter 8 (Finding #8-1).**

  Other key attributes that promote reliability include:

  a) Using a treatment train (as described in **Chapter 9**) with multiple, independent treatment barriers (i.e., redundancy) that meet performance criteria greater than the public health threshold goals for $\log_{10}$ reduction values (LRVs) established for microorganisms.

  b) Ensuring the independent treatment barriers represent a diverse set of processes (i.e., robustness) in the treatment train that are capable of removing particular types of contaminants by different mechanisms. This diversity provides better assurance that if a currently unrecognized chemical or microbial contaminant is identified in the future, there is a greater degree of likelihood it will be removed effectively by the treatment train.

  c) Using parallel independent treatment trains (i.e., resilience and redundancy) and providing sufficient replacement parts, along with trained personnel, to rapidly carryout the most frequently needed repairs.

  d) Encouraging the use of a probabilistic analysis of treatment train performance at the design stage. The analysis should be based on data from other pilot-scale or full-scale facilities. After the full-scale facility is commissioned, the analysis should be updated periodically using actual performance data and operational experience.

  e) Providing the ability to divert advanced treated water that does not meet specifications (i.e., water that is “off spec”).

  f) Implementing a rigorous source control program designed to control the discharge of toxic chemicals and other contaminants to the wastewater collection system that serves the DPR project. The source control program must include stringent sewer ordinances and ongoing surveillance.

g) Providing certified operational personnel who are able to conduct rigorous operation and maintenance (O&M) at advanced water treatment facilities (AWTFs) and drinking water treatment fcailities (DWTFs).

h) Unauthorized short-term peak discharges of chemicals into the wastewater collection system serving a DPR project have the potential to compromise final product water quality; therefore, incorporating a final treatment process (to be specified) after the advanced water treatment train may result in some "averaging" of these potential chemical peaks.

i) Ensuring that the operation and performance of each unit treatment process in the DPR treatment train achieves the proposed and/or anticipated LRVs for pathogens.

j) Ensuring that the chemical and microbial stability of water in the drinking water distribution system will be maintained after introducing advanced treated water, in particular for a DPR system in which the final treated water will directly enter the drinking water distribution system.

- The State Water Resources Control Board (State Water Board) should not codify a specific set of treatment processes as part of developing uniform water recycling criteria for DPR, as it could stifle technological innovation in this growing area of need. The criteria should allow for alternatives to any treatment processes specified in the regulations if it is demonstrated to the State Water Board that the alternatives provide at least an equivalent level of public health protection. See **Chapter 8 (Finding #8-2).**

- The project sponsor needs to show the technical, managerial, and financial (TMF) capacity to reliably implement a DPR project. See **Chapter 10 (Finding #10-1).**

- An approach to stage the introduction of recycled water from a DPR system into a community's drinking water supply should be considered by the State Water Board as part of the review and approval of a project. This approach is consistent with California's regulatory practices for IPR using groundwater replenishment. See **Chapter 8 (Finding #8-3)**.

- A formal process should be established by the State Water Board that includes an internal process to administer the periodic review of the performance of permitted potable reuse projects by an external expert panel on a 5-year cycle. Based on this review process, the State should incorporate new knowledge into potable reuse regulatory permits. See **Chapter 8 (Recommendation #8-1)**.

11.2 Additional Expert Panel Key Findings and Recommendations Related to Topics Investigated

The Expert Panel identified several other key findings that should be conducted to further address possible concerns in the future – some of which would best be supported directly by the State of California, and others that would be better led by national and/or international entities.

A brief summary of findings is presented below. More detail on these findings, as well as other additional findings, are contained at the end of each pertaining chapter in this report.

- AWTFs sometimes employ an oxidant (e.g., ozone, chlorine, chloramines) prior to or after treatment with reverse osmosis. This practice can result in the formation of toxic byproducts, some of which are low molecular weight compounds that are not removed well during reverse osmosis or might remain after subsequent treatment with advanced oxidation. If the water is not subjected to an additional treatment step capable of removing these byproducts, they could be present in the drinking water produced by a DPR system. See **Chapter 4** (**Finding #4-5**).

  For DPR systems that employ oxidants prior to or after reverse osmosis treatment, the State Water Board should require the monitoring of low molecular weight oxidation or disinfection byproducts beyond those for which drinking water standards have already been established. See **Chapter 4** (**Recommendation #4-2**).

- Most AWTFs currently under consideration for DPR in California include reverse osmosis as one of the treatment steps. During reverse osmosis, charged compounds and neutral compounds with molecular weights above approximately 200 grams per mole (g/mol) are almost entirely removed. Uncharged, low molecular weight compounds tend to be poorly rejected by reverse osmosis (e.g., N-Nitrosodimethylamine [NDMA], chloroform, and low-molecular weight aldehydes). Under normal operating conditions, the concentrations of low molecular weight neutral compounds in water produced by DPR systems generally are below the low total organic carbon (TOC) method detection limits observed in reverse osmosis permeate (i.e., typically <0.1 milligram per liter [mg/L]); however, operators of AWTFs have detected short-duration pulses above the TOC method detection limits of acetone and, possibly, other contaminants in reverse osmosis permeate. These contaminants are believed to originate from discharges to the wastewater collection system by commercial and industrial activities. See **Chapter 4** (**Finding #4-6**).

  To minimize the potential for exposure to high concentrations of low molecular weight compounds in drinking water, chemical monitoring plans for DPR systems should include the high-frequency monitoring of TOC or other surrogate parameters capable of detecting pulses of chemicals that are poorly removed by reverse osmosis and subsequent treatment with advanced oxidation. Existing high-frequency TOC analyzers are capable of detecting pulses of elevated concentrations of contaminants in reverse osmosis permeate rapidly enough to allow operators to avoid introducing final product water into the drinking water supply. In the event a pulse of contaminants arrives at the AWTF that is too low to be detected by a high-frequency TOC analyzer, the Expert Panel believes that subsequent removal in later treatment processes (e.g., during advanced oxidation processes) would result in concentrations of contaminants that may not pose unacceptable risks to public health. See **Chapter 4** (**Recommendation #4-3**).

- Bioassays have a potential role in the identification of yet-to-be-discovered contaminants, but the Expert Panel does not recommend the routine use of bioassays in monitoring programs for DPR projects at this time. Bioassay-directed fractionation is a useful research tool for identifying compounds in recycled water that merit further evaluation. For this reason, research efforts that employ bioassays and non-target screening analysis simultaneously are encouraged to be used to discover new contaminants of concern in municipal wastewater and water produced by DPR projects. See **Chapter 5 (Finding #5-1** and **#5-2).**

- Antibiotic resistance is a valid and serious worldwide public health concern that goes well beyond DPR projects. While risk levels associated with antibiotic resistant bacteria (ARB) and

antibiotic resistance genes (ARG) in water have not been determined, concentrations of ARB and ARG in waters subjected to DPR treatment processes would likely be lower than that from current water sources entering DWTFs, suggesting that risk levels would be comparable to, or less than, those associated with current source waters. Further, considering all the available information, a combination of secondary wastewater treatment and advanced water treatment processes (i.e., a sequence of treatment train processes such as microfiltration/ultrafiltration, reverse osmosis, and ultraviolet disinfection/advanced oxidation) leading to a finished potable water is likely to reduce ARB and ARG concentrations in recycled water to levels well below those found in conventional treated drinking water. See **Chapter 7 (Findings #7-1, 7-2,** and **7-9)**.

- The role of public health surveillance is to: (1) establish partnerships, engagement, and communication between water utilities and public health partners; (2) identify sources of data to characterize baseline public health conditions and track trends over time; and (3) help determine if transient treatment failures and contamination events lead to adverse health outcomes. Within the context of potable reuse, local public health partners should be informed when a DPR project is being considered. Points of contact should be identified and available surveillance data sources should be reviewed. In addition, processes for regular engagement, information sharing, and notification should be established with an emphasis on tracking, reporting, and communicating notifiable acute, primarily waterborne diseases. The State Water Board also should work with DPR project sponsors and local health agencies to consider the feasibility of enhanced public health surveillance for communities with DPR systems. Such efforts may include syndromic surveillance, sentinel surveillance, or serological surveys for waterborne infections. See **Chapter 3 (Recommendations #3-1** and **3-2).**

- All current and proposed IPR regulations in the State of California include the use of a regulatory-defined environmental buffer; however there are likely to be potential potable reuse projects where an environmental buffer is available, but does not meet the proposed operational and performance criteria for an IPR project using surface water augmentation (SWA). Notably, the proposed criteria for IPR projects using SWA do not include an alternatives clause (NWRI, 2015b) like that in the regulations for IPR using groundwater replenishment (CCR, 2015), where a project may be allowed to use an alternative to ***any*** requirement if it "assures at least the same level of protection to public health." Consequently, an IPR project for SWA using an environmental buffer that does not meet regulatory criteria would be defined as DPR. This situation creates a regulatory "Gap" between IPR projects with smaller environmental buffers and DPR projects with no environmental buffers. Based on a previous analysis of the environmental buffer conducted by the Expert Panel during the review of proposed criteria for IPR using SWA (NWRI, 2015a,b), the Expert Panel considers IPR projects with a theoretical hydraulic retention time of <2 months in the reservoir to be a DPR project (i.e., the Gap covers IPR-SWA projects with hydraulic retention times of ≥2 months and <4 months). See **Sections 9.1.2** and **9.3.2** in **Chapter 9**. In effect, the Gap represents a transition between the currently proposed criteria for IPR using SWA and DPR. Given the above considerations, the Expert Panel supports the following approach by the State Water Board:

  a) Incorporate an alternatives clause that covers Gap projects into the proposed criteria for IPR using SWA.

b) Require that agencies proposing potable reuse projects failing to meet the criteria for IPR using SWA demonstrate – through hydrodynamic and public health risk modelling – public health protection equivalent to that achieved by full compliance with criteria.

c) Establish a consistent framework as part of the established regulatory process for preparing project-related engineering reports and subsequently reviewing and permitting Gap projects.

d) Conduct a peer review of several Gap project proposals and engineering reports to assist in the establishment of a consistent technical basis for Gap projects.

e) Consider the potential benefits of environmental buffers, irrespective of size, as a means to take advantage of temperature equalization, storage, and peak attenuation.

11.3 Research Recommendations Related to the Development of Direct Potable Reuse Criteria in California

The Expert Panel also was charged to: "…assess what, if any, additional areas of research are needed to be able to establish uniform regulatory criteria for DPR, and recommend an approach for accomplishing the additional needed research in a timely manner." The Expert Panel finds that there is no need for additional research to be conducted to establish uniform water recycling criteria for DPR; however, there are some areas of research that would enhance the understanding and acceptability of DPR in the State of California. The Panel encourages the State Water Board to address Research Recommendations #1 to 6, as discussed below.

The Expert Panel notes that Research Recommendations #1 to 6 could be undertaken either before and/or concurrently with the development of DPR criteria. While the results of the research could be used by the State to inform the development of DPR criteria, the absence of better information is not a barrier to establishing uniform regulatory criteria for DPR.

The Expert Panel notes that applied research has played a significant role in advancing potable reuse. During the 1990s, the State of California Department of Public Health (now the State Water Board's Division of Drinking Water) pioneered the development of analytical methods for monitoring chemical contaminants and identified compounds to be monitored at potable reuse facilities (i.e., the compounds for which notification levels have been established). More recently, the WateReuse Research Foundation (now called the Water Environment & Reuse Foundation) funded research projects on treatment technologies and performance reliability that have been instrumental to advancing DPR (listed in **Appendix 11A**). The Expert Panel is impressed by the research that has been funded by the WateReuse Research Foundation and supports the continuation of such research.

Nonetheless, the Expert Panel has identified important areas not being addressed in the WateReuse Research Foundation's research program related to public health, including efforts to identify new contaminants of concern and develop better monitoring techniques. As such, the Expert Panel believes the State Water Board or other agencies that have expertise in this area (e.g., the Department of Toxic Substances Control) should provide oversight and direction for research efforts designed to address these areas.

Research recommendations of the Expert Panel are as follows:

1. To better inform targeted monitoring for source control and final water quality, the State Water Board should be proactive in monitoring the literature on the potential health risks that could present serious harm to health over short durations of exposure to compounds likely to be present in recycled water. Of specific concern are chemicals that adversely affect the development of fetuses and children. Other compounds that produce such effects will undoubtedly be discovered in the future. This activity could be initiated concurrently with the development of DPR regulations and continued as an ongoing effort. A formal process should be established by the State that includes: (1) an internal process to monitor the literature and (2) an external peer review process to address the results of the internal efforts to maintain a high level of awareness of these issues. See **Chapter 4 (Research Recommendation #4-1)**.

2. The State Water Board should adopt the use of probabilistic quantitative microbial risk assessment (QMRA) to confirm the necessary LRVs of viruses, *Cryptosporidium,* and *Giardia* needed to maintain a risk of infection equal to or less than 10^{-4} per person per year. The State should provide oversight, direction, and funding for implementing probabilistic QMRA. The purpose of using probabilistic QMRA is to provide a better assessment of the performance of DPR treatment trains and to provide an opportunity to identify additional effective DPR treatment trains. Input values for pathogen concentrations should be based on descriptive pathogen statistics resulting from additional review of the literature (as well as information collected from **Research Recommendation #3**). Also, as full-scale DPR systems are built, owners and regulators need to take advantage of these systems to sample and assess actual as-built performance and reliability characteristics. See **Chapter 8 (Research Recommendation #8-1)**.

3. To better inform decisions associated with updating LRVs, as well as conducting probabilistic-based QMRA modeling, the State Water Board should include monitoring requirements in a regulatory permit to measure pathogens (i.e., *Giardia* cysts, *Cryptosporidium* oocysts, and several human viruses) in the raw (untreated) wastewater feeding a DPR system that provide more complete information on concentrations and their variability. Improved methods should be used that will allow for the better characterization and improved precision of concentrations of pathogens. See **Chapter 2 (Research Recommendation #2-1)** and **Chapter 8 (Research Recommendation #8-2)**, as well as **Chapter 6** for more information.

4. The State Water Board should investigate the feasibility of collecting pathogen concentration data for raw wastewater associated with community outbreaks of disease and collect such data where possible. See **Chapter 2 (Research Recommendation #2-2)** and **Chapters 6** and **8** for more information.

5. The State Water Board should encourage short-term research be conducted to identify suitable treatment options for final treatment processes that can provide some “averaging” with respect to potential chemical peaks (in particular, for chemicals that have the potential to persist through advanced water treatment). These options might involve: (1) the use of a buffer tank (clear well) of a sufficient size, potentially blended with an alternative water source prior to release into the drinking water distribution system, or using two tanks feeding into the drinking water distribution system; (2) the removal of volatile contaminants during a degassing step (decarbonization) similar to the approach that is commonly employed after reverse osmosis

treatment in established AWTFs for potable reuse; (3) the use of a biologically active filter after reverse osmosis/advanced oxidation, to provide an additional opportunity for microorganisms (if microorganisms will be able to survive in that environment) to degrade contaminants that may otherwise pass through the filter; or (4) other options. See **Chapter 8 (Research Recommendation #8-3)**.

6. It is important to focus on non-targeted analysis and, furthermore, low molecular weight compounds. For example, the inability of reverse-phase liquid chromatography/mass spectrometry to detect many uncharged, low molecular weight compounds (e.g., halogenated solvents, formaldehyde, and 1,4-dioxane) problematic for potable reuse projects demonstrates the limitations of current analytical approaches for the detection of unknowns that are likely to pass through reverse osmosis membranes. Research is needed to develop more comprehensive methods to identify low molecular weight unknown compounds. It is possible these compounds may be detected by gas chromatography interfaced with time-of-flight mass spectrometers or hydrophilic interaction liquid chromatography coupled with reversed-phase chromatography prior to triple quadrupole mass spectrometry; however, to date, these methods have not been applied to potable reuse projects to detect these compounds. These methods or others need to be developed to increase the understanding of the make-up of the remaining total organic carbon composed of low molecular weight compounds. In addition, these methods also could address the potential vulnerability of AWTF treatment processes to unintended spills or batch releases of chemicals in the sewershed. See **Chapter 4 (Research Recommendation #4-2)**.

11.4 Other General or Research Recommendations

11.4.1 Potential Hazards of Potable Reuse

- Given the large LRVs likely to be assigned to ultraviolet light/advanced oxidation process (UV/AOP) reactors (i.e., up to 6-$\log_{10}$ reduction for all three pathogens), it is important to provide evidence of excellent reactor hydraulics to ensure that short-circuiting does not compromise the efficiency of disinfection. This information should be included as part of the DPR project's engineering report. See **Chapter 2 (Recommendation #2-1).**

- The data for assigning LRVs to each unit process and the total LRV credits for each AWTF should be presented in the DPR project's engineering report. See **Chapter 2 (Recommendation #2-2).**

11.4.2 Public Health Surveillance

- Power calculations to detect changes in waterborne diseases under a range of assumptions should be done to help put the findings of epidemiological analyses of public health surveillance in the proper context. In addition, when epidemiological studies are under consideration, power calculations should also be done to help guide the feasibility and design of epidemiology studies. See **Chapter 3 (Recommendation #3-3).**

- Communities that rely on multiple sources for their drinking water supplies and that deliver these types of water into different pressure zones have an opportunity to develop and conduct a pilot public health surveillance effort for a DPR-augmented service area in comparison to a service area receiving conventional supplies. See **Chapter 3 (Recommendation #3-4).**

11.4.3 Chemical Water Quality

- The operators of DPR systems should be required to develop programs to explain to consumers the implications of excursions of secondary drinking water standards. See **Chapter 4 (Recommendation #4-1).**

- For DPR systems that employ oxidants prior to or after reverse osmosis treatment, the State Water Board should require the monitoring of low molecular weight oxidation or disinfection byproducts beyond those for which drinking water standards have already been established. See **Chapter 4 (Recommendation #4-2)**.

- The potential risks associated with highly toxic contaminants being discharged intermittently by commercial and industrial operations, which would be an infrequent event, are best managed through both (1) a targeted industrial source control program, and (2) more frequent sampling for compounds in this category during the startup phase of new DPR projects. See **Chapter 4 (Recommendation #4-4).**

11.4.4 Bioanalytical Tools

- Define a clear and quantifiable relationship between bioassay results and adverse health outcomes *in vivo*. Research has shown that high-throughput (HTP) single endpoint assays can be applied to testing water (nuclear receptor-activated reporter assays appear the most suitable); however, the challenge is in interpreting the data in terms of the risk of adverse health outcomes in a manner used in the development of maximum contaminant level goals (MCLGs) and public health goals (PHGs). The use of bioassays in routine monitoring should be considered analogous to the monitoring of specific chemicals with identified health risks; therefore, as stated previously, the use of bioassays for routine water quality monitoring requires a more thorough evaluation of the dose-response evaluation of the data in the context of *in vivo* health effects. There needs to be clear descriptions of the meanings that will be attached to positive and negative bioassay results. This issue comes to the fore if the intent is to monitor water intended for human consumption. It must be clear – qualitatively and from a dose-response standpoint – how bioassay results are linked to adverse health outcomes. Before any *in vitro* bioassay is used in the field for this purpose, guidance should be developed for the appropriate technical interpretation of these data relative to health risk and the communication of the results of each bioassay in light of its specific application. The Expert Panel recognizes this very broad task is too large to be addressed by the State of California or the water industry. It is best left to the Federal Programs pursuing these issues. See **Chapter 5 (Recommendation #5-2).**

- Develop Adverse Outcome Pathways (AOPathways) for chemicals with established modes of action, but no formal AOPathway. If a bioassay appears that it might be employed usefully for monitoring, but lacks an "approved AOPathway," it may be possible to develop an appropriate AOPathway. This process would require a review of *in vivo* data in the literature, for which – if sufficient – an AOPathway could be developed by an expert familiar with the use of modes of action in risk assessment. An example would be bioassays based upon an estrogen-receptor/reporter construct (that is, an AOPathway could be developed and subjected to appropriate scientific peer-review in the open risk assessment literature or by a regulatory agency). There is a huge amount of both qualitative and quantitative human and animal data in the biomedical literature on several estrogens that would provide all that is needed to develop

an AOPathway. In these limited cases, an effort focused on the modes of action of chemicals that are known to act primarily by the identified initiating event could be assembled into an AOPathway and coupled with an appropriate pharmacokinetic model to validate the use of the bioassay for monitoring. This work, however, is not an area for amateurs. Expertise within the Office of Environmental Health Hazard Assessment (OEHHA) is available to address this problem, and there are qualified researchers who have specialized in research on modes of action. A technical document for this approach would need to be developed, as well as peer-reviewed in an appropriate journal or by a panel of appropriate experts. See **Chapter 5 (Recommendation #5-3).**

- Develop guidance on the interpretation of bioassay data. Over the last 40 years, bioassays have been used in water analyses to screen for particular biological activities, followed by subsequent identification of the active compounds by chemical analyses (screening and identification). The introduction of HTP bioassays in recent years has greatly expanded the ability to detect biological activities that might contribute to adverse health effects. There are some excellent examples of work of this type and some new efforts are appearing in the literature. The Expert Panel previously concluded that *in vitro* bioassays could be used for the purposes of guiding the identification of chemicals with biological activities of potential health concern in various drinking water sources and finished water. If properly done, such efforts could help to develop public confidence in DPR. Although it is considered less important to validate these assays to the extent required for using them in compliance monitoring, there would be advantages to standardizing methods across the water industry and developing guidelines for presenting the results to the public. Having a group of accepted bioassays for this purpose would help diffuse the potential impact of random reports in the literature using bioassays that have not undergone this level of vetting. See **Chapter 5 (Recommendation #5-4).**

- Undertake research on methods of concentrating organic chemicals in water. It is unlikely that current methods for sampling water are optimal for all bioassays. Currently, little effort has been expended to determine the recovery of compounds in water samples that are active in a specific assay. The compounds that activate one bioassay are likely to have significantly different chemical/physical properties than those that interact with another. Activators of most nuclear receptors are fairly non-polar, whereas compounds that activate the Nrf/KEAP system will have widely varying chemical/physical properties; however, only one concentration technique has been commonly used in published studies of water. The same can be said for many of the so-called non-specific assays. These problems can be addressed by more carefully targeting chemicals of interest and making it standard operating procedure to report the recovery of members of that group of chemicals. See **Chapter 5 (Recommendation #5-5).**

- Conduct investigation(s) on the use of bioassays for the screening and identification of chemicals of potential health concern in water. As previously concluded by the Expert Panel, the use of bioassays for routine monitoring is not recommended primarily because the data generated by bioassays are not themselves clearly relatable to health risk. The standard should be that the bioassay data is linked in a way that allows for risk assessments of the same quality used in the development of MCLGs, PHGs, and maximum contaminant levels (MCLs); however, single bioassays or a collection of bioassays could be assembled to guide the identification of chemicals of health concern. This effort is easily within the reach of the water industry. See **Chapter 5 (Recommendation #5-6).**

- Identify chemicals in water that activate cell-based assays. If cell-based assays continue to be applied in water quality monitoring, it will be necessary to confirm that bioassay results are in fact produced by the intended target analytes and not indirectly by other components in these complex mixtures. It is a particular problem when positive responses are accepted with relatively low thresholds (e.g., $EC_{IR1.5}$). Cells have intricate signaling pathways, and there can be a high degree of interaction among these pathways. Some pathways have the potential of producing a response over background in the absence of targeted agonists and creating a situation that could be difficult to resolve. Utilities need to recognize that the above concerns are a possibility and that measuring this sort of activity could trigger a significant effort in the need to identify such compounds. Chemicals that act via other pathways are unlikely to have the same chemical and physical properties as the known agonists for a receptor. See **Chapter 5 (Recommendation #5-7).**

11.4.5 Microbial Water Quality

- To reduce uncertainty, a major initiative to characterize pathogens in wastewater is encouraged. This effort can be viewed as analogous to the Information Collection Rule for surface waters to characterize risks from viruses, *Giardia*, and *Cryptosporidium*, or the Groundwater Treatment Rule to better characterize risks from viruses. Data should be collected from multiple facilities for several years to capture the variability associated with (1) the disease status of the population in the sewershed, (2) process variability, and (3) seasonal effects. These results can be used to inform engineering reports, permitting, and start-up of DPR projects, as well as for probabilistic QMRA. See **Chapter 6 (Recommendation #6-1).**

- Research and technology transfer workshops are needed to modernize the approach that DPR uses for microbiological pathogen, indicator, and surrogate monitoring in the future. This effort will facilitate the advancement and use of new microbiological methods, datasets, bioinformatics, and instrumentation within the water industry. Technology transfer workshops are one pathway forward, and the Expert Panel encourages the State Water Board to further this effort. See **Chapter 6 (Recommendation #6-2).**

- Large volume samples (i.e., greater than 100 liters using efficient concentration methods) and quantitative polymerase chain reaction (qPCR) or digital droplet quantitative polymerase chain reaction (ddPCR) should be used to assess the removal of actual pathogens by membrane technologies at full-scale installations and to understand the impact of o-ring leaks and membrane integrity. These diagnostic investigations would provide valuable information and assurances in regard to removals. In general, it is now feasible to address the physical barriers to provide better information on the efficacy of these barriers in the treatment train. See **Chapter 6 (Recommendation #6-3).**

- For a DPR system in which the final treated water will directly enter the drinking water distribution system, the impacts on the drinking water distribution system need to be evaluated explicitly. The analysis should explain how the microbial and chemical stability of the water is maintained (or evaluated) with the introduction of the new source of water. The results and analysis should be included in the project's engineering report(s) prepared consistent with California regulations. See **Chapter 6 (Recommendation #6-4).**

- A monitoring program on the drinking water distribution system should be put in place to provide information on any changes that occur once the DPR goes online. One year of an enhanced monitoring program (i.e., more samples, more locations, and more parameters compared to the Total Coliform Rule) for specific data should be collected as a baseline before the introduction of the new water source. This effort would be followed by one year of enhanced monitoring after the introduction of the new source of water. The following microbial parameters should be considered for analysis (in addition to total coliform bacteria and disinfectant residual): HPC, assimilable organic carbon, and specific opportunistic pathogens. Total bacterial counts also may be valuable. For chemical water quality, lead and disinfection byproducts should be included as part of the enhanced monitoring program. The monitoring of premise plumbing also should be considered. See **Chapter 6 (Recommendation #6-5).**

- To the extent possible, microbiology monitoring data should become part of the public record in DPR project proposals, engineering reports, and annual reports. See **Chapter 6 (Recommendation #6-6).**

11.4.6 Antibiotic Resistance

- Additional research is needed to determine the risk to humans associated with ARB and ARG in water relative to other sources of exposure. In particular, research is needed on defining dose-response relationships between ARB and ARG concentrations in water and their ability to be acquired by human pathogens and transferred to environmental microbiota and the gut microbiome. See **Chapter 7 (Recommendation #7-1).**

- Standardized tests to determine ARB and/or ARG concentrations in potable water and wastewater should be developed. These tests should be financially and technologically accessible to a majority of water and wastewater treatment agencies. Ideally, the tests would quantify ARB and ARG that are relevant to humans. Methodology should be developed that also provides an assessment of ARG transferability within water matrices (including biofilms). See **Chapter 7 (Recommendation #7-2).**

- Characterize and evaluate ARB and ARG removal using advanced water treatment processes. Projects practicing DPR should quantitatively determine the removal of ARB and/or ARG and identify the most promising and robust technologies within their treatment trains to reduce antibiotic resistance determinants for potable reuse. See **Chapter 7 (Recommendation #7-3).**

11.4.7 Potable Reuse Regulatory Feasibility Analysis

- A comparison is needed of different waterborne viruses to determine virus risk, including the use of rotavirus (i.e., concentration and dose-response based on culturability) and other viruses like Norovirus GII (i.e., concentration and dose-response based on gene copies determined by qPCR). See **Chapter 9 (Recommendation #9-1).**

- Conduct dose-response studies with *Cryptosporidium* at low doses (<5 oocysts) and re-evaluate dose-response models for low doses. See **Chapter 9 (Recommendation #9-2).**

11.5 References

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BIOGRAPHIES OF THE EXPERT PANEL AUTHORS AND REPORT EDITORS

1. Expert Panel Members

Co-Chair: Adam Olivieri, Dr.PH, P.E. Adam Olivieri has 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 has gained this experience through working as a staff engineer with the California Regional Water Quality Control Board (San Francisco Bay Region), as staff specialist (and Post-doc fellow) with the School of Public Health at the University of California, Berkeley, project manager/researcher for the Public Health Institute, and as a consulting engineer. He is currently the Vice president of EOA, Inc. (Oakland, CA), where he manages a variety of projects, including serving as Santa Clara County Urban Runoff Program's Manager since 1998. Olivieri is also the author or co-author of numerous technical publications and project reports. He received a B.S. in Civil Engineering from the University of Connecticut, an M.S. in Civil and Sanitary Engineering from the University of Connecticut, and both an MPH and Dr.PH in Environmental Health Sciences from University of California, Berkeley.

Co-Chair: James Crook, Ph.D., P.E. Jim Crook is an environmental engineer (based in Boston, MA) with more than 40 years of experience in state government and consulting engineering arenas, serving public and private sectors in the United States and abroad. He has authored more than 100 publications and is an internationally recognized expert in water reclamation and reuse. He has been involved in numerous projects and research activities involving public health, regulations and permitting, water quality, risk assessment, treatment technology, and all facets of water reuse. Crook spent 15 years directing the California Department of Health Services' water reuse program, during which time he developed California's first comprehensive water reuse criteria. He also spent 15 years with consulting firms overseeing water reuse activities and is now an independent consultant specializing in water reuse. He currently serves on several advisory panels and committees sponsored by NWRI and others. Among his honors, he was selected as the American Academy of Environmental Engineers' 2002 Kappe Lecturer and the WateReuse Association's 2005 Person of the Year. Crook received a B.S. in Civil Engineering from the University of Massachusetts and both an M.S. and Ph.D. in Environmental Engineering from the University of Cincinnati.

Michael Anderson, Ph.D. Michael Anderson, a Professor of Applied Limnology and Environmental Chemistry, has taught courses at the University of California, Riverside, since 1990. He also serves as Chair of the Department of Environmental Sciences. His research focus includes water and soil sciences, with particular emphasis in applied limnology and lake/reservoir management; surface water quality and modeling; fate of contaminants in waters, soils, and sediments; and environmental chemistry. Current research projects include laboratory, field, and modeling studies in support of the development of species conservation habitat at the Salton Sea, sponsored by the California DWR and DFG, and a survey of organochlorine pesticides and Polychlorinated Biphenyls (PCBs) in McGrath Lake that is funded by the Los Angeles Regional Water

Quality Control Board. He and his students also recently completed studies quantifying the abundance and distribution of quagga mussel veligers in the reservoirs of the Colorado River Aqueduct, as well as assessing the ecological and biological conditions at Lake Elsinore. In addition, he has served on various panels and workgroups, including as member of the California Department of Water Resource's Salton Sea Hydrologic Technical Workgroup (2007-2008). Anderson received a B.S. in Biology from Illinois Benedictine College, M.S. in Environmental Studies from Bemidji State University, and Ph.D. in Environmental Chemistry from Virginia Tech.

Richard Bull, Ph.D. Since 2000, Richard Bull has been a Consulting Toxicologist with MoBull Consulting (Richland, WA), where he conducts studies on the chemical problems encountered in water for water utilities, as well as federal, state, and local governments. Bull is a Professor Emeritus at Washington State University, where he maintains Adjunct Professor appointments in the College of Pharmacy and the Department of Environmental Science. Formerly, he served as a senior staff scientist at DOE's Pacific Northwest National Laboratory, Professor of Pharmacology/Toxicology at Washington State University, and Director of the Toxicology and Microbiology Division in the Cincinnati Laboratories for the U.S. Environmental Protection Agency. Bull has published extensively on research on central nervous system effects of heavy metals, the carcinogenic and toxicological effects of disinfectants and disinfection by-products, halogenated solvents, acrylamide, and other contaminants of drinking water. He has also served on many international scientific committees convened by the National Academy of Sciences, World Health Organization, and International Agency for Research on Cancer regarding various contaminants of drinking water. Bull received a B.S. in Pharmacy from the University of Washington and a Ph.D. in Pharmacology from the University of California, San Francisco.

Dr.-Ing. Jörg E. Drewes. Jörg Drewes joined the Technical University of Munich (Germany) in 2013, where he currently serves as Chair Professor and Chair of Urban Water Systems Engineering. Prior, he was a professor in the Department of Civil and Environmental Engineering at Colorado School of Mines (CSM), where he taught from 2001 to 2013. While at CSM, he served as the Director of Research for the National Science Foundation's Engineering Research Center *ReNUWIt* (which included Stanford University, University of California Berkeley, New Mexico State University, and CSM). He also served as Co-Director of CSM's Advanced Water Technology Center (AQWATEC). Drewes is actively involved in research in the areas of energy efficient water treatment and non-potable and potable water reuse. Current research interests include treatment technologies leading to potable reuse and the fate and transport of persistent organic compounds in these systems. He has published more than 250 journal papers, book contributions, and conference proceedings, and served on National Research Council Committees on *Water Reuse as an Approach for Meeting Future Water Supply Needs* and *Onsite Reuse of Graywater and Stormwater*. He also currently serves as Chair of the International Water Association (IWA) Water Reuse Specialist Group. Drewes received a Cand. Ing. (B.S.), Dipl. Ing. (M.S.), and Doctorate (Dr.-Ing.) in Environmental Engineering from the Technical University of Berlin, Germany.

Charles Haas, Ph.D. Chuck Haas is the Department Head of the Civil, Architectural, and Environmental Engineering and at Drexel University (Philadelphia, PA) since 1991. He is also the L.D. Betz Professor of Environmental Engineering and Director of the Drexel Engineering Cities Initiative. Prior to joining Drexel, he served on the faculties of Rensselaer Polytechnic Institute and the Illinois Institute of Technology. Haas specializes in water treatment, risk assessment, environmental modeling and statistics, microbiology, and environmental health. He received a B.S. in Biology and M.S. in Environmental Engineering, both from the Illinois Institute of Technology. He also received a Ph.D. in Environmental Engineering from the University of Illinois at Urbana-Champaign.

Walter Jakubowski, M.S. Walt Jakubowski is a practicing private consultant with WaltJay Consulting (Spokane, WA) who serves on various professional committees, panels, and boards. He was a consultant to the World Health Organization on pathogenic intestinal protozoa (for development of the International Drinking Water Guidelines) and the Pan-American Health Organization on environmental virus methods. He was also instrumental in conducting the first international symposium on *Legionella* and Legionnaire's Disease at the Centers for Disease Control. Jakubowski has more than 48 years of experience working with waterborne pathogens, especially enteric viruses, *Giardia,* and *Cryptosporidium.* 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 also 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. In addition, he authored research publications on hospital pharmacy and microorganisms in oysters and clams under the federal Shellfish Sanitation Program, as well as more than 40 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. Jakubowski has degrees in Pharmacy from Long Island University and microbiology from Oregon State University, as well as graduate training in epidemiology from the University of Minnesota.

Perry McCarty, Sc.D. Perry McCarty is the Silas H. Palmer Professor of Civil and Environmental Engineering Emeritus at Stanford University (Stanford, CA). He received the Clarke Prize Award in 1997 for his significant contributions to the areas of water treatment, reclamation, groundwater recharge, and water chemistry and microbiology. He is universally recognized for his research on understanding contaminant behavior in groundwater aquifers and sediments. McCarty has received numerous honors, including being elected to the National Academy of Engineering and American Academy of Arts and Sciences, as well as receiving an honorary doctorate from the Colorado School of Mines. He was also awarded the John and Alice Tyler Prize for Environmental Achievement in 1992 and the Stockholm Water Prize in 2007. McCarty received his B.S. from Wayne State University, and both his M.S. and Sc.D. from Massachusetts Institute of Technology.

Kara Nelson, Ph.D. Kara Nelson is a Professor in Civil and Environmental Engineering at the University of California, Berkeley (Berkeley, CA). She received her B.A. degree in biophysics from U.C. Berkeley, her M.S.E. degree in environmental engineering from the University of Washington, and her Ph.D. in environmental engineering from U.C. Davis. Her research program addresses critical issues at the intersection of public health and the environment, with a focus on reducing the threat posed by waterborne pathogens by improving our engineering infrastructure to make it more effective, affordable, as well as maximize its environmental benefits. Specific research areas include mechanisms of pathogen inactivation, molecular techniques for pathogen detection, optimizing treatment processes, water reuse, and challenges with providing safe drinking water and sanitation in the developing world. Dr. Nelson has published over 50 articles in peer-reviewed journals, including two invited reviews, and one book chapter. She is the Director of Graduate Education at the National Science Foundation Engineering Research Center for Reinventing our Nation's Urban Water Infrastructure (ReNUWIt), the faculty leader of the Research Thrust Area on Safe Water and Sanitation at Berkeley Water Center. Dr. Nelson was awarded the Presidential Early Career Award for Scientists and Engineers (PECASE) at a ceremony in the White House in 2004. This award is the nation's highest honor for scientists in the early stages of their career.

Joan B. Rose, Ph.D. Joan Rose, the Homer Nowlin Endowed Chair for Water Research professor at Michigan State University (East Lansing, MA), has made groundbreaking advances in understanding water quality and protecting public health for more than 20 years and has published over 300 articles. She is widely regarded as the world's foremost authority on the microorganism *Cryptosporidium* and was the first person to present a method for detecting this pathogen in water supplies. She examines full-scale water treatment systems for the removal of pathogens. Among her honors, she received the Stockholm Water Prize in 2016 and the Athalie Richardson Irvine Clarke Prize from NWRI in 2001 for her advances in microbial water-quality issues. She served as the Chair of the Science Advisory Board for the U.S. Environmental Protection Agency's Drinking Water Committee for 4 years, and currently serves on the Science Advisory Board for the Great Lakes. In addition, she is Co-Director of the Center for Water Sciences (which includes work with the Great Lakes and Human Health Center of the National Oceanic & Atmospheric Administration) at Michigan State University, where she is also Director of the Center for Advancing Microbial Risk Assessment. Rose received a B.S. in Microbiology from the University of Arizona, an M.S. in Microbiology from the University of Wyoming, and a Ph.D. in Microbiology from the University of Arizona.

David Sedlak, Ph.D. David Sedlak is the Malozemoff Professor of Civil and Environmental Engineering at the University of California, Berkeley (Berkeley, CA). He is also Co-Director of the Berkeley Water Center and Deputy Director of the National Science Foundation's Engineering Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt). His research focus is on the fate of chemical contaminants, with the long-term goal of developing cost-effective, safe, and sustainable systems to manage water resources. Sedlak's previous experience includes Staff Scientist at ENVIRON Corporation and membership on the National Research Council's Committee on Water Reuse. He has individually or co-authored over 70 peer-reviewed publications, among many other publications and presentations. Sedlak published a book in 2014 called "Water 4.0: The Past, Present, and Future of The World's Most Vital Resource," where he points out that most of the population gives little thought to the hidden systems

that bring us water and take it away and how these marvels of engineering face challenges that cannot be solved without a fundamental change to our relationship with water. Sedlak received a B.S. in Environmental Science from Cornell University and a Ph.D. in Water Chemistry from the University of Wisconsin.

Tim Wade, Ph.D. Tim Wade is the Epidemiology Branch Chief at the United States Environmental Protection Agency (USEPA) in Durham, NC, and Assistant Professor of Epidemiology at the University of North Carolina, Chapel Hill. Wade has been working with the USEPA since 2005, conducting a series of epidemiologic studies to evaluate the health effects of arsenic exposure in well water in Inner Mongolia. As Branch Chief, Wade determines research priorities, directs staff and post-doctoral students, and manages an annual budget of over $1 million annually. In 2011, Wade received the EPA Office of Water Bronze Medal for his exceptional service to the Office of Water in the development of recreational water quality criteria. He received a B.A. in Biological Science from California Polytechnic at Pomona, a B.A. in Psychobiology from Claremont McKenna College, and both an MPH and Ph.D. in Epidemiology from the University of California at Berkeley.

2. Report Editors

Jeffrey J. Mosher. Jeff Mosher has extensive experience in water supply and water resources, including water reuse with an emphasis on indirect and direct potable reuse. For the past 10 years, he has served as executive director of NWRI, a 501c3 nonprofit focused on improving water quality and protecting public health. In this capacity, he oversees project management, strategic planning, financial management, and conference and meeting planning. Under his leadership, NWRI has supported projects, publications, and events focused on potable reuse, desalination, and other areas of advanced water treatment. He also has led more than 30 NWRI independent advisory panels for water, wastewater, and state agencies addressing water quality, treatment options, and the implementation of complex projects and policies; this effort includes administering an expert panel on evaluating the feasibility of developing water recycling criteria for direct potable reuse for the state of California. In addition, he administered a panel to develop a *Direct Potable Reuse Framework* document (2015) for the WateReuse Association, NWRI, and other sponsors; he served as one of the documents editors. Through NWRI, Mosher serves as administrative director for the Southern California Salinity Coalition. His extensive background in association and research foundation management includes previous positions for the WateReuse Association, WateReuse Research Foundation, and Association of Metropolitan Water Agencies. Mosher received a BS in Chemistry from the College of William and Mary and an MS in Environmental Engineering from George Washington University.

Gina Melin Vartanian. Gina Vartanian is an experienced writer and editor, specializing in water resources and technology. Since 1998, she has served as an editor, writer, and project manager for NWRI, a 501c3 nonprofit focused on improving water quality and protecting public health. As communications and outreach manager, she focuses on publications, website and social media development, grant proposals, and program development for conferences, workshops, and others. She has edited hundreds of technical documents for the water industry, including NWRI's *Ultraviolet Disinfection Guidelines for Drinking Water and Water Reuse* and the textbook *Riverbank Filtration: Improving Source-Water Quality*. Vartanian also provides editorial support for NWRI's Independent Advisory Panel program, attending panel meetings and assisting with panel reports. These efforts include panels to review potable reuse projects for agencies like the Orange County Water District (CA), Village of Cloudcroft (NM), and El Paso Water Utilities (TX), as well as an expert panel on evaluating the

feasibility of developing water recycling criteria for direct potable reuse for the state of California. She also edited the document, *Direct Potable Reuse Framework* (2015), developed by an expert panel for the WateReuse Association, NWRI, and other sponsors. Vartanian received a BA in English Literature and a Master of Professional Writing from the University of Southern California.

WRRF PROJECT CONTRIBUTORS

Through the Direct Potable Reuse Research Initiative, the WateReuse Research Foundation invested over $20 million in research projects to assist the Expert Panel in completing its state-mandated charge of investigating the feasibility of developing uniform water recycling criteria for direct potable reuse. Two projects were funded to provide direct support the Expert Panel: WRRF 15-01 on "Potable Reuse Research Compilation: Synthesis of Findings" and WRRF 14-14 on "Feasibility of Establishing a Framework for Public Health Monitoring." Contributors to these projects are listed below.

WRRF 15-01 Project on "Potable Reuse Research Compilation: Synthesis of Findings"

Sponsor	WateReuse Research Foundation
Project Manager	Julie Minton, WateReuse Research Foundation (Alexandria, VA)
Principal Investigator	Jeff Mosher, National Water Research Institute (Fountain Valley, CA)
Report Technical and Editorial Lead	George Tchobanoglous, Ph.D., P.E., University of California, Davis (Davis, CA)
Report Associate Editor	Gina Melin Vartanian, National Water Research Institute (Fountain Valley, CA)
Report Authors	Philip Brandhuber, Ph.D., HDR, Inc. (Denver, CO)
	Debra L. Burris, P.E., DDB Engineering, Inc. (Irvine, CA)
	Jean Debroux, Ph.D., Kennedy/Jenks Consultants (San Francisco, CA)
	Robert W. Emerick, Ph.D., P.E., Robert Emerick Associates (San Francisco, CA)
	Ufuk Erdal, Ph.D., P.E., CH2M (Santa Ana, CA)
	Daniel Gerrity, Ph.D., University of Nevada, Las Vegas (Las Vegas, NV)
	Laura Kennedy, Kennedy/Jenks Consultants (San Francisco, CA)
	Jim Lozier, P.E., CH2M (Tempe, AZ)
	Brian Pecson, Ph.D., P.E., Trussell Technologies, Inc. (Oakland, CA)
	Megan Plumlee, Ph.D., P.E., Orange County Water District (Fountain Valley, CA)
	Channah M. Rock, Ph.D., University of Arizona (Tucson, AZ)
	Andrew Salveson, P.E., Carollo Engineers (Walnut Creek, CA)
	Larry Schimmoller, P.E., CH2M (Englewood, CO)
	Ben Stanford, Ph.D., Hazen and Sawyer (Raleigh, NC)
	Sarah Triolo, Trussell Technologies, Inc. (Oakland, CA)
Project Advisory Committee	Jing-Tying Chao, P.E., State Water Resources Control Board
	Amy Dorman, City of San Diego (San Diego, CA)
	Serge Haddad, Los Angeles Department of Water and Power (Los Angeles, CA)
	Robert Hultquist, P.E., State Water Resources Control Board
	Philip Oshida, U.S. Environmental Protection Agency
	Michael Wehner, Orange County Water District (Fountain Valley, CA)

WRRF 14-14 White Paper on the "Feasibility of Establishing a Framework for Public Health Monitoring"

Sponsor	WateReuse Research Foundation
Project Manager	Kristan Cwalina, WateReuse Research Foundation (Alexandria, VA)
Project Principal Investigator	Jeffrey Soller, Soller Environmental, LLC (Berkeley, CA)
Co-Principal Investigators	Andrew Salveson, P.E., Carollo Engineers (Walnut Creek, CA)
	Mary Schoen, Ph.D., Soller Environmental, LLC (Seattle, WA)
	Edmund Seto, Ph.D., University of Washington (Seattle, WA)
Project Advisory Committee	Brian Bernados, P.E., State Water Resources Control Board (San Diego, CA)
	Joseph Cotruvo, Ph.D., BCES, Joseph Cotruvo & Associates, LLC (Washington, D.C.)
	Karen Levy, Ph.D., MPH, Emory University (Atlanta, GA)
	Kristina Mena, M.S.P.H., Ph.D., The University of Texas Health Science Center at Houston (El Paso, TX)
	Margaret Nellor, P.E., Nellor Environmental Associates, Inc. (Austin, TX)
	Tim Wade, Ph.D., United States Environmental Protection Agency (Durham, NC)

APPENDICES

APPENDIX P1: CALIFORNIA WATER CODE SECTIONS ON POTABLE REUSE

CALIFORNIA WATER CODE
CHAPTER 7.3 DIRECT AND INDIRECT POTABLE REUSE
SECTION 13560-13569

13560. The Legislature finds and declares the following:

(a) In February 2009, the state board unanimously adopted, as Resolution No. 2009-0011, an updated water recycling policy, which includes the goal of increasing the use of recycled water in the state over 2002 levels by at least 1,000,000 acre-feet per year by 2020 and by at least 2,000,000 acre-feet per year by 2030.

(b) Section 13521 requires the department to establish uniform statewide recycling criteria for each varying type of use of recycled water where the use involves the protection of public health.

(c) The use of recycled water for indirect potable reuse is critical to achieving the state board's goals for increased use of recycled water in the state. If direct potable reuse can be demonstrated to be safe and feasible, implementing direct potable reuse would further aid in achieving the state board's recycling goals.

(d) Although there has been much scientific research on public health issues associated with indirect potable reuse through groundwater recharge, there are a number of significant unanswered questions regarding indirect potable reuse through surface water augmentation and direct potable reuse.

(e) Achievement of the state's goals depends on the timely development of uniform statewide recycling criteria for indirect and direct potable water reuse.

(f) This chapter is not intended to delay, invalidate, or reverse any study or project, or development of regulations by the department, the state board, or the regional boards regarding the use of recycled water for indirect potable reuse for groundwater recharge, surface water augmentation, or direct potable reuse.

(g) This chapter shall not be construed to delay, invalidate, or reverse the department's ongoing review of projects consistent with Section 116551 of the Health and Safety Code.

13561. For purposes of this chapter, the following terms have the following meanings:

(a) "Department" means the State Department of Public Health.

(b) "Direct potable reuse" means the planned introduction of recycled water either directly into a public water system, as defined in Section 116275 of the Health and Safety Code, or into a raw water supply immediately upstream of a water treatment plant.

(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) "Surface water augmentation" means the planned placement of recycled water into a surface water reservoir used as a source of domestic drinking water supply.

(e) "Uniform water recycling criteria" has the same meaning as in Section 13521.

13561.5. The state board shall enter into an agreement with the department to assist in implementing this chapter.

13562. (a) (1) On or before December 31, 2013, the department shall adopt uniform water recycling criteria for indirect potable reuse for groundwater recharge.

(2) (A) Except as provided in subparagraph (C), on or before December 31, 2016, the department shall develop and adopt uniform water recycling criteria for surface water augmentation.

(B) Prior to adopting uniform water recycling criteria for surface water augmentation, the department shall submit the proposed criteria to the expert panel convened pursuant to subdivision (a) of Section 13565. The expert panel shall review the proposed criteria and shall adopt a finding as to whether, in its expert opinion, the proposed criteria would adequately protect public health.

(C) The department shall not adopt uniform water recycling criteria for surface water augmentation pursuant to subparagraph (A), unless and until the expert panel adopts a finding that the proposed criteria would adequately protect public health.

(b) Adoption of uniform water recycling criteria by the department is subject to the requirements of Chapter 3.5 (commencing with Section 11340) of Part 1 of Division 3 of Title 2 of the Government Code.

13562.5. Notwithstanding any other law, no later than June 30, 2014, the department shall adopt, by emergency regulations in accordance with Chapter 3.5 (commencing with Section 11340) of Part 1 of Division 3 of Title 2 of the Government Code, requirements for groundwater replenishment using recycled water. The adoption of these regulations is an emergency and shall be considered by the Office of Administrative Law as necessary for the immediate preservation of the public peace, health, safety, and general welfare. Notwithstanding Chapter 3.5 (commencing with Section 11340) of Part 1 of Division 3 of Title 2 of the Government Code, emergency regulations adopted by the department pursuant to this section shall not be subject to review by the Office of Administrative Law and shall remain in effect until revised by the department.

13563. (a) (1) On or before December 31, 2016, the department, in consultation with the state board, shall investigate and report to the Legislature on the feasibility of developing uniform water recycling criteria for direct potable reuse.

(2) The department shall complete a public review draft of its report by September 1, 2016. The department shall provide the public not less than 45 days to review and comment on the public review draft.

(3) The department shall provide a final report to the Legislature by December 31, 2016. The department shall make the final report available to the public.

(b) In conducting the investigation pursuant to subdivision (a), the department shall examine all of the following:

(1) The availability and reliability of recycled water treatment technologies necessary to ensure the protection of public health.

(2) Multiple barriers and sequential treatment processes that may be appropriate at wastewater and water treatment facilities.

(3) Available information on health effects.

(4) Mechanisms that should be employed to protect public health if problems are found in recycled water that is being served to the public as a potable water supply, including, but not limited to, the failure of treatment systems at the recycled water treatment facility.

(5) Monitoring needed to ensure protection of public health, including, but not limited to, the identification of appropriate indicator and surrogate constituents.

(6) Any other scientific or technical issues that may be necessary, including, but not limited to, the need for additional research.

(c) (1) Notwithstanding Section 10231.5 of the Government Code, the requirement for submitting a report imposed under paragraph (3) of subdivision (a) is inoperative on December 31, 2020.

(2) A report to be submitted pursuant to paragraph (3) of subdivision (a) shall be submitted in compliance with Section 9795 of the Government Code.

13563.5. (a) The department, in consultation with the state board, shall report to the Legislature as part of the annual budget process, in each year from 2011 to 2016, inclusive, on the progress towards developing and adopting uniform water recycling criteria for surface water augmentation and its investigation of the feasibility of developing uniform water recycling criteria for direct potable reuse.

(b) (1) A written report submitted pursuant to subdivision (a) shall be submitted in compliance with Section 9795 of the Government Code.

(2) Pursuant to Section 10231.5 of the Government Code, this section is repealed on January 1, 2017.

13564. In developing uniform water recycling criteria for surface water augmentation, the department shall consider all of the following:

(a) The final report from the National Water Research Institute Independent Advisory Panel for the City of San Diego Indirect Potable Reuse/Reservoir Augmentation (IPR/RA) Demonstration Project.

(b) Monitoring results of research and studies regarding surface water augmentation.

(c) Results of demonstration studies conducted for purposes of approval of projects using surface water augmentation.

(d) Epidemiological studies and risk assessments associated with projects using surface water augmentation.

(e) Applicability of the advanced treatment technologies required for recycled water projects, including, but not limited to, indirect potable reuse for groundwater recharge projects.

(f) Water quality, limnology, and health risk assessments associated with existing potable water supplies subject to discharges from municipal wastewater, stormwater, and agricultural runoff.

(g) Recommendations of the State of California Constituents of Emerging Concern Recycled Water Policy Science Advisory Panel.

(h) State funded research pursuant to Section 79144 and subdivision (b) of Section 79145.

(i) Research and recommendations from the United States Environmental Protection Agency Guidelines for Water Reuse.

(j) The National Research Council of the National Academies' report titled "Water Reuse: Potential for Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater."

(k) Other relevant research and studies regarding indirect potable reuse of recycled water.

13565. (a) (1) On or before February 15, 2014, the department shall convene and administer an expert panel for purposes of advising the department on public health issues and scientific and technical matters regarding development of uniform water recycling criteria for indirect potable reuse through surface water augmentation and investigation of the feasibility of developing uniform water recycling criteria for direct potable reuse. The expert panel shall assess what, if any, additional areas of research are needed to be able to establish uniform regulatory criteria for direct potable reuse. The expert panel shall then recommend an approach for accomplishing any additional needed research regarding uniform criteria for direct potable reuse in a timely manner.

(2) The expert panel shall be comprised, at a minimum, of a toxicologist, an engineer licensed in the state with at least three years' experience in wastewater treatment, an engineer licensed in the state with at least three years' experience in treatment of drinking water supplies and knowledge of drinking water standards, an epidemiologist, a limnologist, a microbiologist, and a chemist. The department, in consultation with the advisory group and the state board, shall select the expert panel members.

(3) Members of the expert panel may be reimbursed for reasonable and necessary travel expenses.

(b) (1) On or before January 15, 2014, the department shall convene an advisory group, task force, or other group, comprised of no fewer than nine representatives of water and wastewater agencies, local public health officers, environmental organizations, environmental justice organizations, public health nongovernmental organizations, the department, the state board, the United States Environmental Protection Agency, ratepayer or taxpayer advocate organizations, and the business community, to advise the expert panel regarding the development of uniform water recycling criteria for direct potable reuse and the draft report required by Section 13563. The department, in consultation with the state board, shall select the advisory group members.

(2) Environmental, environmental justice, and public health nongovernmental organization representative members of the advisory group, task force, or other group may be reimbursed for reasonable and necessary travel expenses.

(3) In order to ensure public transparency, the advisory group established pursuant to paragraph (1) shall be subject to the Bagley-Keene Open Meeting Act (Article 9 (commencing with Section 11120) of Chapter 1 of Part 1 of Division 3 of Title 2 of the Government Code).

(c) On or before June 30, 2016, the department shall prepare a draft report summarizing the recommendations of the expert panel.

(d) The department may contract with a public university or other research institution with experience in convening expert panels on water quality or potable reuse to meet all or part of the requirements of

this section should the department find that the research institution is better able to fulfill the requirements of this section by the required date.

13566. In performing its investigation of the feasibility of developing the uniform water recycling criteria for direct potable reuse, the department shall consider all of the following:

(a) Recommendations from the expert panel appointed pursuant to subdivision (a) of Section 13565.

(b) Recommendations from an advisory group, task force, or other group appointed by the department pursuant to subdivision (b) of Section 13565.

(c) Regulations and guidelines for these activities from jurisdictions in other states, the federal government, or other countries.

(d) Research by the state board regarding unregulated pollutants, as developed pursuant to Section 10 of the recycled water policy adopted by state board Resolution No. 2009-0011.

(e) Results of investigations pursuant to Section 13563.

(f) Water quality and health risk assessments associated with existing potable water supplies subject to discharges from municipal wastewater, stormwater, and agricultural runoff.

13567. An action authorized pursuant to this chapter shall be consistent, to the extent applicable, with the federal Clean Water Act (33 U.S.C. Sec. 1251 et seq.), the federal Safe Drinking Water Act (42 U.S.C. Sec. 300f et seq.), this division, and the California Safe Drinking Water Act (Chapter 4 (commencing with Section 116270) of Part 12 of Division 104 of the Health and Safety Code).

13569. The department may accept funds from nonstate sources and may expend these funds, upon appropriation by the Legislature, for the purposes of this chapter.

APPENDIX P2: BACKGROUND ON THE PANEL PROGRAM ADMINISTERED BY THE NATIONAL WATER RESEARCH INSTITUTE

About the National Water Research Institute

For over 20 years, the National Water Research Institute (NWRI) – a science-based 501c3 nonprofit located in Fountain Valley, California – has sponsored projects and programs to improve water quality, protect public health and the environment, and create safe, new sources of water. NWRI specializes in working with researchers across the country, such as laboratories at universities and water agencies, and are guided by a Research Advisory Board (representing national expertise in water, wastewater, and water reuse) and a six-member Board of Directors (representing water and wastewater agencies in Southern California).

Through NWRI's research program, NWRI supports multi-disciplinary research projects with partners and collaborators that pertain to treatment and monitoring, water quality assessment, knowledge management, and exploratory research. Altogether, NWRI's research program has produced over 300 publications and conference presentations.

NWRI also promotes better science and technology through extensive outreach and educational activities, which includes facilitating workshops and conferences and publishing White Papers, guidance manuals, and other informational material.

More information on NWRI can be found online at www.nwri-usa.org.

About the Panel Program

NWRI also specializes in facilitating Independent Advisory Panels on behalf of water and wastewater utilities, as well as local, county, and state government agencies, to provide credible, objective review of scientific studies and projects in the water industry. NWRI Panels consist of academics, industry professionals, government representatives, and independent consultants who are experts in their fields.

The NWRI Panel process provides numerous benefits, including:

- Third-party review and evaluation.
- Scientific and technical advice by leading experts.
- Assistance with challenging scientific questions and regulatory requirements.
- Validation of proposed project objectives.
- Increased credibility with stakeholders and the public.
- Support of sound public-policy decisions.

NWRI has extensive experience in developing, coordinating, facilitating, and managing expert Panels. Efforts include:

- Selecting individuals with the appropriate expertise, background, credibility, and level of commitment to serve as Panel members.
- Facilitating hands-on Panel meetings held at the project's site or location.
- Providing written report(s) prepared by the Panel that focus on findings and comments of various technical, scientific, and public health aspects of the project or study.

Over the past 5 years, NWRI has coordinated the efforts of over 20 Panels for water and wastewater utilities, city and state agencies, and consulting firms. Many of these Panels have dealt with projects or policies involving groundwater replenishment and potable (indirect and direct) reuse. Specifically, these Panels have provided peer review of a wide range of scientific and technical areas related water quality and monitoring, constituents of emerging concern, treatment technologies and operations, public health, hydrogeology, water reuse criteria and regulatory requirements, and outreach, among others.

More information about the NWRI Independent Advisory Panel Program can be found on the NWRI website at http://nwri-usa.org/Panels.htm.

APPENDIX 2A: EXPERT PANEL REVIEW OF CALIFORNIA LOG_{10} REDUCTION VALUES FOR INDIRECT POTABLE REUSE USING GROUNDWATER REPLENISHMENT

The following is a summary of the Expert Panel's review of the assumptions used to develop the log_{10} reduction value (LRV) criteria contained in **Table 2-1** of **Chapter 2** for indirect potable reuse (IPR) groundwater replenishment projects (CCR, 2015), as well as the criteria for IPR projects using surface water augmentation (SWA) being proposed by the Division of Drinking Water of the State Water Resources Control Board (NRWI, 2015a,b) add meeting).

- **Assumption 1:** An acceptable risk of infection of 10^{-4} per person per year has been used consistently in the United States to develop risk-based regulations for drinking water and in California as part of regulating non-potable reuse, and is believed to represent a *de minimis* (or insignificant) level of risk. The actual risk to the public from both endemic waterborne illnesses and non-waterborne outbreaks has been estimated to be much higher (Reynolds et al., 2008), although such estimates are highly uncertain. There is no compelling evidence to support the use of a higher or lower acceptable risk level for direct potable reuse (DPR). It is assumed that by maintaining the risk of infection from enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts below 10^{-4} per person per year, the risk of infection from bacterial pathogens derived from wastewater treated for potable reuse also is maintained below this level because bacterial pathogens are removed or inactivated more effectively than viruses and protozoan cysts by drinking water and wastewater treatment processes.

- **Assumption 2**: The dose-response relationships for enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts are still the most common in use today and form the basis for the assumptions relied upon to develop regulations in the Safe Drinking Water Act. The dose-response relationship used for enteric virus is the same used for rotavirus because rotavirus is the most conservative; however, the concentrations for exposure are based on enteric virus concentrations quantified by cell culture.

  More recent studies investigating the concentrations of enteric viruses in wastewater have used quantitative polymerase chain reaction (qPCR), rather than cell culture values, because non-culturable (and difficult to culture) viruses can be measured with qPCR. An alternative approach for estimating the virus LRV is to use a virus other than enteric virus for which more widespread occurrence data (i.e., concentrations in wastewater based on gene copies) are available, and to use a dose-response relationship that is based on gene copy concentrations. A potential candidate for this analysis is Norovirus GII. Norovirus is the leading cause of acute gastroenteritis infection across all age groups in the United States, causing an estimated 19- to 21-million illnesses each year (Hall et al., 2013). Notably, for these reasons, the Expert Panel does not believe it is appropriate at present to modify the dose-response model used for enteric virus.

  New dose-response relationships recently have been published for *Cryptosporidium* oocysts, based on seven human challenge studies that compared different functional forms for the dose-

response model (Messner and Berger, 2016). Because dosing studies have not been conducted with low oocysts doses (<10 oocysts/L), the low end of the dose-response range has not been observed experimentally. At this time, the Expert Panel does not believe it is appropriate to modify the dose-response model used by the U.S. Environmental Protection Agency (USEPA) to support the national drinking water standards.

- **Assumption 3:** The use of maximum organism concentrations measured in wastewater is intended to provide a conservative point estimate for the risk of infection, as it is assumed that this concentration is constant over the entire year. Actual pathogen concentrations in raw wastewater are highly variable, varying over many orders of magnitude. To assess whether the concentrations used by the State Water Board represent the maximum values reported to date, the Expert Panel reviewed more recent literature from peer-reviewed journals and industry reports. It was found that most reported maximum concentrations were below the values assumed by the State Water Board for all three pathogens, but there were a few higher values. For *Cryptosporidium* oocysts, there were reported values of 4.45×10^4 (Robertson et al., 2006; Norway) and 6×10^4 oocysts per liter (Cantusio Neto et al., 2006; Brazil). For *Giardia* cysts, there was one reported value of approximately 3.75×10^5 cysts per liter (Cantusio Neto et al., 2006; Brazil). No virus concentrations measured by cell culture were found that exceeded the maximum value assumed in **Table 2-1** in **Chapter 2**. Virus concentrations in raw wastewater assessed by qPCR were also reviewed, and concentrations above 10^5 gene copies per liter have been reported (see **Section 2.1.1.3**); however, it is important to note that qPCR is known to detect higher apparent (gene copy) concentrations than culture-based methods. This aspect is discussed in more detail in **Chapter 6** on pathogen analytical methods to assess microbial water quality, but a ratio of up to 1,000 gene copies to one viable infectious virus has been noted and used (e.g., He and Jiang, 2005). More research, however, is needed before virus concentrations determined by qPCR can be combined with dose-response functions to estimate the risk of infection.

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APPENDIX 3A: DESCRIPTION OF SURVEILLANCE SYSTEMS

3A.1. Syndromic Surveillance

Rather than monitor clinical diseases, syndromic surveillance systems track early signals of increased disease frequency in a population (e.g., school or work absenteeism, pharmaceutical sales, emergency room visitations) (Silk and Berkelman, 2013). The CDC (2016) defines syndromic surveillance as "…a process by which public health agencies, hospitals, medical professionals, and other organizations share, analyze, and query health and health-related data in near real-time to make information on the health of communities available to public health and other officials for situational awareness, decision making, and enhanced responses to hazardous events and disease outbreaks."

Syndromic surveillance is characterized by several features, including automated exchange of data originally created for other purposes from clinical electronic health information systems and other sources, the classification of syndromes based on these data sources, and the automation of data scans to detect anomalies and identify potential adverse health events. One example of a long-running syndromic surveillance system is the CDC's BioSense platform, which provides public health officials a common cloud-based health information system with standardized tools and procedures to rapidly collect, evaluate, share, and store information. BioSense "collects ambulatory care data, emergency room diagnostic and procedural information from military and veteran medical facilities, and clinical laboratory test orders and results from LabCorp. BioSense also monitors over-the-counter drug monitoring for 11 syndrome categories including fever, respiratory, gastrointestinal illness, hemorrhagic illness, localized cutaneous lesion, lymphadenitis, neurologic, rash, severe illness and death, specific infection, and botulismlike/botulism" (Chen et al., 2010).

In addition to the federal government, several syndromic surveillance systems have been implemented at the local, county and state levels. One of the first cities to implement this system is the New York City Department of Health and Mental Hygiene (Heffernan et al., 2004). The New York City Syndromic Surveillance System consists of Emergency Department visits, ambulance dispatch calls, retail pharmacy sales, and work absenteeism data. In regard to surveillance for gastrointestinal endpoints, the New York City Syndromic Surveillance System has produced mixed results and failed to accurately identify outbreaks of gastrointestinal illness (Balter et al., 2004). On the other hand, New York's syndromic surveillance identified an increase in diarrhea following a massive power outage in 2003, which was subsequently confirmed by a follow-up case-control study (Marx et al., 2006), and has proved important in providing early warning systems for influenza-like illness, as well as information regarding the trends, health impact, and epidemiology of influenza-associated morbidity (Mostashari et al., 2003; Olson et al., 2007).

While syndromic surveillance holds significant promise as real-time early warning system, there remain challenges with implementation and interpretation massive amounts of data from a wide range of sources with differing quality. These systems require careful interpretation and often sophisticated data processing and analyses to ascertain true disease clusters from noise. Following up on natural data variations that may appear to be outbreaks can result in unnecessary and potentially costly expenses in terms of time and labor (Chen et al., 2010). As the systems and models are being developed, their accuracy can be uncertain. For example, Google Flu Trends was another syndromic surveillance system

that used searches for influenza-like symptoms and terms to identify and track trends in influenza. While there was initial widespread enthusiasm and success, substantial inaccuracies in the model predictions were reported (Olson et al., 2013) and Google Flu Trends was subsequently discontinued, although the models and data continue to be developed and improved by researchers and also are incorporated into HealthMap, an online disease mapping tools (Health Map, 2016).

3A.2 Sentinel Surveillance

Sentinel surveillance programs track key outcomes from a subset of the population, or only track a subset of a population, and then extend the findings to the broader population. Examples of sentinel surveillance are networks of private practitioners reporting cases of influenza or a laboratory-based sentinel system reporting cases of certain bacterial infections among children (Nsubuga et al., 2006). Sentinel surveillance may be useful to identify early trends in common diseases, such as influenza or diarrheal disease, but is ineffective for localized epidemics (Garcia-Abreau et al., 2002).

3A.3 References

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APPENDIX 3B: EPIDEMIOLOGIC STUDIES

As discussed in **Section 3.1** of **Chapter 3**, analytical epidemiology studies have distinctly different goals from public health surveillance. Although public health surveillance can inform descriptive epidemiology regarding the occurrence of a disease in a population, it is not well suited to address formal research questions. Epidemiology is defined broadly as "the study of the distribution and determinants of disease frequency in human populations." Whereas surveillance approaches can provide information regarding the "distribution of disease" in the population, the "determinants of disease" cannot be well studied in the context of surveillance systems and require specific hypothesis-driven studies.

A core focus of epidemiology is causation and causal inference. Because epidemiology often (but not always) focuses on associations determined from observational studies, special care needs to be taken in inferring causality from epidemiologic associations or analyses. Some factors to distinguish causal from non-causal associations include the following:

- Strength of the association.
- Consistency of the association across other studies and different populations.
- Temporality of the association (i.e., the cause or exposure must precede the effect or outcome).
- Dose-response.
- Biological plausibility (Hennekens et al., 1987).

With the exception of temporality, which is a necessary condition for any causal relationship, the factors listed above should be considered guidelines and not criteria, as causal relationships could be present with or without some (or even all) of these factors. Some examples of epidemiological studies focused on waterborne disease are shown in **Table 3B-1.**

3B.1 Epidemiology Study Designs

Epidemiology studies may be experimental or observational. Experimental studies are studies in which subjects are assigned a certain treatment by the investigator. Random assignment to a treatment group is standard to eliminate or reduce bias. Experimental studies include clinical trials where patients with certain disease or conditions are subjects. Field trials or intervention trials are those in which subjects are not patients with disease and are focused on disease prevention. Community intervention trials focus on the application of an intervention or treatment at the community level or on a community-wide basis (e.g., the application of water fluoridation) (Rothman et al., 2008). In contrast, observational studies are those where the researcher does not assign subjects to a treatment group, but categorizes the subjects to various treatments groups or exposure categories based on observed choices or behavior.

3B.1.1 Experimental Designs

Experimental, randomized designs often are considered the gold standard of epidemiological and clinical research because many biases related to self-selection and other confounding factors (i.e., factors

Table 3B-1: Examples of Epidemiology Studies of Waterborne Disease

Lead Author	Design	Exposure/Treatment	Outcome	Location
Colford et al. (2005)	RCT	1-micron filtration plus UV treatment	AGI	Davenport, Iowa, USA
Hellard et al., 2001)	RCT	1-micron filtration plus UV treatment	AGI	Australia
Chen et al. (1985)	Ecologic	Well-water arsenic and Blackfoot disease	Bladder, skin and lung cancer	Taiwan
Smith et al., (1998)	Ecologic	Arsenic in drinking water	Cancer mortality	Chile
Cantor et al. (1987)	Case-control	Drinking water source	Bladder cancer	United States
Nygard et al. (2007)	Cohort	Water main breaks and repairs	AGI	Norway
Schwartz et al. (1997)	Time-series	Drinking water turbidity	Pediatric hospitalizations for AGI	Philadelphia, Pennsylvania, USA

Notes: RCT = Randomized Controlled Trial. UV = Ultraviolet disinfection. AGI = Acute gastrointestinal illness.

related to the exposure and outcome of interest that are unequally distributed across treatment groups) are reduced or eliminated through the randomization process. Other important aspects of experimental design include the "blinding" of subjects to treatment groups, which can sometimes be accomplished through the use of placebos or an alternate treatment not under study. In many cases, however, experimental studies are unfeasible and/or unethical in human populations. The ability to conduct experimental studies on potentially vulnerable groups, like children, pregnant women, and immunocompromised persons, is restricted further due to ethical and safety concerns.

Some examples of experimental studies used in waterborne disease research include randomized trials of in-home drinking water treatments to reduce acute gastrointestinal illness (AGI). These studies were designed to evaluate the fraction of AGI attributable to drinking water and involved the installation of additional water treatment (e.g., reverse osmosis, 1-micron filtration, ultraviolet treatment) in the homes of members of one group, and either no treatment or an identical "placebo" treatment device in the homes of members of the second group (Colford et al., 2002, 2005, 2009; Hellard et al., 2001; Payment et al., 1991). A community intervention study was conducted by Lambertini et al. (2012) where ultraviolet disinfection was installed on the wellheads of selected non-disinfected community groundwater sources for 1 year (Lambertini et al., 2012). Kay et al. (1994) randomized subjects to swim at beach sites that met water quality criteria, but were impacted by fecal contamination, to study the

association between swimming exposures and the risk of AGI (Fleisher et al., 1993; Kay et al., 1994). Experimental designs are widely applied to study the comparative effectiveness, in terms of improving health and water quality, of household water treatments (e.g., boiling, solar disinfection, chlorination) in resource-limited settings without adequate municipal drinking water treatment (Clasen, 2015).

3B.1.2 Observational Designs

Health effects associated with many exposures cannot be studied by experimental design because of ethical concerns; therefore, an observational approach is required. Observational studies may be prospective, retrospective, or cross-sectional in design; these terms refer to the ordering of exposure information and the occurrence of disease.

- ***Prospective studies*** record exposure information prior to the occurrence of disease.
- ***Retrospective studies*** record exposure information after the occurrence of disease.
- ***Cross-sectional studies*** record exposure and outcome information at the same time.

The general types of observational epidemiology studies, which include ecologic studies, case-control studies, cohort studies, and time-series studies, are described as follows.

- ***Ecological studies*** use aggregate measures of health and/or exposures, such as county-level mortality or morbidity rates and county rates of smoking and obesity. Because these studies usually have no individual data and rely on aggregate or group measures, they require careful design and interpretation. "Ecological fallacy" refers to the failure of the group-level associations to reflect true individual level associations. This type of design often is used for hypothesis generation because it is relatively inexpensive and simple to conduct; however, under certain circumstances and assumptions, it can provide valuable insights into the relationships between causal mechanisms of exposure to disease. For example, ecological studies in Taiwan (Chen et al., 1985) and Chile (Smith et al., 1998) provided evidence linking arsenic in well water with lung, bladder, and skin cancer. These studies were successful for several reasons, including excellent and complete records on births and deaths, a population that was demographically and culturally similar, and comparison groups whose only differences were in regard to the levels of arsenic in the well water. In addition, most of the population relied solely on this well water as the primary source of drinking water. Semi- or partial ecological studies can improve on the ecological design by using individual-level data for either the exposure or outcome of interest.

- ***Case-control studies*** compare exposure histories and other characteristics between a group of subjects with a health-endpoint of interest (i.e., "case") and a group of those without the health endpoint of interest (i.e., "controls"). There are many variants and study design considerations of the case-control studies that will not be addressed here. For further reference, see a series of articles by Wacholder et al. (1992a,b.c) that detail theories, assumptions, and variations on this design. Case-control studies are most useful for rare health outcomes, which would likely not be detected in sufficient numbers in a prospective study. With regard to waterborne disease, case-control studies have been used to investigate the association between bladder cancer and chlorinated drinking water sources (Cantor et al., 1987), as well as arsenic (Bates et al., 1995). One major challenge associated with case-control studies is the accurate assessment and

classification of exposure, especially for diseases with a long onset or latency period like cancer. Case-control studies are used widely for outbreak investigations (e.g., foodborne or waterborne outbreaks of an infectious agent) because these studies usually involve an acute health effect in a well-defined population and a short onset to symptoms.

- ***Cohort studies*** follow up on a group of subjects with and without an exposure of interest for a period of time and compare the occurrence of newly developed health outcomes across the exposure groups. Cohort studies are most useful for relatively frequent health endpoints and, unlike retrospective studies, ensure the exposure preceded the development of disease. Cohort studies also can be retrospective in nature when accurate data and records are kept to historically reconstruct a cohort (e.g., occupational settings). Nygard et al. (2007) conducted a cohort study in Norway to investigate the potential health effects associated with drinking water contamination due to water main breaks, repairs, and low-pressure events. They observed an association with these events and the risk of AGI. Prospectively followed cohort studies often are expensive and may be impractical for rare health outcomes like cancer, which could take 20 years or more to develop following exposure. In the case of waterborne infections, cohort studies often use laboratory confirmed, serological, or other clinical tests to acquire evidence of specific infections. Frost et al. (2005) used a cohort design to compare serological evidence of infection to *Cryptosporidium* before and after the introduction of a new water filtration plant.

- ***Time series studies*** incorporate aspects of both ecological studies and cohort studies. In a time series study, a regularly measured exposure series (e.g., daily turbidity, air pollution) is correlated with some health endpoint (e.g., AGI, asthma) within a certain community. Exposures and health outcomes usually are aggregated or ecological measures, such as daily concentrations of airborne particulate matter and cases admitted to the emergency room for asthma. These studies generally are considered stronger and more rigorous than ecological studies. If the community studied is assumed to have remained relatively constant in terms of demographics and other trends, then controlling for many individual characteristics becomes unnecessary. Careful considerations must be made, however, to account for seasonal variations and time trends. In addition, these studies only are possible for acute health effects and transient (i.e., time varying) exposures. The appropriate "lag" between exposure and effect also must be selected and justified in interpreting the results. Schwartz et al. (1997) used time-series analyses to study the association between drinking water turbidity and hospitalizations for AGIs. There are numerous variations of time series studies, including the case-crossover design, which uses similar datasets, but different assumptions and analytical approaches. Case-crossover designs commonly are used in studies of the health effects of air pollution, but also have been used to study the association between flooding and AGI (Wade et al., 2014), heavy precipitation and waterborne outbreaks (Nichols et al., 2009), and weather patterns associated with Legionellosis (Fisman et al., 2005).

3B.2 Planning and Interpretation of Epidemiological Studies

A well-designed analytical epidemiology study should have a clearly stated research question and have considered the appropriate statistical models, sample size, exposures, and outcomes to be studied prior to collecting data and conducting data analysis. Careful consideration must be taken to control for confounding factors, which are factors associated with the exposure and outcome and may bias any association between the exposure and effect. Randomized studies account for confounding by equally distributing (through random allocation) confounding factors to treatment groups in the study design.

Observational studies account for confounding either through design considerations (such as matching in case-control studies) and/or multivariable regression modeling or causal inference techniques. Particularly with observational studies, it is not always possible to control for all known and unknown confounding factor; therefore, some bias may remain in the estimates, known as "residual confounding." Analytical epidemiology studies should be formulated using a causal model that specifies formally the expected association between the exposure and outcome and also postulates relationships between potentially confounding factors. In addition, prior to conducting the study, potential subgroups or stratified analyses should be considered. For example, if there is theoretical justification for differences in the exposure (e.g., outcome association by age, sex, race, or other factors), these factors should be considered prior to data collection so that a large enough sample is collected. Directed acyclic graphs are tools used to guide epidemiology study planning and should be used to guide model design and confounder selection (Greenland et al., 1998).

Guidelines and checklists are available for interpreting and reporting epidemiology studies. A checklist (included in **Appendix 3C)** on "Strengthening the reporting of observational epidemiology studies" (STROBE) provides a thorough review of important items to consider.

3B.3 Sources of Bias and Error

There are two general types of error or bias in epidemiology studies: systematic and random. Random errors result in misclassification in a way that is not dependent on the exposure and/or outcome of interest. In nearly all cases, random misclassification reduces the study power and biases the effect toward "the null," meaning it increases the likelihood that no association will be observed. Examples of random errors in a household drinking water study would include the use of water outside the home or underreporting gastrointestinal symptoms. Most other types of measurement errors – due to instrument error, calibration errors, and the like – are random errors. Random errors impact the ***precision*** of the result, but not usually the ***validity***. In other words, random errors normally will not result in a "false positive" (a spurious association), but rather would bias the study toward a "false-negative" result. Systematic errors are those that impact the validity of the result and cause non-random bias. A confounding factor not adequately controlled for in the design or analysis can be a source of bias. One example of a potentially confounding factor in a cohort study of health outcomes associated with in-home water treatment would be diet, because those individuals who treat their water at home are likely to have a different diet than those who do not, and diet is likely to influence health outcomes. Other types of systematic error include systematic differences in recall by cases and controls in a case-control study (recall bias). For example, a study of a *Cryptosporidium* outbreak among HIV-infected residents in Clark-County, Nevada, may have been impacted by recall bias. Media reports of the outbreak may have influenced cases with *Cryptosporidium* infection to be more likely to report tap water consumption compared to uninfected controls (Craun et al., 2003).

3B.4 Study Power and Sample Size

Epidemiological studies should have adequate sample size to ensure that if there is a true association in the population, it can be observed in the sample studied. Statistical power is defined formally as the probability that a null hypothesis is correctly rejected given that the alternative hypothesis is true, providing protection against false negative findings. Adequate study power usually is considered to be between 70 and 80 percent. Power depends on several factors, including the hypothesized strength of the association, sample size, prevalence of exposure, and frequency of the effect. Very small hypothesized effect sizes will require large samples to observe any association. Inadequate sample size

also can impact the ability to adequately control for other factors in regression models and to conduct subgroup or stratified analyses for subgroups of interest (e.g., by age, sex, or race). Most epidemiology studies should report confidence bounds (e.g., 95-percent confidence intervals) on the effect estimates. If these bounds are very wide, it is an indicator of low precision and, possibly, inadequate sample size.

In contrast with surveillance (where sometimes broad case-definitions may be used to capture population trends), broad, non-specific case-definitions can adversely impact the study power of epidemiology studies. For nearly all diseases and health endpoints, the vast majority of the population is free of the disease under study. As a result, epidemiology studies that use broad case-definitions will incorrectly classify some disease-free people (who are the majority) as disease positive. This error may result in a high overall random misclassification rate, and a reduction in study power to observe an association. In contrast, a specific case definition that minimizes "false-negative" rates and errs on the side of correctly classifying true cases will be preferred to maximize the study power and the ability to observe associations with the exposures under study.

3B.5 Representativeness and Generalizability

Epidemiology studies, especially clinical or randomized trials in a group of subjects, often are done in a population that may not be representative of the broader population base. For example, it may not be valid to extend observations from a group of healthy adults to children or immunocompromised groups.

3B.6 Multiple Comparisons

As statistical software has become increasingly powerful to conduct multiple and nearly unlimited analyses and "big" data has become increasingly available, the ability to conduct numerous analyses on a single set of data has increased considerably. While this approach may be used for hypothesis generation or exploratory analyses, it also has led to the reporting of many results for a single study and increased the possibility of so called "data dredging" (or, "cherry-picking" results so some association is observed, usually to find a statistically significant association). To protect against data dredging, many epidemiology texts advise against rigorous adherence to statistical significance testing and instead encourage reporting only the estimate and confidence bound. Also, if many associations are reported, they should follow logical and coherent trends, and a single "significant" estimate should not be highlighted. Other approaches to dealing with multiple comparisons include reducing the overall alpha or significance level to protect against Type II or "false positive" errors.

3B.7 Sensitivity Analysis

Sensitivity analyses involve assessing the robustness of the results in comparison to assumptions that were made regarding exposure classification, outcome classification, missing data, and data analysis to evaluate whether these assumptions impact the results. Some approaches are complicated, formal, and quantitative, but simpler approaches involve recalculating estimates of "main effects" under different assumptions to see if they impact the results appreciably. If strongly impacted, further justification or examination of the results may be necessary.

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APPENDIX 3C: STROBE CHECKLIST OF ITEMS TO INCLUDE IN REPORTS OF OBSERVATIONAL STUDIES

Table 3C-1: STROBE[a] Statement: Checklist of Items that Should Be Included in Reports of Observational Studies

Item	Item No.	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract.
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found.
Introduction		
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported.
Objectives	3	State specific objectives, including any pre-specified hypotheses.
Methods		
Study design	4	Present key elements of study design early in the paper.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection.
Participants	6	(a) **Cohort study**—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **Case-control study**—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. **Cross-sectional study**—Give the eligibility criteria, and the sources and methods of the selection of participants.
		(b) **Cohort study**—For matched studies, give matching criteria and number of exposed and unexposed. **Case-control study**—For matched studies, give matching criteria and the number of controls per case.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.
Data sources/ measurement	8[b]	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe the comparability of assessment methods if there is more than one group.
Bias	9	Describe any efforts to address potential sources of bias.
Study size	10	Explain how the study size was arrived at.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.

Item	Item No.	Recommendation
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding.
		(b) Describe any methods used to examine subgroups and interactions.
		(c) Explain how missing data were addressed.
		(d) **Cohort study**—If applicable, explain how loss to follow-up was addressed. **Case-control study**—If applicable, explain how matching of cases and controls was addressed. **Cross-sectional study**—If applicable, describe analytical methods taking account of sampling strategy.
		(e) Describe any sensitivity analyses
Results		
Participants	13[b]	Report numbers of individuals at each stage of study (e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed).
		Give reasons for non-participation at each stage.
		Consider the use of a flow diagram.
Descriptive data	14[b]	Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders.
		Indicate number of participants with missing data for each variable of interest.
		Cohort study—Summarize follow-up time (e.g., average and total amount).
Outcome data	15[b]	**Cohort study**—Report numbers of outcome events or summary measures over time.
		Case-control study—Report numbers in each exposure category, or summary measures of exposure.
		Cross-sectional study—Report numbers of outcome events or summary measures.
Main results	16	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95-percent confidence interval). Make clear which confounders were adjusted for and why they were included.
		Report category boundaries when continuous variables were categorized.
		If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.
Other analyses	17	Report other analyses done (e.g., analyses of subgroups and interactions, and sensitivity analyses).
Discussion		
Key results	18	Summarize key results with reference to study objectives.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

Item	Item No.	Recommendation
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.
Generalizability	21	Discuss the generalizability (external validity) of the study results.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.

[a] STROBE = STrengthening the Reporting of OBservational studies in Epidemiology.

[b] Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is used best in conjunction with this article (freely available on the Websites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

APPENDIX 3D: CUSTOMER COMPLAINT SURVEILLANCE PRIMER FOR WATER QUALITY SURVEILLANCE AND RESPONSE SYSTEMS

The following text is taken from the "Customer Complaint Surveillance Primer for Water Quality Surveillance and Response Systems: Customer Complaint Surveillance Primer" prepared May 2015 by the United States Environmental Protection Agency, Office of Water (MC 140) under publication number EPA 817-B-15-002C.

INTRODUCTION

A Water Quality Surveillance and Response System (SRS) provides a systematic framework for enhancing distribution system monitoring activities to detect emerging water quality issues and respond before they become problems. An SRS consists of six components grouped into two operational phases, surveillance and response. The surveillance components are designed to provide timely detection of water quality incidents in drinking water distribution systems and include: Online Water Quality Monitoring, Enhanced Security Monitoring, Customer Complaint Surveillance and Public Health Surveillance. The response components include Consequence Management and Sampling & Analysis, which support timely response actions that minimize the consequences of a contamination incident. The *Water Quality Surveillance and Response System Primer* provides a brief overview of the entire system (USEPA, 2015).

This document provides an overview of the Customer Complaint Surveillance (CCS) component of an SRS. It presents basic information about the goals and objectives of CCS in the context of an SRS. This primer covers the following four topics:

Topic 1: What is CCS?

Topic 2: What are the major design elements of CCS?

Topic 3: What are common design goals and performance objectives for CCS?

Topic 4: What are cost-effective approaches for CCS?

TOPIC 1: WHAT IS CCS?

CCS consists of information management systems, processes and procedures that collectively compile, track and analyze water quality-related customer complaints indicative of a water quality incident.

Figure 1 illustrates the *Funnel/Filter/Focus* surveillance approach of CCS. First, all complaints are *funneled* into one location, such as a call management system, to ensure that complaints are not missed. Next, water quality complaints are *filtered* out from non-water quality complaints by Customer Service Representatives (CSR) or other water utility staff. Finally, water quality specialists *focus* on the remaining complaints to assess whether the complaints are related to a water quality incident or to system operations, such as main breaks or maintenance. CCS can track the time, number and location

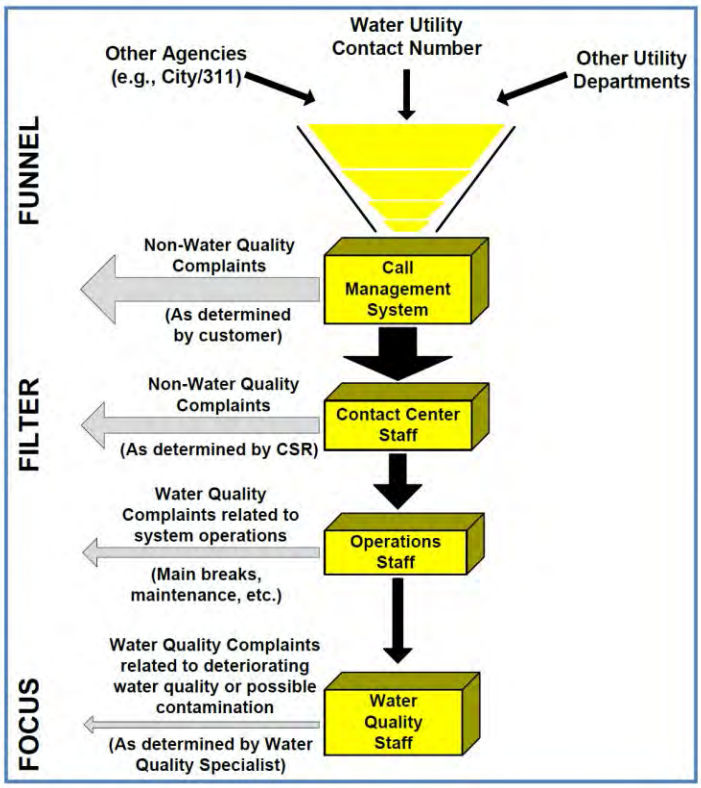


Figure 1: Recommended Funnel/Filter/Focus Approach for Utility-managed Customer Calls.

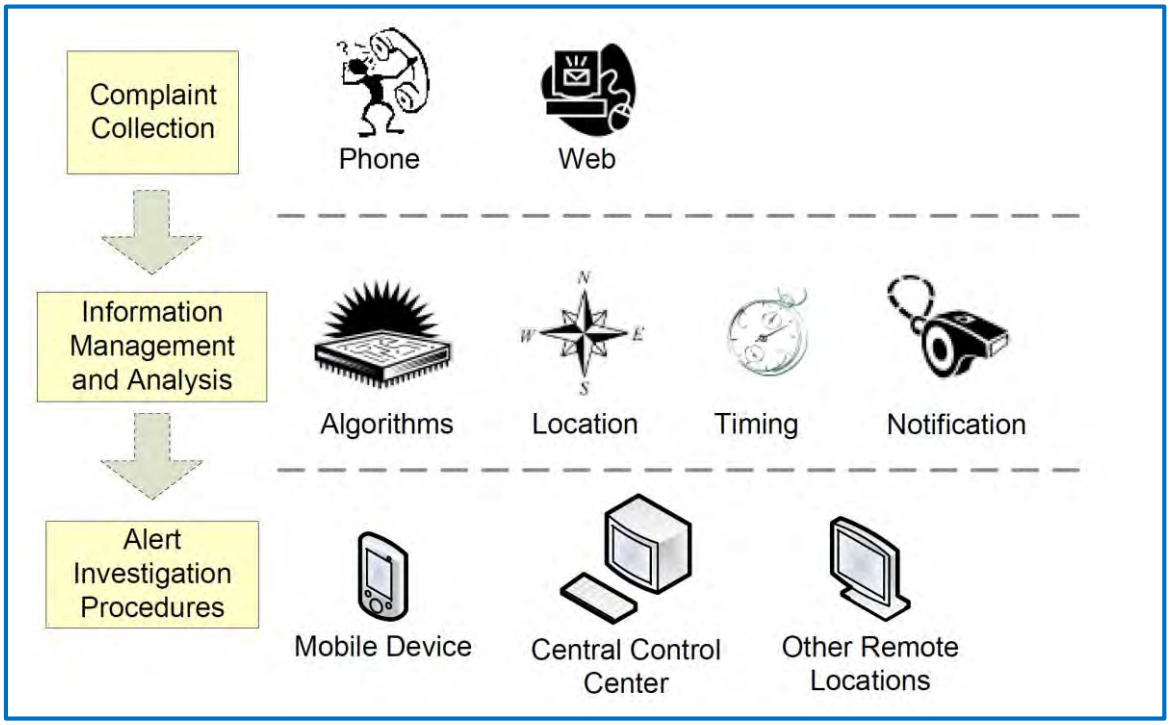


Figure 2: CCS Design Elements.

of complaints that are entered into call or work management systems, and alert utility personnel of unusually high call volumes or spatially-clustered complaints.

DID YOU KNOW?

Many utilities with CCS utilize a 311 system for complaint collection. This allows them to build on an existing system, with only minor procedural changes needed to implement the design element.

TOPIC 2: WHAT ARE THE MAJOR DESIGN ELEMENTS OF CCS?

The major design elements for CCS are shown in **Figure 2** and described under the remainder of this topic.

Complaint Collection

A variety of methods are available to funnel all customer calls to one point of contact. For example, a unified call center with a widely publicized telephone number helps to ensure that the majority of complaints are captured.

Additionally, procedures should be put in place for water quality- related complaints that are initially received by external agencies, such as a city-wide call center or a 311 system. These procedures funnel calls to the CCS centralized complaint management system, ensuring robust surveillance by accounting for all calls.

Complaint collection has two design sub-elements:

- **Communicating Water Quality Concerns**: Activities implemented to ensure that customers are aware of how to report their water quality concerns to the utility.
- **Consolidating Water Quality Complaints**: Systems or procedures that filter water quality complaints to a central location, facilitating timely and efficient data analysis.

Information Management and Analysis

A key requirement of CCS is the ability to systematically track water quality complaints from receipt to closure. Existing customer complaint processes used by a utility can typically be leveraged to develop a CCS information management and analysis system. One of the most important decisions when implementing CCS is determining which datastreams should be incorporated. Most of the CCS tracking mechanisms work behind the scenes, limiting interference with day-to-day operations. Complaints are continuously analyzed for information indicative of a water quality incident in the distribution system, such as an unusually high numbers of calls or clustering of complaint locations. CCS information management and analysis has six design sub-elements:

- **Complaint Descriptive Data and Categories**: Capture and categorize complaint descriptions for the purpose of data analysis and alert investigations.
- **Detecting Abnormally High Complaint Volumes**: Develop processes to identify complaint volumes attributable to a significant change in water quality.
- **Timeliness of Detection**: Ensure data is reviewed with sufficient regularity to identify potential water quality incidents as they develop.
- **Establishing Thresholds**: Develop alerting thresholds that are insensitive to normal variation in call volume, yet low enough to detect potential water quality incidents.

- **Spatial Clustering Analysis**: Use spatial analysis to determine whether an unusually high volume of calls is clustered, and to determine the area of the distribution system impacted by a possible water quality incident.
- **Alert Notifications**: Develop reliable processes for informing utility personnel when alerting thresholds are exceeded.

DID YOU KNOW?

Many utilities with CCS modified their existing data management systems by establishing water quality-related complaint categories and then tracking the resolution of these complaints through their customer service process.

CCS can take advantage of existing information management systems used in a typical call management process by filtering water quality-related complaints. Utilities without formal call management systems or software may still reap the benefits of CCS by: 1) streamlining the manner in which water quality- related complaints are managed, and by 2) instituting frequent checks of the number of water quality-related calls received over time.

Water quality-related customer complaint data that is collected should be analyzed in a timely manner for conditions indicative of a water quality incident in the distribution system. This involves identifying when the total number of water quality-related complaints is unusual compared to an established baseline. The anomaly detection process can be automated using simple counting algorithms, which automatically track the number of calls over a defined period of time. When the number of calls exceeds a pre- determined threshold value, an alert is generated and utility personnel notified.

DID YOU KNOW?

USEPA's SRS Program has published a *Threshold Analysis Tool* that performs statistical analysis of complaint data to guide the development of threshold values.

If spatial data is available, the frequency of complaints within hydraulically related areas, such as pressure zones or service areas, can also be evaluated. In addition, mapping the location of complaints can highlight clustering, which focuses investigation and response actions.

An example of a detection timeline is illustrated in **Figure 3**. The delay times for detection shown in the middle row provide a sense of how quickly CCS data is available. For this example, data is extracted from existing call and work management data systems and analyzed in near real time (every 15 minutes or less) using a simple counting algorithm. Upon generation of an alert, notifications are sent to investigators using an existing email server.

Alert Investigation Procedures

CCS alerts need to be promptly investigated by utility personnel to determine whether the alerts can be explained by known factors, such as distribution system work near the area of the complaint locations. Example steps performed during CCS alert investigations are described below.

1. A CCS investigation begins following receipt of an alert, signifying an anomaly in one or more CCS datastreams.
2. Utility personnel use a CCS alert investigation checklist to guide them through a predetermined procedure to determine if the complaints are related to a water quality incident in the distribution system.

3. If it is determined the alert is not related to a water quality incident in the distribution system, the investigation is closed and logged.
4. If a water quality incident cannot be ruled out, the investigation continues according to procedures in the drinking water utility's Consequence Management Plan.

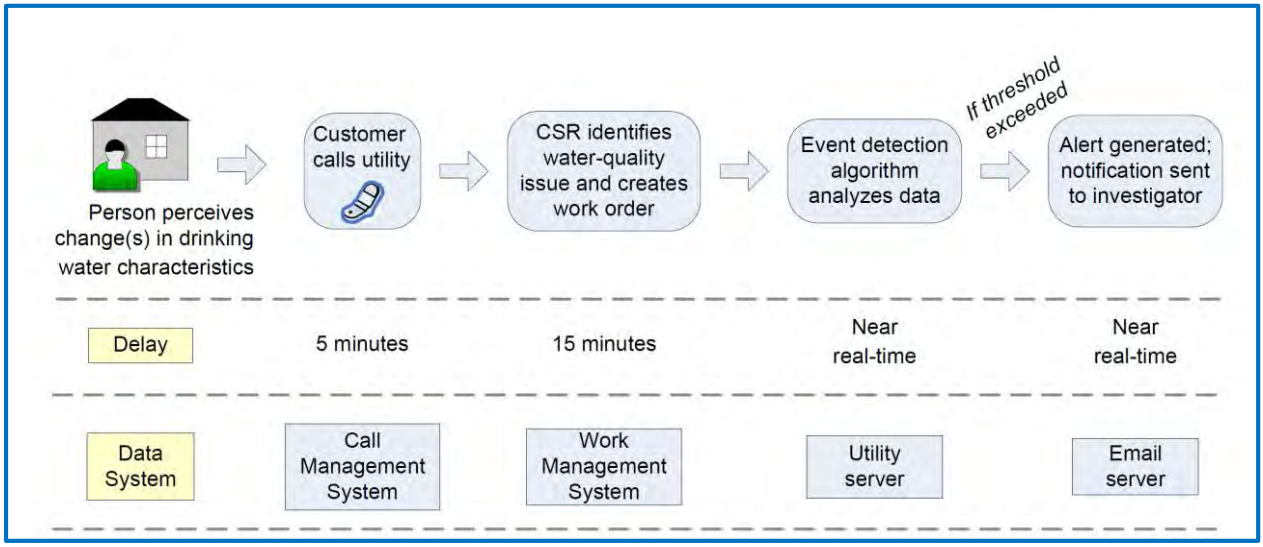


Figure 3: Example of a CCS Detection Timeline.

TOPIC 3: WHAT ARE COMMON DESIGN GOALS AND PERFORMANCE OBJECTIVES FOR CCS?

The design goals and performance objectives established for CCS by the utility provide the basis for the design of an effective component.

CCS Design Goals

Design goals are the specific benefits that utilities expect to achieve by implementing CCS. A fundamental design goal of an SRS is the ability to detect and respond to water quality anomalies in the distribution system. In addition to this fundamental SRS design goal, other CCS-specific design goals such as improving the level of customer service can be realized. Examples of common CCS design goals are listed **in Table 1**.

CCS Performance Objectives

Performance objectives are measurable indicators of how well the SRS meets the design goals established by the utility. Throughout design, implementation and operation of the SRS or its components, the utility can use performance objectives to evaluate the added value of each capability, procedure or partnership. While specific performance objectives should be developed by each utility in the context of its unique design goals, general performance objectives for an SRS were defined in the *Water Quality Surveillance and Response System Primer* (USEPA, 2015) and are further described in the context of CCS as follows.

Table 1: Examples of Common CCS Design Goals

Design Goal	Description
Detect water contamination	CCS provides an early indicator of water contamination which may impact the health of customers or utility infrastructure.
Monitor the impact of system operations on customers	Some utility operations, such as changing sources and chlorine feed levels, can impact the aesthetics of the drinking water. CCS can alert the utility if these changes are noticed by customers.
Increase the level of customer service	CCS can alert the utility to distribution issues, such as main breaks, through customer complaints. This can reduce utility response time while providing the latest information to CSRs receiving customer complaints.
Improve the response to water quality complaints	Developing CCS procedures can streamline and standardize a utility's decision-making process when investigating customer water quality complaints.

- **Incident coverage**: Detect and respond to a broad spectrum of water quality incidents. CCS is limited to detection of contaminants which alter the taste, odor or appearance of drinking water. Within this subset of contaminants, CCS can detect incidents regardless of the source.
- **Spatial coverage**: Achieve spatial coverage of the entire distribution system. Theoretically, CCS has the ability to cover every customer in the distribution system. Spatial coverage is improved by educating customers about how to contact the utility.
- **Timeliness of detection**: Detect water quality incidents in sufficient time for effective response. This performance objective is dependent upon how quickly data is available for analysis and how often the analysis is performed.
- **Operational reliability**: Minimize downtime for equipment, personnel and other support functions necessary for the component to meet the other performance objectives. Operational reliability for CCS is achieved by ensuring that information management and analysis systems continue to operate.
- **Alert occurrence**: Minimize the number of invalid alerts, which are not caused by abnormal water quality, while maintaining the ability of the system to detect true water quality anomalies. The balance between reducing the rate of invalid alerts while maintaining detection capabilities is primarily a function of the quality of the data monitored by the system and the data analysis method(s) used.
- **Sustainability**: Provide value to day-to-day utility operations and distribution system management that exceeds the cost to deploy and operate the component. Because CCS involves little to no physical equipment, it is relatively easy to sustain.

TOPIC 4: WHAT ARE COST-EFFECTIVE APPROACHES FOR CCS?

Utilities can take the following simple steps to develop the foundation for CCS:

- Review historical customer complaint data, and estimate a threshold for the number of calls or work orders that could be indicative of a water quality incident.

- Evaluate the daily volume of water quality complaints relative to the threshold, and manually plot the locations of calls on a map with 'push-pins' to identify clusters.
- Establish procedures for investigating water quality complaint clusters and train staff on their execution.

NEXT STEPS

Visit the Water Quality Surveillance and Response Website at http://water.epa.gov/infrastructure/watersecurity/lawsregs/initiative.cfm for more information about SRS practices. The Website contains guidance and tools that will help a utility to enhance surveillance and response capabilities, as well as case studies that share utility experiences with SRS implementation and operation.

REFERENCES

USEPA (2015). *Water Quality Surveillance and Response System Primer*, 817-B-15-002.

APPENDIX 3E: PUBLIC HEALTH SURVEILLANCE PRIMER FOR WATER QUALITY SURVEILLANCE AND RESPONSE SYSTEMS

The following text is taken from the "Public Health Surveillance Primer for Water Quality Surveillance and Response Systems" prepared May 2015 by the United States Environmental Protection Agency, Office of Water (MC 140) under publication number EPA 817-B-15-002D.

INTRODUCTION

A Water Quality Surveillance and Response System (SRS) provides a systematic framework for enhancing distribution system monitoring activities to detect emerging water quality issues and respond before they become problems. An SRS consists of six components grouped into two operational phases, surveillance and response. The surveillance components are designed to provide timely detection of water quality incidents in drinking water distribution systems and include: Online Water Quality Monitoring, Enhanced Security Monitoring, Customer Complaint Surveillance and Public Health Surveillance. The response components include Consequence Management and Sampling & Analysis, which support timely response actions that minimize the consequences of a contamination incident. *The Water Quality Surveillance and Response System Primer* provides a brief overview of the entire system (USEPA, 2015a).

This document provides an overview of the Public Health Surveillance (PHS) component of an SRS. It presents basic information about the goals and objectives of PHS in the context of an SRS. This primer covers the following four topics:

Topic 1: What is PHS?

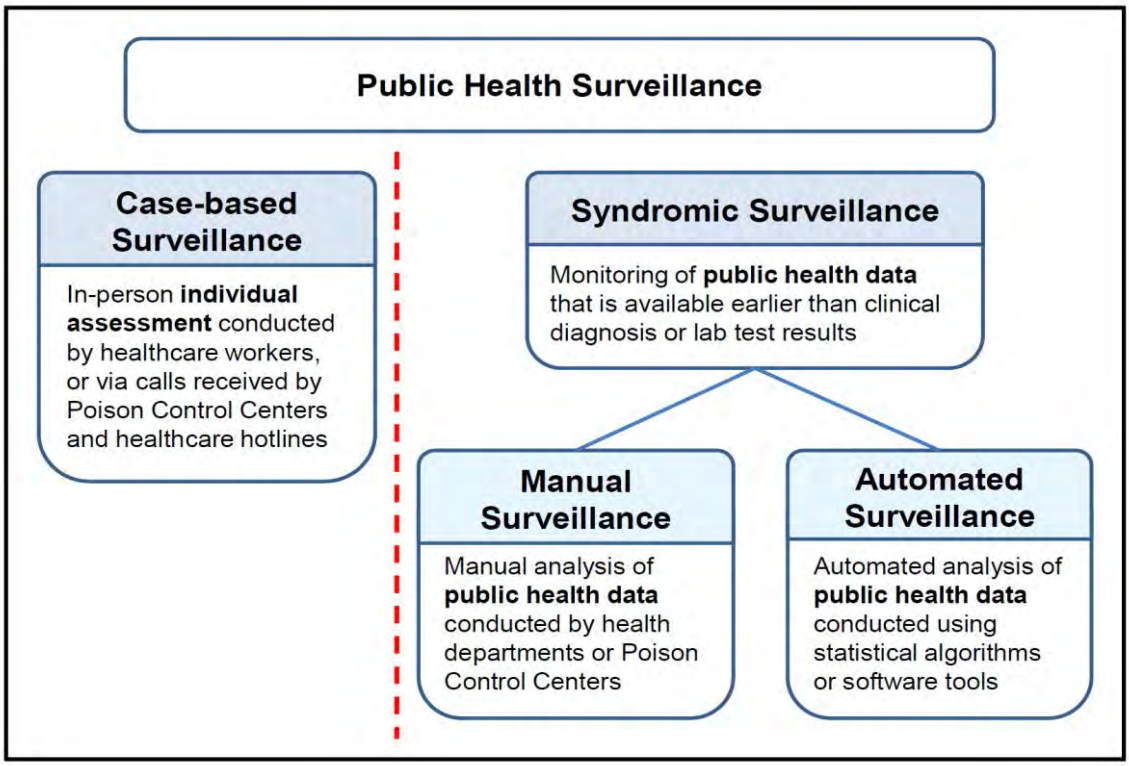
Topic 2: What are the major design elements of PHS?

Topic 3: What are common design goals and performance objectives for PHS?

Topic 4: What are cost-effective approaches for PHS?

TOPIC 1: WHAT IS PHS?

PHS is the ongoing, systematic collection, analysis and interpretation of public health data. The main goal of PHS is to detect changes in the health status of a community in sufficient time to allow for intervention to mitigate the consequences of an emerging threat to public health. **Figure 1** shows two broad types of PHS, case-based and syndromic surveillance. ***Case-based surveillance*** involves an assessment of public health status based on in-person observation of individual patients. ***Syndromic surveillance*** involves the monitoring of aggregated public health data. Each type of surveillance provides community-level health information. Public health partners can increase the potential to detect a change in the health status of a community by conducting both case-based and syndromic surveillance.



PHS is unique in that it is generally monitored by public health partners, whereas other SRS components are monitored by water utility personnel. Communication between water utilities and public health partners has often been insufficient to provide timely detection and response to waterborne disease outbreaks. Incorporating PHS into an SRS helps ensure that data acquisition, analysis and information sharing is coordinated between the drinking water utility and public health partners, resulting in earlier detection of possible drinking water contamination incidents.

TOPIC 2: WHAT ARE THE MAJOR DESIGN ELEMENTS OF PHS?

The major design elements for PHS are shown in **Figure 2** and described under the remainder of this topic.

Integration of Public Health Surveillance Capabilities

A well designed PHS component is able to provide both timely and detailed information to investigators. Potential public health datastreams include:

- 911 calls
- Emergency medical services runs
- Poison control center calls
- National Poison Data System
- Emergency department data

- Over-the-counter medication sales
- Direct observation by healthcare professionals

For purposes of an SRS, these datastreams are analyzed in order to detect patterns that may signal a public health incident that could be related to drinking water contamination. Many of these datastreams include geographic identifiers that can be used to investigate spatial clustering of cases. Factors to consider when deciding which datastreams to leverage for PHS may include:

- Availability of datastreams
- Frequency of data analysis
- Level of confidence in the datastream as an indicator of illness or disease in the population
- Types of contamination incidents that could be identified through this datastream
- Availability of methods to automate data collection and analysis
- Availability of underlying case data, such as patient information such as symptoms, age and location of the exposure, during alert investigations

Ideally, PHS should include surveillance of datastreams that would provide detection capability for contaminants with both rapid and delayed symptom onset, thus covering a wide range of potential contaminants.

Figure 2. PHS Design Elements.

PHS Communication and Coordination

Communication and coordination involves identifying relevant public health partners, engaging them during planning activities, and working with them during the investigation of PHS alerts in a manner that facilitates efficient data sharing and consensus building. An SRS can only be successful if the water utility and public health partners communicate and share information. Public health experts provide information that might not otherwise be available to utilities and which is useful for investigating possible water contamination incidents.

PHS Alert Investigation Procedures

PHS alert investigation procedures describe how public health partners use PHS datastreams and other resources to investigate PHS alerts and determine whether or not they are caused by possible drinking water contamination. In general, PHS alert investigations are conducted jointly by the utility and public health partners. **Figure 3** illustrates how an investigation into a possible water contamination incident can begin with either a PHS alert or a utility alert.

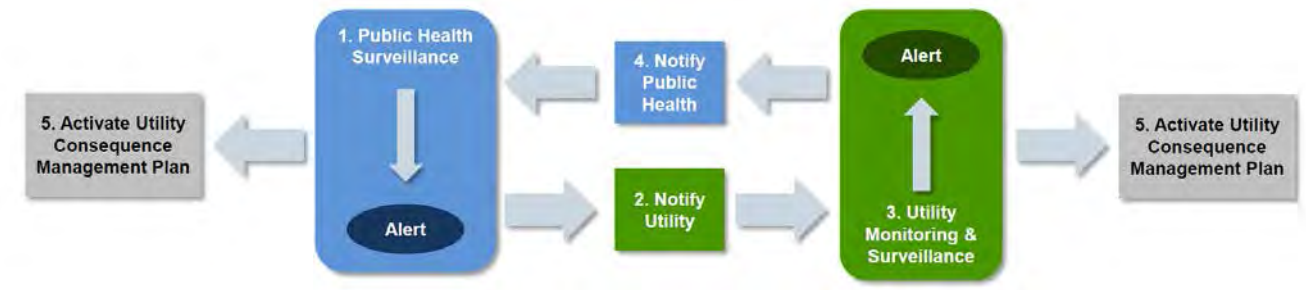


Figure 3. Example of a PHS Alert Investigation Process.

In the example shown in **Figure 3**, the investigation is assumed to begin with the generation of a PHS alert, and illustrates the communication pathways between the utility and public health agencies during the investigation. The numbered steps in this figure are briefly described below:

1. The investigation begins following receipt of a PHS alert generated through one of the surveillance methods. Public health personnel review underlying case data related to the alert in order to determine whether the alert is valid.
2. If public health partners determine that the PHS alert is valid, and if investigators cannot rule out possible water contamination as the cause of the alert, the public health investigator notifies the water utility.
3. Once notified of the PHS alert, the utility reviews data from other SRS components, such as customer complaint surveillance, along with other utility information that can help investigators determine whether or not the PHS alert is related to drinking water quality.
4. The utility shares the results of their investigation with public health partners. If information provided by the utility is sufficient to rule out drinking water contamination, the investigation is closed.

5. If contamination cannot be ruled out following the utility investigation, water contamination is considered possible and the consequence management plan is activated.

TOPIC 3: WHAT ARE COMMON DESIGN GOALS AND PERFORMANCE OBJECTIVES FOR PHS?

The design goals and performance objectives established for PHS by the utility and its public health partners provide the basis for the design of an effective component.

PHS Design Goals

Design goals are the specific benefits that utilities expect to achieve by implementing PHS. A fundamental design goal of an SRS is the ability to detect and respond to water quality anomalies in the distribution system. In addition to this fundamental SRS design goal, other PHS-specific design goals such as improved coordination between the utility and public health partners can be realized. Examples of common PHS design goals are listed in **Table 1**.

Table 1. Examples of Common PHS Design Goals

Design Goal	Description
Detect water contamination incidents	Training healthcare providers and creating systems to monitor for symptoms related to waterborne disease outbreaks, such as those resulting from Cryptosporidium and norovirus infections. Training is also provided to improve recognition of exposures to toxic chemicals via contaminated drinking water.
Increase awareness of the relationships between public health protection and drinking water quality	Establishing a joint public health and utility workgroup will encourage collaboration to effectively address water quality issues that may impact public health.
Monitor for other community health issues of interest	Beyond possible drinking water contamination, PHS systems used in an SRS can support other public health goals such as monitoring for low-level but potentially harmful background environmental exposures.

PHS Performance Objectives

Performance objectives are measurable indicators of how well the SRS meets the design goals established by the utility. Throughout design, implementation and operation of the SRS or its components, the utility can use performance objectives to evaluate the added value of each capability, procedure or partnership. While specific performance objectives should be developed by each utility in the context of its unique design goals, general performance objectives for an SRS were defined in the Water Quality Surveillance and Response System Primer (USEPA, 2015a) and are further described in the context of PHS as follows.

- **Incident coverage:** Detect and respond to a broad spectrum of water quality incidents. PHS is limited to detection of contaminants that result in illness. It should be noted that while some contaminants do not result in short or long term health impacts, they may elicit physiological

effects such as nausea or headaches if they have strong aesthetic characteristics such as a foul odor. Thus, these contaminants may prompt exposed individuals to seek healthcare, and potentially generate a PHS alert.

- **Spatial coverage:** Achieve spatial coverage of the entire distribution system. Theoretically, PHS has the ability to cover every customer in the distribution system, but the actual spatial coverage achieved by PHS may be impacted by the degree to which public health data can be effectively collected in real-time throughout the distribution system.
- **Timeliness of detection:** Detect public health incidents in sufficient time for effective response. This performance objective is impacted by how quickly data is available for analysis and how often the analysis is performed.
- **Alert occurrence:** Minimize the number of invalid alerts while maintaining the ability of the system to detect true alerts based on pre-established thresholds of syndrome and case frequencies. This performance objective is primarily impacted by the accuracy of data generated and the data analysis method(s) used.
- **Sustainability:** Maintain surveillance systems and relationships between the utility and public health partners. The effectiveness of PHS requires maintenance of relationships and communication pathways across multiple agencies.

TOPIC 4: WHAT ARE COST-EFFECTIVE APPROACHES FOR PHS?

Utilities can take the following simple steps to develop the foundation for PHS:

- Meet with local public health partners to establish relationships, exchange contact information, and learn how public health partners could support detection of and response to contaminated drinking water. USEPA has developed a Public Health Assessment Interview Form that utilities can use to engage their local public health partners (USEPA, 2015b).
- Evaluate PHS datastreams currently monitored by public health partners to determine if they have the potential to provide timely detection of contaminated drinking water.
- Establish procedures for the joint utility and public health investigation of PHS alerts that might be indicative of contaminated drinking water.

NEXT STEPS

Visit the Water Quality Surveillance and Response Website at http://water.epa.gov/infrastructure/watersecurity/lawsregs/initiative.cfm for more information about SRS practices. The Website contains guidance and tools that will help a utility to enhance surveillance and response capabilities, as well as case studies that share utility experiences with SRS implementation and operation.

REFERENCES

USEPA. (2015a). Water Quality Surveillance and Response System Primer, 817-B-15-002.

USEPA. (2015b). Public Health Surveillance Assessment Interview Form, 817-B-15-001.

APPENDIX 4A: MAXIMUM CONTAMINANT LEVELS AND DRINKING WATER STANDARDS

Appendix 4A-1: Federal and State Maximum Contaminant Levels (Updated 07/01/14)

Constituent	USEPA		California	
	MCL (mg/L)*	Effective Date[a]	MCL (mg/L)	Effective Date
Inorganics				
Aluminum	0.05 to 0.2[b]	1/91	1 0.2[b]	2/25/89 9/8/94
Antimony	0.006	7/92	0.006	9/8/94
Arsenic	0.05 0.010	eff: 6/24/77 eff: 1/23/06	0.05 0.010	1977 11/28/08
Asbestos	7 MFL[c]	1/91	7 MFL[c]	9/8/94
Barium	1 2	eff: 6/24/77 1/91	1	1977
Beryllium	0.004	7/92	0.004	9/8/94
Cadmium	0.010 0.005	eff: 6/24/77 1/91	0.010 0.005	1977 9/8/94
Chromium	0.05 0.1	eff: 6/24/77 1/91	0.05	1977
Copper	1.3[d]	6/91	1b 1.3[d]	1977 12/11/95
Cyanide	0.2	7/92	0.2 0.15	9/8/94 6/12/03
Fluoride	4 2[b]	4/86 4/86	2	4/98
Hexavalent Chromium	-	-	0.010	7/1/14
Lead	0.05[e] 0.015[d]	eff: 6/24/77 6/91	0.05[e] 0.015[d]	1977 12/11/95
Mercury	0.002	eff: 6/24/77	0.002	1977
Nickel	Remanded		0.1	9/8/94
Nitrate	(as N) 10	eff: 6/24/77	(as $N0_3$) 45	1977
Nitrite (as N)	1	1/91	1	9/8/94
Total Nitrate/ Nitrite (as N)	10	1/91	10	9/8/94
Perchlorate	-	-	0.006	10/18/07
Selenium	0.01 0.05	eff: 6/24/77 1/91	0.01 0.05	1977 9/8/94
Thallium	0.002	7/92	0.002	9/8/94
Radionuclides				

Constituent	USEPA		California	
	MCL (mg/L)*	Effective Date[a]	MCL (mg/L)	Effective Date
Uranium	30 µg/L	12/7/00	20 pCi/L 20 pCi/L	1/1/89 6/11/06
Combined Radium - 226+228	5 pCi/L	eff: 6/24/77	5 pCi/L 5 pCi/L	1977 6/11/06
Gross Alpha particle activity (excluding radon and uranium)	15 pCi/L	eff: 6/24/77	15 pCi/L 15 pCi/L	1977 6/11/06
Gross Beta particle activity	4 millirem/yr	eff: 6/24/77	50 pCi/L[f] 4 millirem/yr	1977 6/11/06
Strontium-90 now covered by Gross Beta	8 pCi/L	eff: 6/24/77	8 pCi/L[f] 8 pCi/L[f]	1977 6/11/06
Tritium now covered by Gross Beta	20,000 pCi/L	eff: 6/24/77	20,000 pCi/L[f] 20,000 pCi/L[f]	1977 6/11/06
Volatile Organic Compounds				
Benzene	0.005	6/87	0.001	2/25/89
Carbon Tetrachloride	0.005	6/87	0.0005	4/4/89
1,2-Dichlorobenzene	0.6	1/91	0.6	9/8/94
1,4-Dichlorobenzene	0.075	6/87	0.005	4/4/89
1,1-Dichloroethane	-	-	0.005	6/24/90
1,2-Dichloroethane	0.005	6/87	0.0005	4/4/89
1,1-Dichloroethylene	0.007	6/87	0.006	2/25/89
cis-1,2-Dichloroethylene	0.07	1/91	0.006	9/8/94
trans-1,2-Dichloroethylene	0.1	1/91	0.01	9/8/94
Dichloromethane	0.005	7/92	0.005	9/8/94
1,3-Dichloropropene	-	-	0.0005	2/25/89
1,2-Dichloropropane	0.005	1/91	0.005	6/24/90
Ethylbenzene	0.7	1/91	0.68 0.7 0.3	2/25/89 9/8/94 6/12/03
Methyl-tert-butyl ether (MTBE)	-	-	0.005[b] 0.013	1/7/99 5/17/00
Monochlorobenzene	0.1	1/91	0.03 0.07	2/25/89 9/8/94
Styrene	0.1	1/91	0.1	9/8/94
1,1,2,2-Tetrachloroethane	-	-	0.001	2/25/89
Tetrachloroethylene	0.005	1/91	0.005	5/89
Toluene	1	1/91	0.15	9/8/94
1,2,4 Trichlorobenzene	0.07	7/92	0.07 0.005	9/8/94 6/12/03

Constituent	USEPA		California	
	MCL (mg/L)*	Effective Date[a]	MCL (mg/L)	Effective Date
1,1,1-Trichloroethane	0.200	6/87	0.200	2/25/89
1,1,2-Trichloroethane	0.005	7/92	0.032 0.005	4/4/89 9/8/94
Trichloroethylene	0.005	6/87	0.005	2/25/89
Trichlorofluoromethane	-	-	0.15	6/24/90
1,1,2-Trichloro-1,2,2-Trifluoroethane	-	-	1.2	6/24/90
Vinyl chloride	0.002	6/87	0.0005	4/4/89
Xylenes	10	1/91	1.750	2/25/89
Soluble Organic Compounds				
Alachlor	0.002	1/91	0.002	9/8/94
Atrazine	0.003	1/91	0.003 0.001	4/5/89 6/12/03
Bentazon	-	-	0.018	4/4/89
Benzo(a) Pyrene	0.0002	7/92	0.0002	9/8/94
Carbofuran	0.04	1/91	0.018	6/24/90
Chlordane	0.002	1/91	0.0001	6/24/90
Dalapon	0.2	7/92	0.2	9/8/94
Dibromochloropropane	0.0002	1/91	0.0001 0.0002	7/26/89 5/3/91
Di(2-ethylhexyl)adipate	0.4	7/92	0.4	9/8/94
Di(2-ethylhexyl)phthalate	0.006	7/92	0.004	6/24/90
2,4-D	0.1 0.07	eff: 6/24/77 1/91	0.1 0.07	1977 9/8/94
Dinoseb	0.007	7/92	0.007	9/8/94
Diquat	0.02	7/92	0.02	9/8/94
Endothall	0.1	7/92	0.1	9/8/94
Endrin	0.0002 0.002	eff: 6/24/77 7/92	0.0002 0.002	1977 9/8/94
Ethylene Dibromide	0.00005	1/91	0.00002 0.00005	2/25/89 9/8/94
Glyphosate	0.7	7/92	0.7	6/24/90
Heptachlor	0.0004	1/91	0.00001	6/24/90
Heptachlor Epoxide	0.0002	1/91	0.00001	6/24/90
Hexachlorobenzene	0.001	7/92	0.001	9/8/94
Hexachlorocyclopentadiene	0.05	7/92	0.05	9/8/94
Lindane	0.004 0.0002	eff: 6/24/77 1/91	0.004 0.0002	1977 9/8/94

Constituent	USEPA		California	
	MCL (mg/L)*	Effective Date[a]	MCL (mg/L)	Effective Date
Methoxychlor	0.1 0.04	eff: 6/24/77 1/91	0.1 0.04 0.03	1977 9/8/94 6/12/03
Molinate	-	-	0.02	4/4/89
Oxamyl	0.2	7/92	0.2 0.05	9/8/94 6/12/03
Pentachlorophenol	0.001	1/91	0.001	9/8/94
Picloram	0.5	7/92	0.5	9/8/94
Polychlorinated Biphenyls	0.0005	1/91	0.0005	9/8/94
Simazine	0.004	7/92	0.010 0.004	4/4/89 9/8/94
Thiobencarb	-	-	0.07 0.001[b]	4/4/89 4/4/89
Toxaphene	0.005 0.003	eff: 6/24/77 1/91	0.005 0.003	1977 9/8/94
2,3,7,8-TCDD (Dioxin)	3×10^{-8}	7/92	3×10^{-8}	9/8/94
2,4,5-TP (Silvex)	0.01 0.05	eff: 6/24/77 1/91	0.01 0.05	1977 9/8/94
Disinfection Byproducts				
Total Trihalomethanes	0.100 0.080	11/29/79 eff: 11/29/83 eff: 1/1/02[g]	0.100 0.080	3/14/83 6/17/06
Haloacetic acids (five)	0.060	eff: 1/1/02[g]	0.060	6/17/06
Bromate	0.010	eff: 1/1/02[g]	0.010	6/17/06
Chlorite	1.0	eff: 1/1/02[g]	1.0	6/17/06
Treatment Technique				
Acrylamide	TT[h]	1/91	TT[h]	9/8/94
Epichlorohydrin	TT[h]	1/91	TT[h]	9/8/94

Source: Adapted from CCR Title 22, Division 4, Chapter 15. *Concentrations in milligrams per liter (mg/L) if not noted otherwise.

[a] "eff." = Indicates the date the MCL took effect; any other date provided indicates when the USEPA established (i.e., published) the maximum contaminant level (MCL).

[b] Secondary MCL.

[c] MFL = Million fibers per liter, with a fiber length of >10 microns.

[d] Regulatory Action Level; if the system exceeds, it must take certain actions, such as additional monitoring, corrosion control studies, and treatment and, for lead, a public education program; replaces MCL.

[e] The MCL for lead was rescinded with the adoption of the regulatory action level described in Footnote d.

[f] Gross beta MCL is 4 millirem per year annual dose equivalent to the total body or any internal organ; Sr-90 MCL = 4 millirem per year to bone marrow; tritium MCL = 4 millirem per year to total body.

[g] Effective for surface water systems serving more than 10,000 people; effective for all others on 1/1/04.

[h] TT = Treatment technique, because an MCL is not feasible.

Table 4A-2: Secondary Drinking Water Standards

"Consumer Acceptance Contaminant Level" Constituents	**Maximum Contaminant Levels/Units**
Aluminum	0.2 mg/L
Color	15 Units
Copper	1.0 mg/L
Foaming Agents (MBAS)	0.5 mg/L
Iron	0.3 mg/L
Manganese	0.05 mg/L
Methyl-*tert*-butyl ether (MTBE)	0.005 mg/L
Odor—Threshold	3 Units
Silver	0.1 mg/L
Thiobencarb	0.001 mg/L
Turbidity	5 Units
Zinc	5.0 mg/L

Source: Adapted from CCR Title 22, Division 4, Chapter 15, Article 16, Section 6449.

Table 4A-3: Drinking Water Notification Levels

Notes[a]	Chemical	Notification Level (milligram per liter)
1	Boron	1
2	n-Butylbenzene	0.26
3	sec-Butylbenzene	0.26
4	tert-Butylbenzene	0.26
5	Carbon disulfide	0.16
6	Chlorate	0.8
7	2-Chlorotoluene	0.14
8	4-Chlorotoluene	0.14
9	Diazinon	0.0012
10	Dichlorodifluoromethane (Freon 12)	1
11	1,4-Dioxane	0.001
12	Ethylene glycol	14
13	Formaldehyde	0.1
14	HMX	0.35
15	Isopropylbenzene	0.77
16	Manganese	0.5
17	Methyl isobutyl ketone (MIBK)	0.12
18	Naphthalene	0.017
19	N-Nitrosodiethylamine (NDEA)	0.00001
20	N-Nitrosodimethylamine (NDMA)	0.00001
21	N-Nitrosodi-n-propylamine (NDPA)	0.00001
22	Propachlor	0.09
23	n-Propylbenzene	0.26
24	RDX	0.0003
25	Tertiary butyl alcohol (TBA)	0.012
26	1,2,3-Trichloropropane (1,2,3-TCP)	0.000005
27	1,2,4-Trimethylbenzene	0.33
28	1,3,5-Trimethylbenzene	0.33
29	2,4,6-Trinitrotoluene (TNT)	0.001
30	Vanadium	0.05

Source: Adapted from CCR Title 22 (more information can be found at http://www.swrcb.ca.gov/drinking_water/certlic/drinkingwater/NotificationLevels.shtml.

[a] Additional notes are included with the reference on toxicological endpoint, references, history, and other information.

APPENDIX 5A: BIOASSAYS REFERENCED IN THE STUDY BY ESCHER ET AL.

Table 5A-1: Bioassays Referenced in Figure 5-3 (from Chapter 5, Which Was Reproduced from Escher et al. (2014a,b)

No.[a]	Bioassay
1	PXR-cisFACTORIAL
2	PXR-transFACTORIAL
3	HG5LN PXR
4	CAR-transFACTORIAL
5	CAR-yeast
6	PPARα-transFACTORIAL
7	PPARγ-transFACTORIAL
8	HELN-PPARγ
9	CALUX-PPARα
10	CALUX-PPARγ2
11	MCF7-PPAR
12	PPARγ-GeneBLAzer
13	Anti-PPARγ-GeneBLAzer
14	AhR-yeast
15	RECETOX CAFLUX
16	ECETOX H4IIEluc
17	MCF7DRE
18	AhR-cisFACTORIAL
19	Cyp1a induction
20	Algae photosynthesis inhibition
21	Acetylcholinesterase inhibition
22	IWW ER-CALUX
23	E-SCREEN
24	UA YES
25	hER yeast
26	medER yeast
27	HELN-ERα
28	HELN-ERß
29	ERE-cisFACTORIAL
30	RECETOX hERα-HeLa-9903
31	MCF7-ERE
32	ERα-transFACTORIAL
33	Steroidogenesis (estrogens)
34	DART cyp19a1b 77 63
35	USF, UCR, SCCWRP ERα-GeneBLAzer

36	Anti ER-CALUX
37	AR-CALUX
38	HELN-AR
39	MCF7-ARE
40	YAS
41	GeneBLAzer
42	AR-transFACTORIAL
43	RECETOX MDA-kb2
44	RECETOX Anti-MDA-kb2
45	Anti-AR-CALUX
46	CSIRO GR-CALUX 66 33
47	GR Switchgear
48	GR-transFACTORIAL
49	RECETOX GR-MDA-kb2
50	SCCWRP GR-GeneBLAzer
51	Anti-GR-GeneBLAzer
52	Anti-GR-CALUX
53	PR-GeneBLAzer
54	PR-CALUX
55	Anti-PR-CALUX
56	Steroidogenesis (progesterone)
57	Steroidogenesis (17α OH-progesterone)
58	TR-CALUX
59	T-SCREEN
60	THRα1-transFACTORIAL
61	HELN-TR
62	MCF7-RARE
63	P19/A15
64	RORß-transFACTORIAL
65	hRAR-Yeast Assay
66	umuC TA1535/pSK1002
67	umuC TA1535/pSK1002 +S9
68	umuC NM5004
69	RECETOX SOS chromotest
70	Ames TA98 94 IWW
71	Ames TA98+ S9
72	Ames TAmix
73	Ames TAmix +S9
74	Ames TA100 94
75	Micronucleus assay
76	ROS formation RTG2
77	Protein damage *E. coli*
78	HSE-cisFACTORIAL

79	hspb11 induction DART
80	HIF-1a-cisFACTORIAL
81	Hypoxia-Switchgear
82	NF-kB-cisFACTORIAL
83	NF-kB-Geneblazer
84	NF-kB-CALUX
85	Jurkat E6.1 IkB
86	AREc32
87	Nrf2-keap
88	Nrf2/ARE-cisFACTORIAL
89	Nrf2-CALUX
90	p53-cisFACTORIAL
91	p53-CALUX
92	p53-CALUX +S9
93	p53-GeneBLAzer
94	AREc32 cell viability
95	Caco 2 NRU
96	RTG2MTT
97	DART 48h lethality
98	DART 120h sublethal
99	SK-N-SH cytotoxicity
100	THP1 cytokine
101	Algae growth inhibition
102	Vibrio fischeri (Microtox)
103	Photobacterium phosphoreum

[a] Numbers herein are equivalent to the numbers provided in **Figure 5-3** from **Chapter 5.**

References:

Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P. Busch, W., Crago, J., Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Medez, J.E., Poulsen, A., Procazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraish, F., Snyder, S., Su, G.Y., Tang, J.Y.M., van der Berg, B/. van der Linden, S.C., Werner, I., Westerheide, S.D., Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X.W., and Leusch, F.D.L. (2014a). "Benchmarking organic micropollutants in wastewater, recycled water, and drinking water with *in vitro* bioassays." *Environ. Sci. Technol*., 48: 1940-1958.

Escher, B.I., Tang, J.Y.M., Poulsen, A., Leusch, F.D.L., Snyder, S.A., and Ja, A. (2014b). *Development of Bioanalytical Techniques to Assess the Potential Human Health Impacts.* Final Report WateReuse Foundation Project Nl. WRRF-10-07

APPENDIX 7A: HALTING ENVIRONMENTAL ANTIMICROBIAL RESISTANCE DISSEMINATION

NSF-PIRE: Halting Environmental Antimicrobial Resistance Dissemination (HEARD)

NSF Org:	OISE Office Of International Science &Engineering
Initial Amendment Date:	September 17, 2015
Latest Amendment Date:	September 17, 2015
Award Number:	1545756
Award Instrument:	Continuing grant
Program Manager	Elizabeth Lyons OISE Office Of International Science &Engineering O/D Office Of The Director
Start Date:	October 1, 2015
End Date:	September 30, 2020 (Estimated)
Awarded Amount to Date:	$634,239.00
Investigator(s):	Peter Vikesland, pvikes@vt.edu (Principal Investigator) Pedro Alvarez (Co-Principal Investigator) Diana Aga (Co-Principal Investigator) Amy Pruden (Co-Principal Investigator) Krista Wigginton (Co-Principal Investigator)
Sponsor:	Virginia Polytechnic Institute and State University Sponsored Programs 0170 BLACKSBURG, VA 24061-0001 (540)231-5281
NSF Program(s):	PIRE
Program Reference Code(s):	021E, 5914, 5919, 5927, 5937, 5950, 5976, 6194, 9200
Program Element Code(s):	7742

ABSTRACT

Antimicrobial resistance (AMR), which occurs when disease-causing organisms no longer respond to the drugs commonly used to treat them, is a worldwide public health crisis and as such has been proclaimed to be one of the greatest threats to human wellbeing of the 21st Century. Halting AMR is a complex task because natural background levels of AMR vary worldwide, there are many ways that humans impact AMR, and because natural and human impacts interact in different ways around the world to influence how multi-antimicrobial resistant "super-bugs" arise and are transmitted. Although substantial effort has focused on lessening hospital-derived resistance, the spread of AMR has continued to accelerate, thus creating new attention to diminishing the spread and/or transmission of AMR in the wastewater environment. Wastewater treatment plants are a logical focus because they serve as collection points for resistant organisms and antimicrobial compounds from a wide variety of sources (i.e., hospitals, industries, households) and they are potential breeding grounds for environmental dissemination of AMR. Antimicrobial drugs and other chemical stressors (e.g., heavy metals, biocides) regularly enter wastewater treatment plants and may select for resistant organisms, while also stimulating them to produce and share the DNA elements responsible for resistance. This PIRE project, Halting Environmental Antimicrobial Resistance Dissemination [HEARD], will 1) quantify how wastewater treatment processes affect different aspects of AMR (e.g., the antimicrobial drugs, AMR organisms, and the DNA elements underlying AMR) across a global transect of wastewater treatment plants, 2) determine how the characteristics of wastewater treatment plants and the receiving environment (e.g., river, lake, or pipe network) interact to affect the spread of AMR, and 3) develop and test novel approaches to stop the spread of AMR originating from wastewater treatment plants. The international team assembled for this PIRE project includes researchers from four U.S. institutions and six other countries (China, India, Philippines, Portugal, Sweden, and Switzerland). The international dimensions of this project are essential because 1) the propagation of AMR is of global concern, 2) the use and disposal of antimicrobials and wastewater management practices differ significantly from one society to another, and 3) international research collaboration prepares U.S. students to be part of a globally engaged U.S. science and engineering workforce.

Three overarching hypotheses drive HEARD:

- Hypothesis 1: Wastewater treatment plant influents can be monitored to gauge the impacts of local antimicrobial use and disposal practices on the prevalence of resistant organisms and resistance elements.
- Hypothesis 2: A broad gradient of antimicrobial resistance elements and resistant bacteria are present in wastewater effluents across the globe.
- Hypothesis 3: Wastewater treatment processes and receiving environments can be chosen or modified to mitigate the spread of antimicrobial resistance.

To address these hypotheses and answer these questions, we have developed a comprehensive research plan organized around three research thrusts:

- Thrust 1: Global Reconnaissance of Antimicrobial Drugs, Antibiotic Resistant Bacteria, and Resistance Element Fate During Wastewater Treatment.

- Thrust 2: The Relative Roles of Wastewater Treatment Plants and Receiving Environments in Resistance Dissemination.
- Thrust 3: Advancing Wastewater Treatment Technologies for Antimicrobial Drug, Antibiotic Resistant Bacteria, and Resistance Element Removal.

HEARD brings together four U.S. universities and 10 international academic institutions. The project's initial focus will be to globally track and quantify the concentrations of a select group of target resistance elements (e.g., NDM-1, intI1, blaTEM, vanA, and sul1) within wastewater treatment plant influents and effluents in the U.S., Asia, and Europe. In parallel, the project members will utilize metagenomics to detect nontarget resistance elements and bacteria. The metagenomic information will then direct and refine future targeted sampling efforts across the global transect of field sites. To develop solutions to the threat of wastewater mediated resistance dissemination, the team will examine both at field and laboratory scale how changes in wastewater treatment plant operational variables (e.g., F/M ratio, solids retention time, and aerobic/anaerobic conditions) affect both resistant bacteria and resistance elements. The project's international partners synergistically provide the U.S.-based PIRE students with intracultural context, international research experience with access to world-class collaborators and facilities, and unique expertise in antimicrobial resistance and the global threat of resistance dissemination.

This award is co-funded by the Division of Chemical, Bioengineering, Environmental, and Transport Systems of NSF's Directorate for Engineering.

APPENDIX 7B: OCCURRENCE, PROLIFERATION, AND PERSISTENCE OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE DURING WASTEWATER TREATMENT

WERF 1C15

This Water Environment Research Foundation (WERF) project is led by Dr. Daniel Gerrity from the University of Nevada, Las Vegas and a team of researchers from the University of Las Vegas and the University of Arizona. Other collaborative partners of this research include WateReuse Association & Research Foundation, Water Research Foundation, American Cleaning Institute, Merck & Company, Inc., and four wastewater and water utilities in the United States and Singapore.

OCCURRENCE, PROLIFERATION, AND PERSISTENCE OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE DURING WASTEWATER TREATMENT

Drs. Dan Gerrity and Jacimaria Batista
University of Nevada, Las Vegas
Las Vegas, Nevada

Dr. Channah Rock
University of Arizona
Tucson, Arizona

Dr. Eric Dickenson
Southern Nevada Water Authority
Las Vegas, Nevada

Final Report: April 2016

1.0 Project Background

1.1 Project Purpose

In 2011, the World Health Organization (WHO) identified antibiotic resistance (AR) as “one of the three greatest threats to human health” (WHO, 2011). Recent scientific studies have also established a link between recycled water and environmental occurrence of AR (Fahrenfeld et al., 2013). In fact, wastewater effluent has been identified as one of the “leading reservoirs” of AR in the environment (Novo and Manaia, 2010). This may be attributable to the selective pressure of residual antibiotics, which are ubiquitous in wastewater, coupled with gene transfer between microorganisms during wastewater treatment; however, there is a need to better characterize the role of wastewater treatment in either proliferating or mitigating antibiotic resistance in treated effluent. Wastewater agencies must try to allay public and regulatory concerns, despite limited knowledge of the severity of the problem or the efficacy of potential mitigation strategies.

The emergence of bacterial AR illustrates the power of evolution and selection. While antibiotics provide effective means to treat and cure bacterial diseases, their use leads to the selection and transmission of genes conferring resistance. The use of alternative or more powerful antibiotics precipitates new rounds of selection and evolution, which only augments the genetic reservoir of AR. Potential alternatives to mitigate the emergence of AR include slowing down the evolutionary process or using novel strategies to prevent horizontal gene transfer. This requires an understanding of how gene transfer occurs in natural and engineered environments and how environmental systems may promote mutagenesis within the microbial genome. Developing solutions to this problem in the water/wastewater industry requires a comprehensive research approach, similar to the public, regulatory, and scientific communities' coordinated response to the increasing awareness of pharmaceuticals in water.

This project specifically seeks to understand how the following variables affect the occurrence and potential proliferation of antibiotics and antibiotic resistance in wastewater:

- Unit operations within a wastewater treatment train.
- Operational conditions, specifically solids retention time, in activated sludge systems.
- Influent wastewater quality, specifically influent antibiotic concentrations.

Note: Due to matrix interference effects during sample analysis, this project will focus on primary effluent as a surrogate for wastewater influent. This also provides a better representation of the concentrations to which bacteria are exposed during biological treatment.

This project will provide a better understanding of how unit processes and operational conditions within wastewater treatment plants impact AR. This information will equip stakeholders with knowledge and tools that can be used to address concerns related to public and environmental health. In particular, wastewater agencies will have a better understanding of how to optimize their biological treatment systems to simultaneously reduce concentrations of trace organic compounds (TOrCs), including antibiotics, and also mitigate the proliferation of AR within the microbial community. With respect to beneficial use of treated wastewater, this will hopefully facilitate the future growth of the water reuse industry by elucidating critical knowledge gaps.

1.2 Project Objectives

The primary objective of this research is to characterize the impact of solids retention time and influent antibiotic concentrations in activated sludge systems on the quantity and extent of AR in treated effluent (Task 2). The quantity of AR will be based on the number of culturable Gram-positive bacteria that are resistant to a suite of single and combined antibiotics at standard clinical concentrations. AR will also be quantified based on the detection of antibiotic resistance genes in the samples. Using these cultural and molecular data, ratios of antibiotic resistant to total bacteria will be determined. The extent of AR will be based on the concentration at which prescreened antibiotic resistant isolates are no longer able to grow. The hypothesis is that increased solids retention times or higher influent antibiotic concentrations select for bacteria that are increasingly resistant to antibiotics. Secondary goals of the research include preparation of a literature review on occurrence, methodology, and implications of AR in wastewater matrices (Task 1), adaptation of existing microbiological methods for the specific goals of

this project (Task 1), and characterization of the efficacy of conventional and advanced wastewater treatment processes for mitigating the potential impacts of AR in environmental discharge, non-potable reuse, and potable reuse applications (Task 3). The experimental components of the research will be performed at two different full-scale wastewater treatment facilities. The first facility will serve as the site of the laboratory-scale sequencing batch reactors, and the second facility will serve as the study site for the full-scale occurrence study.

1.3 Applicability of Study Results

This project is by no means intended to provide a definitive conclusion regarding the environmental or public health relevance of antibiotics and AR in treated wastewater or recycled water. This emerging issue is highly analogous to the increased perceived risk regarding trace organic compounds in the environment. Particularly in the early 2000s, more advanced analytical instrumentation, studies indicating potential adverse impacts of endocrine disrupting compounds on aquatic species, and increased media attention resulted in a dramatic expansion of TOrC-related research in the water, wastewater, and water reuse industries. Similarly, the ability to detect antibiotics at the part-per-trillion, or ng/L level, coupled with advancements in molecular methods for the detection of antibiotic resistance genes has also led to a greater emphasis on AR. In contrast with the general TOrC issue, for which environmental health has been identified as the primary risk, AR is particularly concerning because of the recent rise in multidrugresistant infections in clinical settings; however, it is entirely unclear whether there is a direct link between AR in clinical settings and AR in water, wastewater, and recycled water. As indicated earlier, a multi-pronged approach is needed to fully characterize the risks (or lack thereof) of AR in water. As with the TOrC research, information related to occurrence, treatability, and environmental/public health must be developed simultaneously over many years by interdisciplinary teams of researchers, scientists, medical professionals, etc.

The goal of this research is to expand the industry's understanding of the occurrence and treatability of AR in wastewater applications. The conclusions from this study will hopefully aid in characterizing the overall risks posed by this issue. In addition to expanding the knowledge base of AR, this study also identifies research questions that should be addressed in future studies.

The project outcomes will specifically benefit wastewater treatment facilities considering treatment plant modification/upgrades and or the inclusion of advanced treatment to mitigate trace organic compounds. Little is currently understood about the prevalence, persistence, fate, and public health relevance of antibiotic resistant bacteria and antibiotic resistance genes in wastewater and recycled water; therefore, water managers must make decisions about the use and safety of treated wastewater under considerable uncertainty. This project will provide information related to the impact of changes to treatment plant operations, specifically in relation to the secondary biological treatment process, on trace organics and the microbial community in the treated wastewater. While a major undertaking, it should be noted that this study will not provide information related to the mechanism of gene transfer or the impacts on public health, but the project will characterize the impact of operational changes on the prevalence of AR.

WRF 4536

This Water Research Foundation (WRF) project, which started in 2014, is led by Andrew Salveson of Carollo Engineers. Subcontractors of this research include Virginia Polytechnic Institute and State University, Trussell Technologies, Inc., Southern Nevada Water Authority, and AQUAlity, Inc. Also participating are seventeen water and wastewater utilities from the states of Arizona, California, Colorado, Florida, Oklahoma, Nevada, Texas, and Virginia. One component of this project involves investigating the potential regrowth and spread of antibiotic resistance genes (ARG) in water distribution systems.

BLENDING REQUIREMENTS FOR WATER FROM DIRECT POTABLE REUSE TREATMENT FACILITIES

Principal Investigator: Mr. Andrew Salveson, P.E., Carollo Engineers, Inc.

1. Project Objective

To provide recommendations and guidance for the appropriate use of blending as part of a direct potable reuse (DPR) project, including evaluations of treatment, impact of different water qualities and corrosion control issues, and impact on engineered storage, blending location, and blending percentages.

2. Excerpt from the First Periodic Report (dated November 1, 2014 – January 31, 2015)

Antibiotic Resistant Microorganisms

Antibiotic resistance is a growing public health concern and it has been argued that, at the current pace, antibiotics may soon cease to be functional for fighting and preventing deadly infections (Smith and Coast 2013, Carlet et al. 2011). There is growing attention on the potential for water reuse to contribute to this problem as well, including ARG persistence or even amplification through wastewater treatment (Rizzo et al. 2013, Zhang et al. 2009, Schlüter et al. 2007). Few studies have directly examined the potential for reclaimed water to contribute to the spread of antibiotic resistance (Hong et al. 2013), and one recent study observed likely regrowth of several ARGs in two U.S. RWDSs (Fahrenfeld et al. 2013).

Results of other studies that have traced ARG abundance as reclaimed water flows through other natural and engineered systems; such as aquifers (Böckelmann et al. 2009), soils (Knapp et al. 2011), constructed wetlands (Nõlvak et al. 2013, Anderson et al. 2013, Sidrach-Cardona and Bécares 2013), and aerobic and anaerobic digesters (Burch et al. 2013, Ma et al. 2011). The overall abundance of ARGs tends to decrease as water passes through such systems, but there have also been instances in which ARGs amplified by > 3 logs (Knapp et al. 2011, Nõlvak et al. 2013, Burch et al. 2013, Ma et al. 2011). In several studies ARG amplification has been linked to redox conditions, presence of specific ionic constituents, such as copper, availability of nutrients, and temperature (Knapp et al. 2011, Nõlvak et al. 2013, Ma et al. 2011, Hoffman et al. 2010, Diehl and LaPara 2010). Proposed mechanisms of ARG attenuation under anoxic conditions include shifting of the dominant bacteria carrying ARGs (Ma et al. 2011, Diehl and LaPara 2010, Pei et al. 2007) and efforts of bacteria to conserve metabolism and thus rid themselves of fitness costs associated with carrying ARGs (Rysz et al. 2013).

Information on ARG removal through advanced treatment is limited, but processes that destroy or remove dissolved organics would be expected to be effective in ARG control.

3. Virginia Tech Lab Analysis

The laboratory at Virginia Tech will analyze the regrowth potential of ARG in the distribution system. This effort will be jointly directed by Dr. Marc Edwards and Dr. Amy Pruden.

As noted in the draft document entitled *Virginia Tech Protocols (Revision 0 – January 7, 2015)* for WRF 4536, the following will be undertaken:

> *Quantification of Antibiotic Resistance Genes (ARGs).* Antibiotic resistance genes (ARGs) are analyzed routinely in Dr. Pruden's laboratory, and methods have been optimized for various matrices, including wastewater, drinking water, soil, manure, and river sediments. In this study, antibiotic resistance gene targets will include a subset that encode resistance to antibiotics that are critically important to human health, including: vancomycin (*van*A, Schwartz et al. 2003); cephalosporins (*bla*CTX, Marti et al. 2013); and fluoroquinolones (*qnr*A, Marti et al. 2013). The *int*I gene, which plays a crucial role in the ability of bacteria to share ARGs and is thought to be a key factor in the spread of antibiotic resistance in the environment (Gillings, et al. 2015; Wellington et al. 2013; Gaze et al. 2011), will also be quantified.

4. References

Edwards, M., A. Pruden, and J. Parks (2015). Draft *Virginia Tech Protocols* for Water Reuse Foundation Project 4536: Blending Requirements for Water from Direct Potable Reuse Treatment Facilities (Revision 0 – January 7, 2015).

Salveson, A. (2015). *First Periodic Report* (November 1, 2014 – January 31, 2015) for Water Reuse Foundation Project 4536: Blending Requirements for Water from Direct Potable Reuse Treatment Facilities.

APPENDIX 7C: WASTEWATER AS A SOURCE OF CARBAPENEM-RESISTANT *ESCHERICHIA COLI*

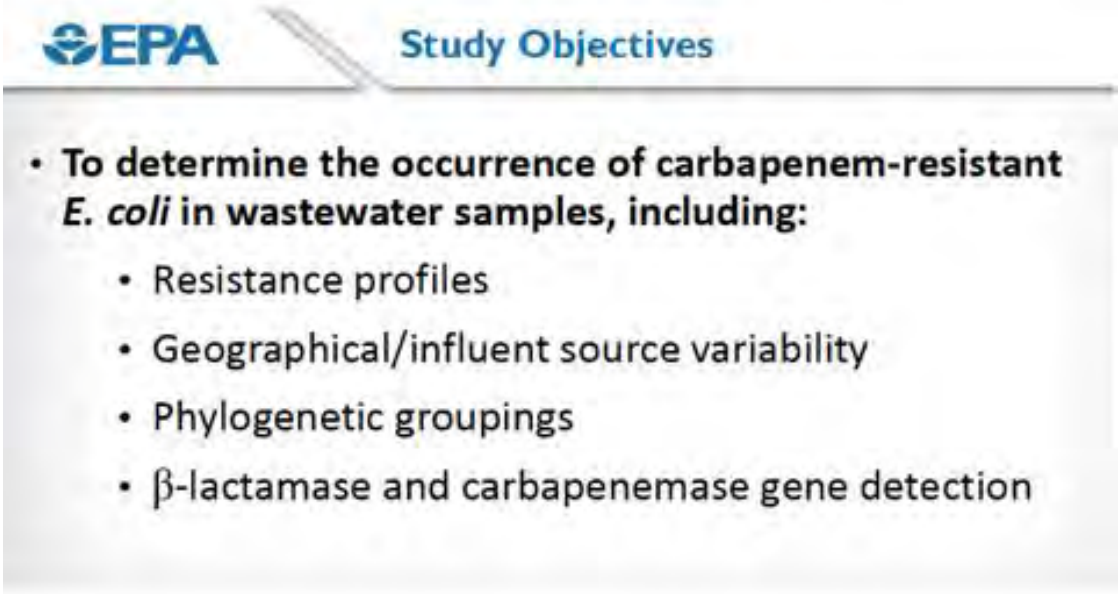
The following abstract and accompanying poster were presented at the September 17-21, 2015, meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). This work was the basis for an article on ARB in wastewater published by the* LA Times *on March 7, 2016. NOTE: All samples were primary treated wastewater collected after the clarifier.

WASTEWATER AS A SOURCE OF CARBAPENEM-RESISTANT *ESCHERICHIA COLI*

J.M. Hoelle, E.W. Rice, L.A. Boczek, H. Ryu, J.J. Johnson, B.D. Johnston; US EPA, Cincinnati, OH, University of MN, Minneapolis, MN

Clinical studies have reported that the occurrence of carbapenem-resistant *E. coli* is on the rise. This is of concern because carbapenem antibiotics are typically reserved for treating infections caused by bacteria resistant to other classes of antibiotics. Current literature states that wastewater effluents serve as a reservoir of antibiotic resistant genes (ARGs). In this study, we sought to determine the occurrence of carbapenem-resistant *E. coli* in wastewater samples from seven geographically dispersed locations during the summer and winter seasons in the United States between 2012 and 2013. A total of 353 *E.coli* isolates were recovered using mFC agar supplemented with antibiotics. *E. coli* isolates were confirmed biochemically using BBL Crystal™. All isolates had MICs as determined by E-Test™ strip confirming intermediate or full resistance according to the CLSI 2012 guidelines to one or more of imipenem, cefotaxime, ceftazidime, or ciprofloxacin. Phylogenetic grouping of the isolates was performed using a quadraplex PCR assay. PCR assays targeting nine carbapenemase and extended-spectrum β-lactamase (ESBL) genes were performed against 88 isolates classified as non-susceptible to imipenem. The resistance profiles of *E. coli* most prevalent in all samples combined was to cefotaxime (66 percent), followed by ciprofloxacin (65 percent), ceftazidime (60 percent), and imipenem (17 percent). Of 353 *E. coli* isolates, twenty three percent were nonsusceptible to imipenem, and resistant to cefotaxime and ceftazidime, meeting the Centers for Disease Control and Prevention's definition of Carbapenem Resistant *Enterobacteriaceae* (CRE). The prevalence of imipenem resistant *E. coli* per facility was greater in effluents from urban wastewater treatment plants treating domestic waste (18 percent), than in effluents from rural plants treating a mixture of domestic and agricultural waste (8 percent). Phylogenetic groupings showed that group D was the most prevalent (27 percent), followed by groups A (21 percent), B2 (20 percent), B1 (14 percent), F (11 percent), C (10 percent), and E (<1 percent). Global phylogenetic analyses have demonstrated that extraintestial pathogenic *E. coli* (ExPEC) belong to groups B2 and D, which accounted for nearly half of the study isolates. Sixty percent of *E. coli* isolates had positive PCR reactions for at least one ARG, and 27 percent were positive for two or more ARGs. This study demonstrates that the occurrence of CRE *E. coli* is widespread in wastewaters in the United States.


EPA
Office of Research and Development
Wastewater as a Source of Carbapenem-Resistant
Escherichia coli
J. Hoelle[1,2], E. Rice[1], H. Ryu[1], B. Kinkle[2], D. Grogan[2], B. Johnston[3], J. Johnson[3]
[1]US EPA, Office of Research and Development, Cincinnati, OH
[2]University of Cincinnati, Department of Biological Sciences, Cincinnati, OH
[3]VA Medical Center and University of Minnesota, Minneapolis, MN


EPA
Study Objectives
• To determine the occurrence of carbapenem-resistant E. coli in wastewater samples, including:
• Resistance profiles
• Geographical/influent source variability
• Phylogenetic groupings
• β-lactamase and carbapenemase gene detection

CRE Definition

Resistance Profiles

- CRE- Carbapenem-resistant *Enterobacteriaceae*
- CDC definition of CRE (used in this study):

  "Non-Susceptible to one of the following carbapenems: doripenem, meropenem, or imipenem AND resistant to all third generation cephalosporins tested (cefotaxime and ceftazidime)."

- Newly adapted CDC definition (January 2015):

  "Resistant to imipenem, meropenem, doripenem, or ertapenem OR documentation that the isolate possesses a carbapenemase."

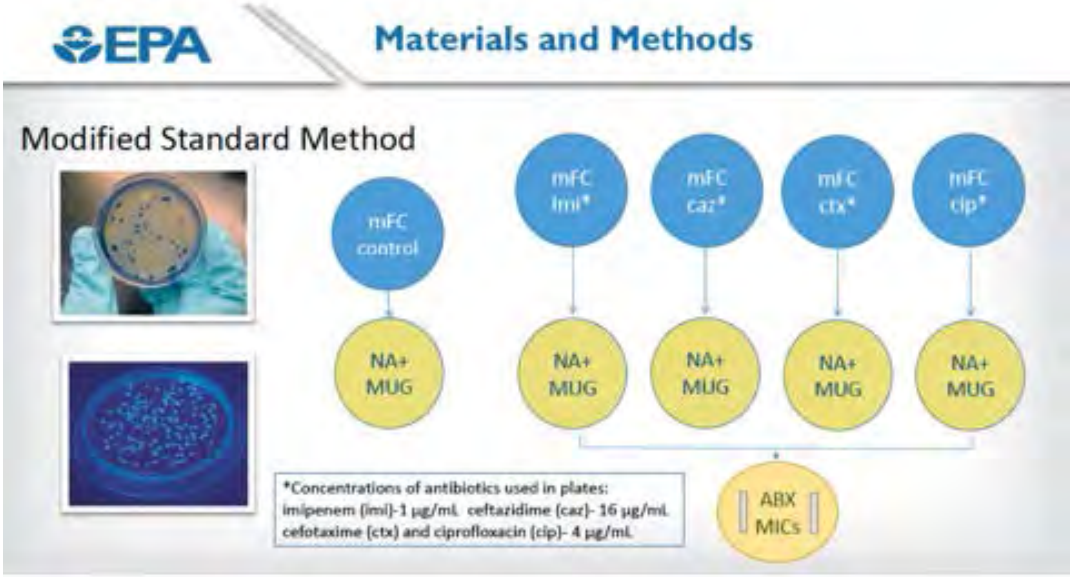
Materials and Methods

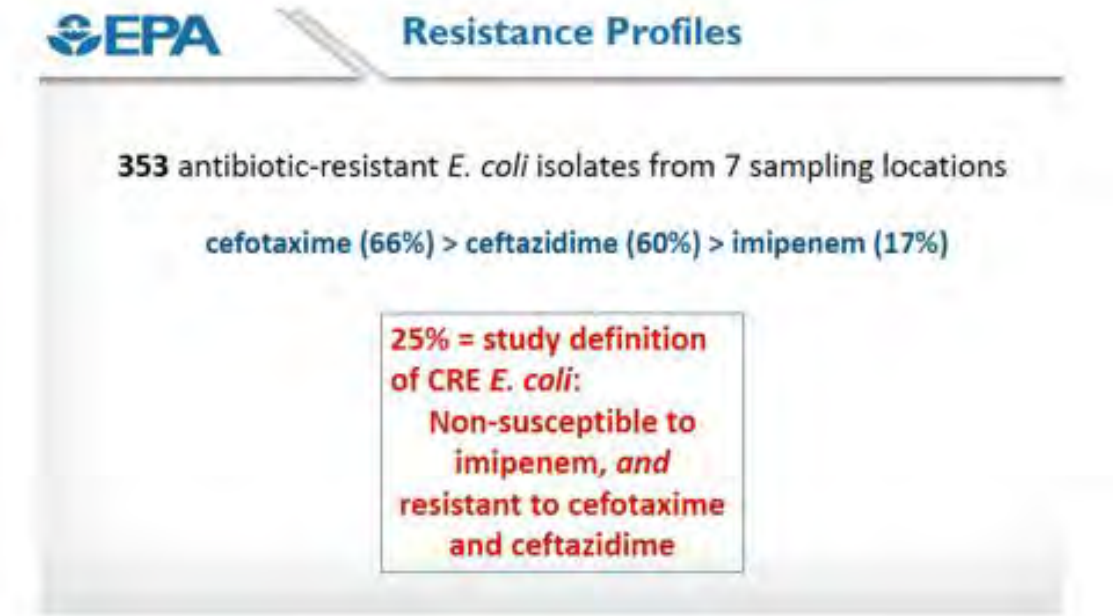
Isolated resistant *E. coli* using modified Standard Method

Determined antibiotic-resistance profile using E-Test Strips™

Determined phylogenetic groupings of all *E. coli* isolates using PCR

Screened 88 *E. coli* isolates non-susceptible to imipenem for 9 most common β-lactamase and carbapenemase genes using qPCR


EPA
Materials and Methods
Modified Standard Method
mFC control
mFC Imi*
mFC caz*
mFC ctx*
mFC cip*
NA+ MUG
NA+ MUG
NA+ MUG
NA+ MUG
NA+ MUG
*Concentrations of antibiotics used in plates: imipenem (imi)-1 µg/mL ceftazidime (caz)- 16 µg/mL cefotaxime (ctx) and ciprofloxacin (cip)- 4 µg/mL
ABX MICs

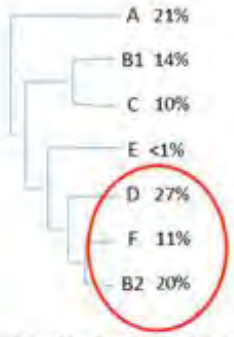

EPA
Resistance Profiles
353 antibiotic-resistant E. coli isolates from 7 sampling locations
cefotaxime (66%) > ceftazidime (60%) > imipenem (17%)
25% = study definition of CRE E. coli:
Non-susceptible to imipenem, and resistant to cefotaxime and ceftazidime

Geographical/Influent Source Variability

Location	CRE *E. coli*	Potential influences reported by plant
N. California (suburban)	2/22 (9%)	Agriculture, Domestic
S. California (urban)	18/128 (14%)	Agriculture, Domestic, Industrial
Colorado (urban)	11/36 (31%)	Domestic, Agriculture, Industrial
Maryland (suburban)	4/34 (12%)	Domestic
New York (Urban)	17/93 (18%)	Domestic
Ohio (urban)	15/60 (25%)	Domestic
Texas (rural)	1/24 (4%)	Agriculture, Domestic

*Range of *E. coli* found in wastewater: 10^4-10^5

Phylogenetic Analysis



Relatedness Between *E. coli* Phylotypes

Clermont Phylotyping

- Global phylogenetic analyses show that extraintestinal pathogenic *E. coli* (ExPEC) belong to groups B2 and D
- Group F: recently defined sister group to B2 and D
- ***Among present wastewater isolates, 58% of antibiotic-resistant E. coli isolates and 41 % of CRE E. coli belong to groups B2, D, and F***

Antibiotic Resistance Gene Detection

Gene Target	Gene Product	Number of Positives n=88
kpc	*Klebsiella pneumoniae* carbapenemase	4 (5%)
imp	IMP-type carbapenemase	1 (1%)
vim	Verona integron-mediated metallo-β-lactamase	40 (45%)
ndm	New Delhi metallo-β-lactamase	0 (<1%)
ctx	CTX-M βeta-lactamase	7 (8%)
oxa	Oxacillinase group of β-lactamases	0 (<1%)
tem	Extended spectrum β-lactamase	27 (31%)
shv	Extended spectrum β-lactamase	2 (2%)
ges	Carbapenem-hydrolyzing β-lactamase	0 (<1%)

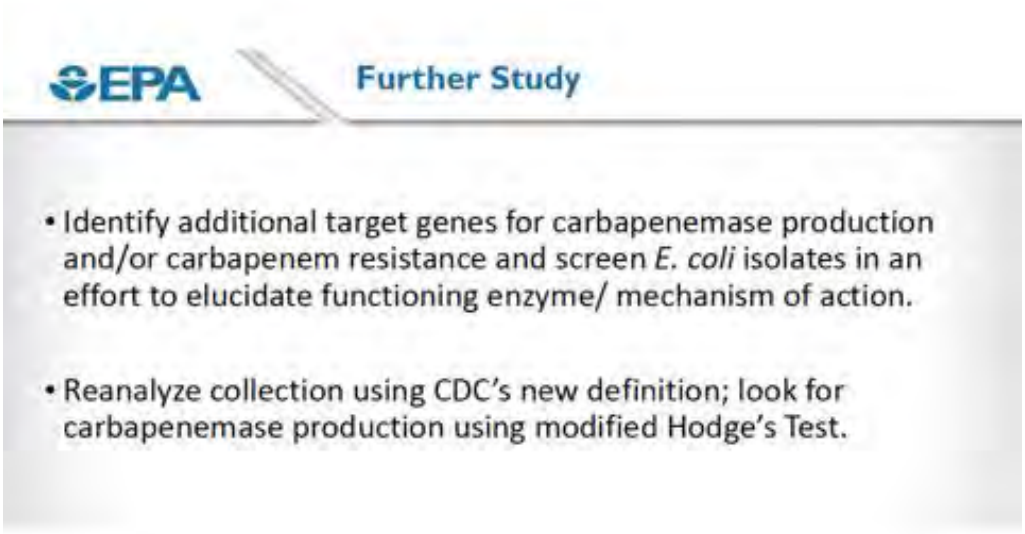
88 CRE *E. coli* isolates

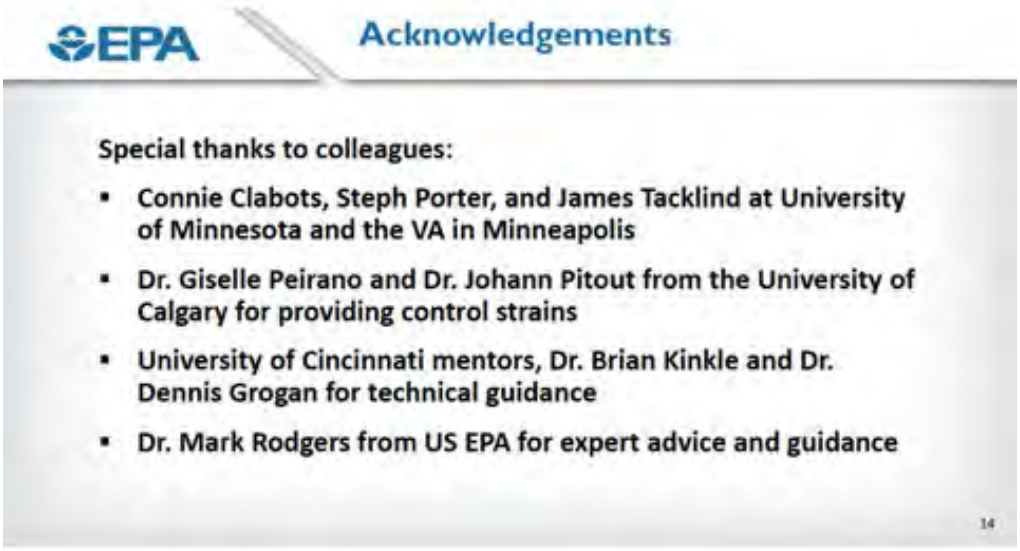
- *60% isolates: ≥ 1 gene*
- *27% isolates: ≥ 2 genes*

39% negative for all target genes tested

Summary

- CRE *E. coli* are widespread in wastewaters in the US.
- 25% of antibiotic-resistant *E. coli* were CRE.
- CRE *E. coli* prevalence per utility was higher in wastewater from urban plants treating primarily domestic waste.
- 41% of CRE *E. coli* isolates belonged to phylotypes B2, D, and F.
- 39% of CRE *E. coli* were negative for all target genes, indicating the possibility of other widespread carbapenemase genes.


EPA
Further Study
• Identify additional target genes for carbapenemase production and/or carbapenem resistance and screen E. coli isolates in an effort to elucidate functioning enzyme/ mechanism of action.
• Reanalyze collection using CDC's new definition; look for carbapenemase production using modified Hodge's Test.


EPA
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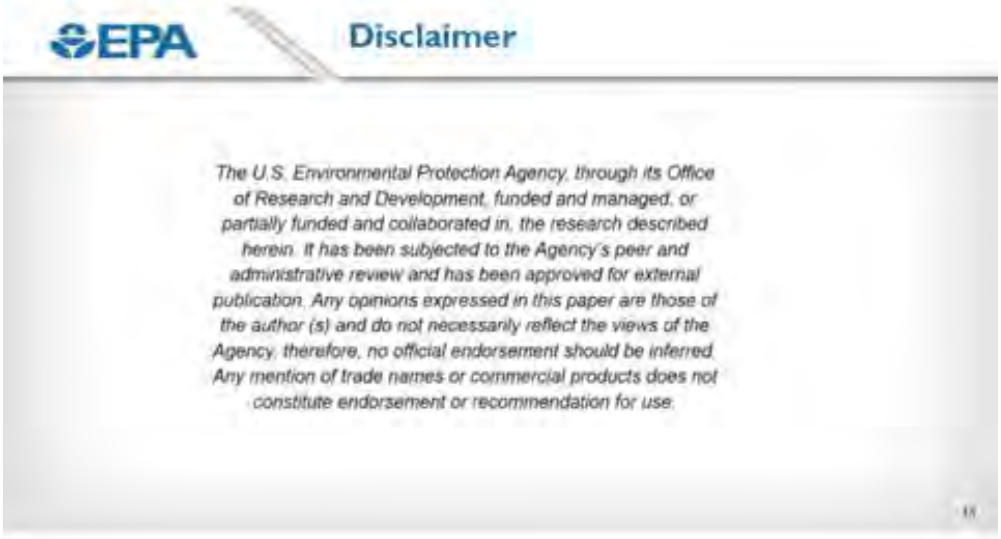
Dr. Mark Rodgers from US EPA for expert advice and guidance
14


EPA
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EPA
Contact Information
Jill Hoelle
US EPA/ORD/NRMRL
email: Hoelle.jill@epa.gov
Phone: (513) 569-7911

APPENDIX 7D: STRATEGIC RESEARCH AGENDA: JOINT PROGRAMMING INITIATIVE ON ANTIMICROBIAL RESISTANCE

The following material is taken from the Strategic Research Agenda of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR).

Executive Summary

Unlike the therapeutic advances through which acute diseases have been transformed into chronic diseases (e.g. anti-retrovirals for HIV patients), antibiotics are true miracle drugs that can completely cure patients who suffer from life-threatening illnesses. Antibiotics have saved millions of lives from once-deadly diseases. But antibiotics are misused in both humans and animals[A]. Every dose of antibiotics creates selective evolutionary pressures, which can result in bacteria becoming resistant to multiple antibiotics. These resistant bacteria can then spread pandemically over the entire planet. Infections with multi-drug resistant bacteria are a major threat to human health since correct antibiotic therapy may not be started in time or because there are very few antibiotics that can be used for the successful treatment of infections with these bacteria. The World Health Organization currently considers antibiotic resistance[B] one of the three greatest threats to human health. A return to the pre-antibiotic era would not only mean that classical bacterial epidemics would again become a major threat to public health but it would also threaten some of the most valuable therapies of modern medicine, such as transplantation programmes and immunosuppressive chemotherapy, which would be impossible to undertake without antibiotics as supportive treatments. The global and multifaceted problem of antimicrobial resistance demands comprehensive and creative solutions, which require action from many sectors of society.

The Joint Programming Initiative

Joint Programming is the process by which Member States define, develop and implement a common strategic research agenda based on an agreed vision on how major societal challenges can be addressed, that no individual Member State is capable of handling independently. The Joint Programming Initiative on AMR has 19 participating countries[C]. The JPI on AMR will develop integrated approaches to pursue unique world-class research on AMR that will be translated into new prevention and intervention strategies that improve the public health and wellbeing of populations, and delivers economic and societal benefit throughout Europe and beyond. An important element of the mission of the JPI AMR

[A] Irresponsible usage of antibiotics includes the use of dosages that are either too high or too low, the use of the wrong type of antibiotic (including the use of antibiotic to treat infections that are not caused by bacteria), and poor-quality antibiotics.

[B] WHO refers to AMR in the broad sense, namely bacterial, viral, and parasitic resistance.

[C] Belgium, Canada, Czech Republic, Denmark, Finland, France, Germany, Greece, Israel, Italy, the Netherlands, Norway, Poland, Romania, Spain, Sweden, Switzerland, Turkey, and the United Kingdom.

will be to connect to and collaborate with the different stakeholders involved in its mission. As well as the research community, the JPI AMR will invite industry to discuss their needs in terms of scientific support to stimulate their interest in the development of novel antimicrobials and alternatives to antibiotics. Healthcare service organisations and professionals will be invited to provide their experiences and to frame the questions to be responded to by this JPI. Public administrations will provide their input on policies related to pharmaceutical treatments, patient safety, and international collaboration in surveillance, public health and education.

The Strategic Research Agenda

This Strategic Research Agenda provides a framework of opportunities for countries involved in the JPI AMR and those who are willing to participate in joint actions. Joint actions will be implemented through co-operative activities that realign or link national investments in order to achieve increased impact and the provision of new funding.

Recommendations and Priority Topics

To reduce the threat of antimicrobial resistance:

- Antibiotics should be used prudently in people and animals.
- The development of new antibiotics and alternatives for antibiotics, such as vaccines, should be stimulated. In addition, novel, high-quality diagnostic tools are needed to promote the efficacious use of these new medications.
- A warning system should be created to enable better risk assessments to support effective policy measures to contain resistance in hospital, care, community and agricultural settings. To achieve this, surveillance systems on antibiotic use and on resistance (in humans, animals, food and the larger environment) should be standardised, improved and extended.
- Interventions are needed to prevent infection and transmission of resistant bacteria. A better, quantitative understanding of the transmission routes of AMR between bacterial populations and between different (animal, human, food, environment) reservoirs is needed to support the development of strategies and interventions to minimize the spread of resistance. Research on the effectiveness of intervention strategies and how they can most effectively be implemented is also required.

Political and societal awareness on the threat of AMR is crucial to stimulate the implementation of measures to fight the misuse of antibiotics and to stimulate innovation. Knowledge transfer and intensive collaborations between scientists and policy makers is important for the successful adaptation of measures that positively impact on AMR, have social support and are cost effective.

So far, most efforts to tackle AMR have mostly looked at the medical and human angle. Environmental risk factors for the spread of resistant bacteria have not been assessed. There is a lack of systematic analysis of food contamination, in particular in relation to environmental contamination. It is also unclear how current practices in food production could contribute to the spread of AMR. Plus there is no systematic programme that addresses the impact of sewage on resistance in the environment, animals, food contamination and potable water. A holistic assessment of the contributions of pollution on the

environment with antibiotics, antibiotic residues and resistant bacteria is a must. This will lead to the development of strategies to minimize environmental contamination by antibiotics and resistant bacteria.

Under the umbrella of the Strategic Research Agenda, JPIAMR, with its collaborative activities (calls, workshops, international collaboration, etc.), aims to:

- Perform risk assessment studies to estimate the various transmission pathways from the environment to humans.
- Perform a meta-analysis of current national and international activities that are aimed at reducing the contamination of the environment by human and animal waste and by human activity with antibiotics and resistant bacteria.
- Determine the exact role of various environmental reservoirs (e.g., surface water, soil, air) on the emergence and dissemination of AMR.
- Understand the basic biological process that underlies these phenomena to develop remediate and preventative measures.

APPENDIX 7E: DEVELOPMENT AND EVALUATION OF MONITORING FOR SURVEILLANCE: ANTIMICROBIAL RESISTANT BACTERIA IN LEÓN, NICARAGUA AND CHAPEL HILL, NORTH CAROLINA

Ongoing work at the University of North Carolina, Chapel Hill, was reflected in the following poster, which was presented at the UNC Water & Health Conference in October 2015.

DEVELOPMENT AND EVALUATION OF MONITORING METHODS FOR SURVEILLANCE: ANTIMICROBIAL RESISTANT BACTERIA IN LEÓN, NICARAGUA, AND CHAPEL HILL, NORTH CAROLINA

Katy M. Brown[1], Claudia Perez[2], Erick Amaya[2], Daniel Reyes[2], Lydia Abebe[1], Sylvia Becker-dreps[3], Samuel Vilchez[2], and Mark Sobsey[1]

[1] Gillings School of Global Public Health, Dept. of Environ. Sci. Eng., UNC-Chapel Hill, USA; [2] Dept. Microbiology, Faculty of Medical Sciences, University of Nicaragua, León, Nicaragua; and [3] Dept. Family Med., UNC-Chapel Hill, USA

Average *E. Coli* as Seen in CHROMagar Orientation™ and Proportion of *E. Coli* ESBL and KPC Producers by Sites (León and Chapel Hill)

Water Sample Origin	León Sites	*E. Coli* CFU/100 mL[a]	% ESBL *E. Coli*	% KPC *E. Coli*
Raw sewage waters	Hospital sewage	5.13×10^6	31.4	0.2
	Raw sewage Cocal (urban)	2.19×10^7	3.95	--
	Secondary effluent Cocal	3.12×10^6	4.19	--
	Raw sewage Sutiaba (urban and hospital)	2.59×10^7	4.66	0.02
	Secondary effluent Sutiaba	1.61×10^6	1.86	0.01
	Raw sewage San Isidro (rural)	6.44×10^6	4.02	--
Recreational waters	Upstream Chiquito River	2.54×10^7	3.11	--
	Downstream Chiquito River (Cocal and Sutiaba)	1.79×10^5	2.84	--
	Downstream Quezalhauque River (San Isidro)	2.66×10^4	2.56	--
Water Sample Origin	**Chapel Hill Sites**	***E. Coli* CFU/100 mL**	**% ESBL *E. Coli***	**% KPC *E. Coli***
Raw sewage waters	Hospital sewage	2.00×10^6	18	12.1
	Raw sewage	3.5×10^6	22	17
	Secondary effluent	2.20×10^4	15	3
Recreational waters	Upstream Morgan Creek	4.30×10^2	19	1.3
	Downstream Morgan Creek	3.80×10^2		

[a] CFU = Colony forming unit. mL = Milliliter.

APPENDIX 7F: DRAFT PAPER ON DEVELOPING A RESEARCH AGENDA RELATED TO WATER, SANITATION, AND HEALTH WITH RESPECT TO ANTIMICROBIAL RESISTANCE AND THE WHO GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE

The following excerpts are from a draft paper on research needs related to water, sanitation, and health relative to antimicrobial resistance. The paper is expected to be submitted to the IWA Journal of Water and Health.

TOWARDS A RESEARCH AGENDA FOR WATER, SANITATION, AND ANTIMICROBIAL RESISTANCE

Susanne Wuijts[1], Harold van den Berg[1], Jennifer Miller[2], Lydia Abebe[3], Mark Sobsey[3], Antoine Andremont[4], Kate Medlicott[5], Ana Maria de Roda Husman[1,6], and workshop participants

[1]National Institute for Public Health and the Environment (RIVM); PO Box 1; 3720 BA Bilthoven, The Netherlands
[2] Virginia Tech, USA
[3] University of North Carolina, USA
[4] Diderot Medical School and Bichat Hospital Bacteriology Laboratory, France
[5] World Health Organization (WHO), Switzerland
[6] Institute for Risk Assessment Sciences (IRAS) of Utrecht University, The Netherlands
Corresponding author: susanne.wuijts@rivm.nl

Abstract

Clinically relevant antimicrobial resistant (AMR) bacteria are abundant at exposure-relevant sites suggesting risk for human exposure. Exposure-relevant sites become contaminated with these AMR bacteria and also antibiotic residues and resistance genes originating from wastewater and manure. Intervention strategies targeted at these sources of contamination could therefore limit emission of AMR bacteria to the environment.

In May 2015, WHO developed a Global Action Plan on AMR. To identify knowledge gaps with respect to the role of the environment in the spread of AMR bacteria, genes and residues and transmission to humans, a strategic research agenda was developed. To gather input, a workshop was organized by WHO in Lisbon, September 18, 2015, which was attended by scientists and other stakeholders in this domain.

Guidance is needed to reduce the spread of AMR to humans via the environment and to introduce effective intervention measures. Therefore, the health impact of exposure of humans to AMR in the environment should be quantified. Moreover, such impact needs to be compared to other exposures such as to humans directly, animals and through the consumption of food. Knowledge on the cost-effectiveness of possible interventions throughout the clinical, veterinary and environmental domains is required. Research in these areas is warranted at the earliest point in time.

Research Needs on AMR and WaSH (Water, Sanitation, and Health)

Research	Description
Identification and quantification of sources, occurrence, and transport	• Quantify total loads from humans and animals to the environment. • Identify and quantify AMR bacteria, genes and residues in different (water) exposure routes (drinking water, (treated) waste water, sludge and sludge application to land, irrigation water, grey water, etcetera) and determine the importance of each route in order to focus research capacity and to support advice to stakeholders and the public on adequate actions. Identify, localize and quantify sources that are hotspots of emission such as health care facilities. • Set up a uniform approach for this identification and quantification (HACCP as a suggestion). • Perform meta-analysis to determine persistence of AMR bacteria, genes and residues in different environmental matrices. • Determine the persistence of AMR bacteria, genes and residues in the environment.
Understanding the risk to human health	• Provide guidance on waste and wastewater management for the reduction of AMR bacteria, genes and residues in e.g. clinical, agricultural, household settings. • Quantify the role of horizontal gene transfer in waste and wastewater. • Identify ways of optimizing on site waste treatment for reduction of AMR bacteria, genes and residues. • Determine reduction efficiencies for applied water and waste treatment processes for AMR bacteria, genes and residues and other water facilities like household water treatment processes, reuse practices and new sanitation concepts.
Efficiency of water and wastewater treatment technologies	• Identify simple measurements (indicators) for system verification. • Incorporate AMR bacteria, genes and residues in WHO Water Safety Plan and Sanitation Safety Planning concepts. Which critical control points should be taken into account? • Develop guidance to water utilities and wastewater treatment plants on the reduction efficiencies of their applied treatment processes and overall treatment for AMR bacteria, genes and residues. • Develop guidance to different communities such as rural communities on the reduction efficiencies of AMR bacteria, genes and residues by local waste and wastewater practices.
Development of practical risk management systems and tools	• Exchange and link information, data and experiences from the environmental domain to industrial, clinical and veterinary domains and vice versa (epidemiology). • Identify target communities such as septic tank communities in rural settings and health care nurses in clinical and rural settings for awareness raising on what to do with waste and wastewater with respect to AMR and produce community specific educational materials. • Collect and provide evidence based information to the public on safety of drinking water with respect to AMR.

Research	Description
Identification of policies, practices, and tools	• Perform systematic review of the literature on regional/national policies and risk governance. • Determine effectiveness of regulations not specifically directed at reduction of AMR genes, bacteria and residues and develop indicators for monitoring of policy measures. • Derive treatment targets (Log Reduction Values) and other health based targets for exposure to AMR bacteria, genes and residues to determine the need for reduction.
Development of monitoring strategies, surveillance, and regulatory agents	• Provide guidance for the different purposes, methods and targets of AMR surveillance (use of tiered approach) and interpretation of AMR data and actions to be taken. • Select an index parameter and standard method to identify and quantify AMR bacteria, genes, and residues in the environment that also is applicable in low resource settings.

APPENDIX 8A: OPERATION AND MAINTENANCE PLAN

Table 8A-1: Components of an Operation and Maintenance Plan for a Direct Potable Reuse System

Component	Description
Staffing (i.e., for daily operations and emergencies)	• The direct potable reuse system will operate all day every day; therefore, appropriately trained staff will be needed to ensure it is operated properly and routine periodic maintenance is performed. • Water and/or wastewater operators are needed to manage day-to-day plant operations, allowing for the continuity of operation in the event of illness or vacation. • A wide range of skills and experience are required to operate the plant; therefore, it may be difficult to hire the required personnel. An alternative would be to use a contracted turnkey service provider to operate the plant with appropriately trained personnel. • Remote sensing capability is necessary to provide 24/7 surveillance. • A summary of the various tasks to be performed, along with the appropriate hours, can provide insight into the number of operators that would be needed to perform all of the required maintenance, sampling, and monitoring.
Operator training and certification	• The lead operators of a direct potable reuse system will need the highest level of certifications (Level 4 for either water or wastewater). It would be useful if the operators had both water and wastewater certifications. • Operators must be trained in and demonstrate an understanding of advanced treatment system operations for potable reuse. • The State Water Board should create a training program for each specific advanced treatment technology to be used for potable reuse, as well as a general training program to define the broader picture of public health protection, pathogen and pollutant targets, and so on. The training program could require a minimum of 16 hours per year to maintain a pool of higher level operators and advance the knowledge of advanced treatment systems throughout the State. • A separate direct potable reuse (advanced treatment) certification program could be developed, or an "endorsement" for direct potable reuse (advanced treatment) could be applied to a water or wastewater certificate.
Checklists for operations procedures (daily, weekly, and monthly)	• Use checklists developed with information provided by manufacturers to ensure routine procedures and duties are performed. • Checklists should include water quality sampling and monitoring to document treatment performance. • Incorporate monthly or other water quality sampling for compliance with State Water Board requirements.
Routine maintenance	• The most important aspect of operations is periodic maintenance of equipment and monitoring systems.

Component	Description
of equipment	• Identify routine maintenance as recommended by equipment manufacturers, and verify that online meters are properly integrated for each critical control point. • Determine the amount of hours and type of work needed to perform periodic maintenance and incorporate this information into the annual startup and shutdown plans. • Regularly perform the monitoring and calibration of online instruments to ensure they are functioning properly.
Critical spare parts and failure training	• Identify a list of critical spare parts needed onsite in the event of system failure. • Recommend periodic "failure" drills to verify that staff is trained and parts are available to make rapid repairs to equipment.
Control system (e.g., SCADA, shutdown procedures, and alarms)	• Operators need to be connected to the Supervisory Control and Data Acquisition (SCADA) system to constantly monitor system operations. • Program the SCADA system to alert operators when the system is not operating properly and to shut down the system if performance is compromised. • A phone, internet, or cloud-based messaging system could be used to notify operators during non-working hours if an alarm goes off. • The types of alarms that would generate these phone calls need to be determined to ensure operators respond swiftly to the situation. • System shutdown criteria need to be developed to automatically stop the system from allowing out-of-specification water to enter into the drinking water supply distribution system. These systems should be checked at least once per year.
Process monitoring and control	• Operators must know proper procedures for the calibration of online instruments, sampling and testing, and sensor testing. • Additional spare units may be needed to allow for easy change out if the instrument fails or calibration requires that the system be shut down for extended periods of time. • Develop process control during initial startup and verify with vendors, contractors, and operations staff.
Regulatory compliance	• Address regulatory compliance monitoring, including online instruments, daily sampling, monthly compliance sampling and testing, and others. • The State Water Board will need to determine the number and types of sampling required with online monitoring. • The State Water Board will need to determine the type and frequency of monitoring used to demonstrate compliance.
Frequency of monitoring	• Process monitoring is needed to monitor the performance of individual equipment or a collection of equipment. • Process monitoring should be based on manufacturer recommendations to ensure the proper operation and performance of equipment. • Process monitoring should be a combination of online instruments and water quality sampling. • Use the initial startup period to familiarize operators with equipment and various

Component	Description
	methods of process monitoring. • Employ the Supervisory Control and Data Acquisition (SCADA) system as a means of monitoring online instruments and processes during non-working hours. • The State Water Board will need to determine the frequency and types of monitoring used to demonstrate the protection of public health.
Drinking water supply distribution system	• Include periodic sampling of the drinking water supply distribution system during initial startup to determine chemical compatibility between existing drinking water supplies and advanced treated water. • Implement these tests prior to bringing the direct potable reuse project online and on a regular basis during operation. • Consider simple water quality testing comparing existing supplies to advanced treated water (or blend of the two), including pH, hardness, alkalinity, total ions and cations.
Response time to treatment failures or non-compliant water quality	• Operators should be available 24-hours a day, 7 days per week.

Source: Adapted from Crook, J., J.A. Cotruvo, A. Salveson, J.M. Stomp, and B.M. Thomson (2016). *Final Report of an NWRI Independent Advisory Panel: Recommended DPR General Guidelines and Operational Requirements for New Mexico*. J. Mosher and G.M. Vartanian, Eds. National Water Research Institute, Fountain Valley, CA.

APPENDIX 8B: ELEMENTS OF ENHANCED SOURCE CONTROL FOR DIRECT POTABLE REUSE

Table 8B-1: Principal Elements of an Enhanced Source Control Program for Direct Potable Reuse (Tchobanoglous et al., 2015)

Element	Description
Regulatory Authority	
Legal authority	Ensure that the source control program has sufficient legal authority to develop and implement source control measures, including authority for oversight/inspection, as well as plan and review new connections to the collection system.
Discharge permits	Ensure that industrial wastewater discharge permits and other control mechanisms can effectively regulate and reduce the discharge of COCs.
Enforcement	Ensure that the enforcement response program can identify and respond rapidly to discharges of COCs.
Alternative control programs	Consider alternative control mechanisms, such as BMPs or self-certification for zero discharge of pollutants, for classes of industries or commercial businesses.
Monitoring and Assessment of the Wastewater Collection System Service Area (Sewershed)	
Routine monitoring program	The influent to the WWTP and secondary or tertiary effluent sent to the AWTF are monitored routinely for regulated constituents and other COCs that may be discharged into the collection system service area.
Constituent prioritization program	COCs are identified and short-listed using results from the routine monitoring program. It may be necessary to develop separate monitoring programs for the constituents of greatest concern.
Evaluation of technically based local limits	Regulated constituents and other COCs are evaluated for their potential to cause interference, pass through an AWTF, or affect human and environmental health and safety. For the development of local limits, consider including a broader spectrum of COCs, such as (1) regulated and nonregulated constituents that are relevant for DPR (e.g., drinking water contaminants) or (2) CECs.
Source Investigations	
Industrial and commercial business inventory	Develop and maintain a frequently updated, comprehensive inventory of industries and businesses that may use products or chemicals containing COCs or generate intermediate COCs. For agencies with large service areas, multiple communities, or industrial flows coming from other wastewater entities, it may be desirable to link the inventory to a service area mapping tool such as a geographic information system network.
WWTP-AWTF joint response plan	The response plan includes a flow chart showing key responsibilities and decision points to either investigate or mitigate COCs being discharged into the collection system.
Maintenance of Current Inventory of Chemicals and Constituents	
Chemical inventory program	Develop and maintain a database of the chemicals stored and inventory volumes used annually by industrial and commercial producers and manufacturers in the service area. Potential sources of this type of information include the industries themselves, State Emergency Response Commission, Local Emergency Response Commission, or local fire departments.

Element	Description
Waste hauler monitoring program	A program is needed to monitor and track discharges of septic wastes or other wastewater delivered to the collection system by truck. Haulers should be permitted and required to provide chemical inventory and discharge information to the wastewater treatment authority before being allowed to discharge. Consideration should be given to requiring waste haulers to deliver to a different treatment facility.
Chemical fact sheets	Maintain a database of fact sheets for COCs encountered within the service area.
Public Outreach Program	
Industrial discharges	Provide (1) public outreach information on DPR to industries; (2) source control practices; and (3) compliance assistance and permit assistance to support the DPR program. Develop a program that encourages commercial and industrial dischargers to be partners in protecting the sewershed, such as environmental stewardship programs or award programs for consistent compliance. Assist and encourage industries and businesses that use chemicals that contain COCs to identify source control options, such as chemical substitution.
Service area pollution prevention partnership program	Develop a cooperative program with cities, counties, or other jurisdictions within the WWTP service area to disseminate information to the public about COCs and acceptable discharges to the sewer.
Public education and outreach program	Provide outreach to the public regarding the proper disposal of pharmaceuticals and household products containing chemicals that may be difficult to treat (e.g., what to flush and not flush). Consider developing a household hazardous waste collection program.
Education program	Develop school educational programs for grades 1 through 12 that address source control issues related to potable reuse.
Response Plan for Identified Constituents	
Interagency collaboration	The success of a source control program will depend on strong interagency cooperation and responsiveness between the WWTP and AWTF. For DPR projects that receive industrial waste from outside the service area, ensure that the agreement to accept the waste is consistent with source control program requirements. For DPR projects where the agency that administers the source control program is not the agency that operates the AWTF, consider entering into a memorandum of understanding or other contractual agreement so that appropriate source control actions can be taken, if necessary, to protect water quality.
Response to water quality deviations	Develop an action plan for responding to water quality deviations. For example, if a specific chemical constituent is detected at the AWTF, review operation and calibration records for online meters and any analytical methods that may be involved. If a problem is not identified, then notify the WWTP to initiate a review and inspection of the WWTP for possible sources of the constituent. If no source is found at the WWTP, then initiate a wastewater collection system sampling program. If a problem is identified, the action plan should include procedures for the operations staff to notify the source control staff to respond to and correct the issue and, if necessary, procedures for bypassing or shutting down the facility.

Notes: AWTF = Advanced water treatment facility. BMP = Best management practice. CEC = Constituent of emerging concern. COC = Constituent of concern. DPR = Direct potable reuse. WWTP = Wastewater treatment plant.

Sources: USEPA (2011) and APAI (2015).

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Tchobanoglous, G., J. Cotruvo, J. Crook, E. McDonald, A. Olivieri, A. Salveson, and R.S. Trussell (2015). *Framework for Direct Potable Reuse*, WateReuse Association, Alexandria, VA. http://www.twdb.texas.gov/publications/reports/contracted_reports/doc/1248321508_Vol2.pdf (accessed 9/3/2015).

USEPA (2011). *Introduction to the National Pretreatment Program*, (EPA-833-B-11-001). Office of Wastewater Management: U.S. Environmental Protection Agency: Washington, DC, 2011. http://water.epa.gov/polwaste/npdes/pretreatment/upload/pretreatment_program_intro_2011.pdf (accessed 9/3/2015).

APPENDIX 9A: INDIRECT POTABLE REUSE: DEFINING THE ROBUSTNESS OF THE ENVIRONMENTAL BUFFER

Prepared by Expert Panel Member Michael A. Anderson, Ph.D., University of California, Riverside

The defining characteristic of indirect potable reuse (IPR) is the presence of a substantive environmental buffer. The environmental buffer provides the following three important benefits:

- Storage of advanced treated water (ATW) for subsequent potable use.
- Attenuation of any contaminants that evade treatment resulting from the production of "off-spec" water.
- Time to respond to treatment plant upsets during production.

9A.1 Indirect Potable Reuse via Groundwater Recharge

From a regulatory perspective, IPR projects presently are limited to the use of groundwater basins as the environmental buffer. IPR via groundwater recharge can provide a substantial opportunity for the storage and use of ATW subject to the availability of a suitable groundwater basin. The interaction with and transport of ATW through soil and aquifer matrices also provides for the significant attenuation of contaminants via dilution and dispersion, removal of microbial contaminants via adsorption, straining and inactivation, and removal of chemical contaminants through adsorption and other reactions. Moreover, the slow rate of groundwater flow (often mm s^{-1}) further allows for ample time to react to a treatment plant upset before potentially inadequately treated water is extracted for potable use. Recently adopted groundwater recharge regulations defined the required level of robustness of the aquifer/environmental buffer in terms of both attenuation and time, specifically requiring at least 2-$\log_{10}$ virus reduction assuming 1-$\log_{10}$ per month inactivation or removal, and a minimum of 2-months elapsed time before 2 percent of treated water is extracted (t_2 value).

The capacity of the aquifer to serve as an effective environmental buffer providing both attenuation and time to respond, when compared with a direct connection between an advanced water treatment facility (AWTF) effluent and a surface water treatment plant or potable distribution line, is illustrated through the solution of the one-dimensional advection-dispersion-reaction equation (Thomann and Mueller, 1987):

$$C(x,t) = \frac{C_0}{2}\exp\left(\frac{-kx}{U}\right)\left[erf\frac{x-U(t-\tau)(1+\eta)}{\sqrt{4D_x(t-\tau)}} - erf\frac{x-Ut(1+\eta)}{\sqrt{4D_x t}}\right] \quad \text{(Equation 9A-1)}$$

where C is concentration, t is time, U is groundwater velocity, x is the longitudinal position, D_x is the longitudinal dispersion coefficient, k is the first order loss rate constant, τ is the pulse duration, and η =

kD_x/U^2. The dispersion coefficient is a product of the dispersivity of the media (α) and groundwater velocity.

In this example, it was assumed that the distance between the injection wells and extraction wells was 10 kilometers, groundwater velocity was 0.2 cm s^{-1}, dispersivity of the aquifer was 500 meters, and k was 0 d^{-1} (i.e., conservative contaminant) or 0.077 d^{-1} (equivalent to 1-$\log_{10}$ $month^{-1}$). The concentration of off-spec water exiting the AWTF and injected into the aquifer was assumed to be 100 for a duration of 1 day and, ignoring any dispersion or reaction in the pipeline, also would represent the pulse delivered via direct potable reuse (DPR) (**Figure 9A-1**, red line).

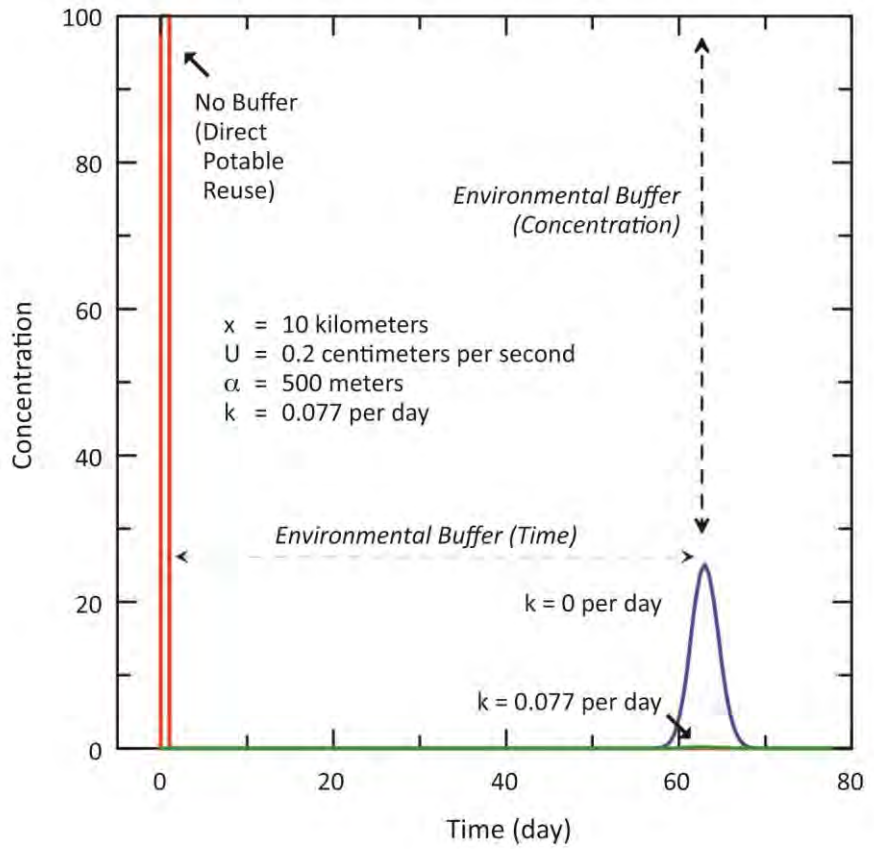

Figure 9A-1: Predicted concentration delivered to surface water treatment plant or distribution system following a 1-day treatment plant failure, comparing scenarios with no environmental buffer (i.e., direct potable reuse) (red line) and indirect potable reuse via groundwater recharge. Notes: Conservative contaminant = blue line. Reactive contaminant = green line.

In addition to providing storage of ATW, the groundwater basin also substantially reduces the concentrations delivered to downgradient extraction wells (see **Figure 9A-1**). For a conservative contaminant with negligible retention or reaction (i.e., k = 0 d^{-1}), in this example, dispersion lowers the peak concentration from 100 to approximately 25 (arbitrary units). The aquifer attenuated the concentration of any contaminants in off-spec water and lowered the concentration delivered to a treatment plant or consumer. In this example, the aquifer also provided significant time to respond:

60.6 days elapsed before 2-percent of the off-spec water reached the extraction well, while 50-percent of the off-spec water later reached the extraction well within a short timeframe (i.e., 63.8 days following its injection into the aquifer in). The duration of off-spec water at the extraction well was short, with 99 percent of the off-spec water delivered over a period of 8 days (see **Figure 9A-1**).

Allowing for reaction (e.g., inactivation of virus particles) further reduces the concentrations at the extraction well (see **Figure 9A-1**, green line). At this scale, it is difficult to see, but a very low concentration eluted after about 63 days and reached a maximum concentration of 0.2, corresponding to a 2.7-$\log_{10}$ reduction (i.e., 2-$\log_{10}$ reduction due to inactivation and 0.7-$\log_{10}$ reduction due to dispersion). Importantly, the aquifer serves as an environmental buffer by providing storage, attenuation of contaminants, and reduction in downstream concentration, and by providing for time to respond to treatment plant upsets.

9A.2 Indirect Potable Reuse via Surface Water Augmentation

As illustrated above, the groundwater basin serves as an effective environmental buffer for IPR projects where a suitable aquifer exists. In many regions, however, an adequate groundwater basin may not be available, either through insufficient storage capacity, poor hydraulic characteristics, contamination, or other factors. In such regions, an alternative approach is needed. A logical alternative is to supplement source supply or emergency storage reservoirs with ATW (i.e., surface water augmentation [SWA]).

Notably, surface water reservoirs inherently are more complex than groundwater basins. Whereas groundwater moves in response to spatial gradients in gravitational potential energy at velocities modulated by the hydraulic conductivity of the aquifer matrix, water movement in surface waters is driven by wind-forcing, convective mixing, and advective movement due to the momentum flux of inflowing and outflowing water. Wind velocities and directions can vary dramatically over the course of the day; strong diurnal changes in temperature and relative humidity also can drive convective mixing and transport processes. Advective motion due to inflows and outflows is strongly influenced by flow rates, velocities, depths, and the locations of inflow and outflow, as well as by bathymetry and other factors. Moreover, the thermal stratification present in most reservoirs during the summer limits vertical mixing and transport, with turbulent kinetic energy inputs driving motion that is effectively constrained to the epilimnion, followed by the deepening of the thermocline and subsequent mixing of the water column during the fall or winter.

In general, surface water reservoirs also have a number of beneficial uses (beyond serving as a municipal supply [MUN]) that are protected by the Clean Water Act. For example, many surface water reservoirs also provide habitat for wildlife (WILD) and warm-water fishery (WARM), as well as recreation (REC2 and, in some cases, REC1).

Defining the properties of a reservoir that constitute a robust environmental buffer is a principal challenge in developing regulatory criteria for SWA. As with IPR via groundwater recharge, the surface reservoir is expected to provide:

- Storage of ATW.
- Attenuation of contaminants and reduction in downstream concentrations relative to an input pulse of off-spec water.
- Time to respond to treatment plant upsets.

Thermal stratification offers an effective way to isolate warm inflowing water from cooler hypolimnetic water, allowing the selective withdrawal of bottom water (although the use of the thermocline as a barrier is restricted to the period of the year when the lake is thermally stratified). In many warm monomictic reservoirs in Southern California, this restriction can extend 8 to 10 months or more each year, while well-mixed conditions often are present for 1 to 3 months during the winter. Reservoir operation strongly affects these dynamics, and a reservoir with a moderate hydraulic residence time and withdrawal of water below the thermocline will weaken stratification and hasten mixing (Anderson et al., 2014). For a reservoir operated in this manner with a theoretical hydraulic residence of 4 to 6 months, one could reasonably expect the well-mixed period to extend for 6 months or more each year.

It is during this time that the intake at the drinking water treatment facility (DWTF) is most vulnerable; this period also overlaps with the greatest availability of recycled water. As a result, the well-mixed period is considered to be the greatest challenge for a reservoir to function as a sufficiently robust environmental buffer. Assuming that, during this time, the reservoir can be approximated as a continuous flow stirred-tank reactor (Thomann and Mueller, 1987), a relatively simple analytical expression allows for the calculation, under volumetric steady-state, of the concentration in the reservoir at time t (C_t) following a pulse input of off-spec water (Chapra, 1997; NWRI, 2015a):

$$°C_t = \frac{QC_{in}\,\Delta t}{V} e^{-\left(\frac{Q}{V}+k\right)t} \qquad \text{(Equation 9A-2)}$$

where Q is the volumetric flow rate in (and out) of the reservoir, C_{in} is the concentration of a contaminant in the influent (as noted above, assumed here to be 100), Δt is the duration of a pulse of off-spec water (1 day), V is the volume of the reservoir, and k is the first-order loss rate constant (0 or 0.077 d^{-1} for conservative and reactive contaminants, respectively). Because the reservoir is assumed to be well-mixed, the concentration in the reservoir at time t is equivalent to that exiting the reservoir and delivered to a surface drinking water treatment plant. For these calculations, the reservoir was assumed to have a volume of 10,000 acre-feet and Q was 27 million gallons per day (83 acre-feet per day), corresponding to an average hydraulic residence time (t_r) of 4 months.

Subject to these assumptions, a dramatic reduction occurred in the concentration in the reservoir (and delivered to a DWTF) when a pulse of off-spec water is routed to the surface reservoir, lowering the concentration from 100 to 0.83 (**Figure 9A-2**). The reservoir, therefore, serves as a very effective environmental buffer with respect to concentration by diluting pulse inputs of both conservative and reactive contaminants (see **Figure 9A-2**). It differs from IPR via groundwater recharge, where the concentration of the conservative contaminant was reduced only modestly via dispersion from 100 to 25 (see **Figure 9A-1**). In this example, the surface water reservoir was 30 times more effective as an environmental buffer for an unreactive contaminant compared to the aquifer. For a reactive contaminant with a k value of 0.077 d^{-1}, rapid mixing of the off-spec water into the reservoir yielded an equivalent peak concentration of 0.83, a value slightly higher than that for the aquifer (i.e., 0.2) (see **Figure 9A-1**), but (in both cases) achieving greater than 2-$\log_{10}$ reduction in concentration relative to no environmental buffer (DPR).

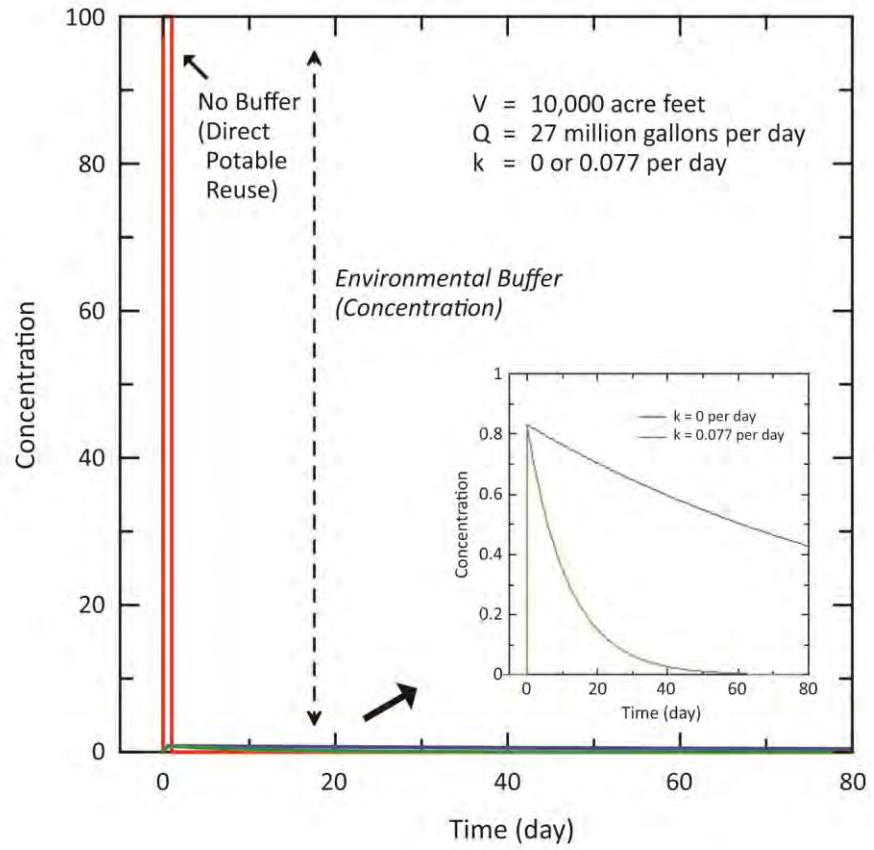

Figure 9A-2: Predicted concentration delivered to surface water treatment plant or distribution system following a 1-day treatment plant failure, comparing scenarios with no environmental buffer (i.e., direct potable reuse) (red line) and indirect potable reuse via surface water augmentation. Notes: Conservative contaminant = blue line. Reactive contaminant = green line.

The effectiveness of the reservoir as an environmental buffer (i.e., attenuating the concentration of any contaminants resulting from a pulse of off-spec water when ATW represents the inflow) can be shown to be related to the average hydraulic residence time. Immediately following the discharge of a pulse of off-spec water into the reservoir (i.e., elapsed time t=0), **Equation 9A-2** reduces to:

$$C = \frac{QC_{in}\Delta t}{V} \qquad \text{(Equation 9A-3)}$$

that can be further rearranged to:

$$\frac{C_{in}}{C} = \frac{V}{Q\Delta t} \qquad \text{(Equation 9A-4)}$$

Because C_{in}/C is equivalent to the dilution factor (DF) for off-spec water within the reservoir and V/Q is the hydraulic residence time (t_r) (i.e., the average length of time water and contaminants reside in the reservoir), **Equation 9A-4** can be reduced further to:

$$DF = \frac{t_r}{\Delta t} \quad \text{(Equation 9A-5)}$$

Consequently, for reservoirs receiving ATW as the sole inflow and operated at volumetric steady-state, the amount of dilution achieved for a pulse of off-spec water is directly proportional to the t_r value and, for a 1-day pulse of off-spec water, is equivalent to the t_r value. Thus, a SWA project using a reservoir with a t_r value of 2-months would, under winter well-mixed conditions receiving a 1 day pulse of off-spec water, achieve an ideal dilution of 60, a t_r value of 4 months would achieve an ideal dilution of 120, and so on (**Table 9A-1**).

Table 9A-1: Properties of Surface Water Reservoir Represented as Continuous Flow Stirred-Tank Reactor during the Winter Well-Mixed Period Subject to a 1-Day Pulse of Off-Specification Advanced Treated Water in an Indirect Potable Reuse Project

t_r (months)	Dilution Factor[a]	% *In Situ* Removal	t_2 (days)	t_{50} (days)
1	30	69.8	0.7	21
2	60	82.2	1.3	42
3	90	87.4	2.0	63
4	120	90.2	2.7	84
5	150	92.0	3.3	104
6	180	93.3	4.0	125

[a] Assumes the advanced treated water flow constitutes total flow through the system.

The dilution factors presented in **Table 9A-1** represent idealized values assuming a 1-day pulse of off-spec water that is completely mixed in the reservoir. In practice, dilution values during the winter likely will be lower than these values due to incomplete mixing and short-circuiting; the amount of deviation from ideal values will be a function of siting the inlet and outlet, bathymetry, meteorological conditions, and other factors. In contrast, concentrations in water drawn from the hypolimnion during the summer (not shown) would yield a larger apparent dilution of off-spec water. Notwithstanding, it is useful to note that a reservoir at approximate volumetric steady-state, receiving ATW as the primary inflow, and with a t_r value of 4 months, achieves much greater attenuation of unreactive contaminant concentrations as compared to groundwater recharge (30 times), while providing similar (>2-$\log_{10}$) attenuation of reactive contaminant concentration (see **Figure 9A-1**).

In situ removal of contaminants in SWA under idealized mixing conditions also can be compared to groundwater recharge. The concentration of unreactive contaminants in the surface reservoir decreases over time due only to flushing (see **Equation 9A-2** and **Figure 9A-2**, inset-blue line), while reactive contaminants are removed through both flushing and reaction (NWRI, 2015a). The relative

importance of these two processes can be calculated from their respective rate constants and also are reflected in the amount of *in situ* removal (e.g., via inactivation) (see **Table 9A-1**). As a result, about 70 percent of the reactive contaminant in a reservoir with a 1-month mean hydraulic residence time is removed via inactivation or another reaction (and 30 percent is exported), while 90 percent (1-$\log_{10}$) of reactive contaminant is removed via reaction in a reservoir with a t_r value of 4 months. The percent of *in situ* removal increases with increasing t_r, albeit in a non-linear way, with much slower increases at larger t_r values. Much of the removal occurs within 1 to 2 months such that there is comparatively less advantage gained with respect to the percent of *in situ* removal with increasing t_r values beyond 2 to 3 months for reactive contaminants with large k values (although removal extends for a longer period of time for contaminants with lower k values) (NWRI, 2015a).

Surface water augmentation does differ in a complicated way from groundwater recharge with respect to the time to respond. IPR projects with groundwater recharge are required to have a t_2 value (i.e., elapsed time for 2-percent extraction) of at least 2-months. As noted above, comparatively little additional time elapses before 50 percent of the off-spec water (or contaminant mass within that water) reaches the extraction well (i.e., t_{50} in the above groundwater example was only 3.2-days longer than the t_2 value). With advection being the principal transport mechanism in groundwater systems, flow typically is laminar and quite slow. In contrast, much more rapid turbulent flow is in place in surface water reservoirs; therefore, for the winter well-mixed reservoir condition, a much shorter period of time is expected to elapse before 2 percent of the off-spec water or contaminants are exported from the reservoir, while a much longer period of time is required before 50 percent is exported (see **Table 9A-1**). Unlike the groundwater basin in which off-spec water travels in a well-defined pulse with little relative difference between t_2 and t_{50} values (see **Figure 9A-1**), off-spec water (or contaminants in that water) are flushed out more slowly from a surface water reservoir, with months elapsing between the time that 2 percent and 50 percent of off-spec water or contaminant mass are exported (see **Figure 9A-2** and **Table 9A-1**).

The use of t_r as a measure of robustness holds for reservoirs receiving any combination of AWT, imported water, and local watershed flows. The value of C_{in} in reservoirs receiving flow from a number of different sources simply is the flow-weighted mean concentration for any contaminant or tracer, and t_r, t_2 (and t_{50}), and percent *in situ* removal values are all equivalent to the values reported in **Table 9A-1** as reservoir volume V, outflow rate Q, and reaction rate constant k are the key parameters (see **Equation 9A-2**). The only difference is with respect to the amount of dilution achieved for off-spec ATW, with the amount of dilution *increasing* relative to the values calculated using **Equation 9A-5** or reported in **Table 9A-1**. Consequently, for a reservoir in which a fraction (f) of total flow is derived from an AWTF, the amount of dilution of off-spec ATW comprising a fraction of inflow (DF_f) would be given by:

$$DF_f = \frac{t_r}{f\Delta t} \qquad \text{(Equation 9A-6)}$$

For a reservoir with a t_r value of 2 months receiving one-half of its flows from an AWTF and one-half from imported water, the amount of dilution of off-spec water would be 120, a value two times that compared with a system where ATW flows comprise essentially all the flows delivered to the reservoir (see **Table 9A-1**). As previously noted, other attributes are unchanged because total flow and reservoir volume remain the same (e.g., as given by **Equation 9A-2**).

This analysis indicates that the routing of ATW through a surface water reservoir can provide benefits comparable to groundwater recharge, and further suggests that the robustness of the surface water reservoir as an environmental buffer can be described conveniently by the theoretical hydraulic residence, t_r. In turn, the t_r value provides a simple measure of the average amount of dilution that might be expected during well-mixed conditions, amount of contaminant removal via inactivation or other loss processes, and amount of time that a given fraction of a contaminant is exported from the reservoir. A t_r value of 12 months was included in preliminary SWA concept criteria (Hultquist, 2014), although following review, the Expert Panel recommended a value of 4 to 6 months (NWRI, 2015b). Subsequently, a value of 6 months was incorporated by the State Water Resources Control Board into draft criteria.

9A.3 References

Anderson, M.A., A. Komor, and K. Ikehata (2014). "Flow routing with bottom withdrawal to improve water quality in Walnut Canyon Reservoir, California." *Lake Reserv. Manage,* 30: 1321-142.

Chapra, S.C. (1997). *Surface Water Quality Modeling,* McGraw-Hill, New York, NY.

Hultquist, B. (2014). *Surface Water Augmentation: Draft Criteria Elements.* Presented at an NWRI-Expert Panel meeting held on July 28, 2014, in San Diego, CA.

NWRI (2015a). *Final Panel Meeting Report #4: Surface Water Augmentation – IPR Criteria Review* (Based on Panel Meeting Held March 11-12, 2015). National Water Research Institute, Fountain Valley, CA.

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Thomann, R.V., and J.A. Mueller (1987). *Principles of Surface Water Quality Modeling and Control,* Harper & Row, New York, NY.

APPENDIX 9B: DIRECT POTABLE REUSE DEMONSTRATION PROJECT IN SAN DIEGO, CALIFORNIA

For the development of the process performance distribution functions used in **Chapter 9**, the following treatment and water quality conditions (Pecson et al., 2016) were assumed, as summarized in **Tables 9B-1** to **9B-6**.

9B-1 Summary of Design Criteria for Unit Processes at the Direct Potable Reuse Demonstration Facility in San Diego, California

Table 9B-1: Design Criteria for Ozone System

Ozone Generation and Injection System	
Design Flow	1.6 million gallons per day (1,100 gallons per minute)
Generator Capacity	190 pounds per day at 10 percent
Maximum Applied Dose	14.3 milligrams per liter (1,100 gallons per minute at 10 percent)
Manufacturer	Wedeco
Ozone Contactor (Pipeline)	
Pipeline Diameter	24 inches
Pipeline Length	6 × 60 foot segments
Total Volume	7,800 gallons
Contact Time	7.5 minutes at 1.5 million gallons per day
Design CT[a]	14 milligram-minutes per liter
Baffling Efficiency	97.8 percent T_{10}/T_{HRT}[b]
T_{10}	6.85 minutes at 1,100 gallons per minute

[a] CT = Residual disinfectant concentration, C (in milligrams per liter), multiplied by the contact time, T (in minutes).

[b] Based on tracer study approved by the Division of Drinking Water of the State Water Resources Control Board.

Table 9B-2: Design Criteria for Biologically Activated Carbon (BAC)

BAC Filters	
Number of Filters	2
Area per Filter	180 square feet
Media Depth	6.5 feet
Filter Loading Rate	3.05 gallons per minute per square foot at 1,100 gallons per minute 1.53 gallons per minute per square foot at 550 gallons per minute

BAC Filters	
Empty Bed Contact Time	15 minutes at 1,100 gallons per minute 30 minutes at 550 gallons per minute
Filter Media	Granular activated carbon 31 tons of 8 × 20 mesh (Effective Size of 0.8 to 1.0 millimeter)
Air Scour Rate	720 standard cubic feet per minute
Backwash Rate	12 gallons per minute per square foot (2,160 gallons per minute)

Table 9B-3: Design Criteria for Microfiltration and Ultrafiltration Systems

Microfiltration	
Net Product Flow	0.625 million gallons per day
Nominal Pore Size	0.1 micrometer
Number of Modules	50
Area per Module	538 square feet
Instantaneous Flux	29 gallons per square foot per day
Recovery	93 percent
Enhanced Flux Maintenance, Backwash Chemicals	None
Chemical Cleaning Frequency	>3 months
Manufacturer	Pall Corporation
Ultrafiltration	
Net Product Flow	0.625 million gallons per day
Nominal Pore Size	0.015 micrometer
Number of Modules	33
Area per Module	775 square feet
Instantaneous Flux	33 gallons per square foot per day
Recovery	95 percent
Enhanced Flux Maintenance, Backwash Chemicals	None
Chemical Cleaning Frequency	>3 months
Manufacturer	Toray (modules) H2O Innovation (system)

Table 9B-4: Design Criteria for Reverse Osmosis System

Reverse Osmosis Train A	
Net Product Flow	0.5 million gallons per day
Membrane Manufacturer and Type	Hydranautics ESPA2 LD
Number of Elements	105
Area per Element	400 square feet
Number of Elements per Vessel	7
Number of Vessels	10 (Stage 1); 5 (Stage 2)
Instantaneous Flux	12 gallons per square foot per day
Recovery	75 to 80 percent
Chemical Cleaning Frequency	>3 months
Manufacturer (System)	EnAqua
Reverse Osmosis Train B	
Net Product Flow	0.5 million gallons per day
Membrane Manufacturer and Type	Toray TML20-400
Number of Elements	108
Area per Element	395 square feet
Number of Elements per Vessel	6
Number of Vessels	10 (Stage 1) 5 (Stage 2) 3 (Stage 3)
Instantaneous Flux	12 gallons per square foot per day
Recovery	75 to 80 percent
Chemical Cleaning Frequency	>3 months
Manufacturer (System)	EnAqua

Table 9B-5: Design Criteria for Ultraviolet Light/Advanced Oxidation Process System

Ultraviolet Light /Advanced Oxidation Process	
Design Flow	1.0 million gallons per day
Number of Lamps	72
Watts per Lamp	240 watts
Total Power	17.3 kilowatts
Design Ultraviolet Transmission	95 percent
Electrical Energy per Order (EE/O) for N-Nitrosodimethylamine (NDMA)	0.18 kilowatt hours per 1,000 gallons
Electrical Energy per Order (EE/O) for 1,4-Dioxane	0.46 kilowatt hours per 1,000 gallons
Manufacturer	TrojanUV
Oxidant for Advanced Oxidation Process	Hydrogen Peroxide

9B-2 Water Quality Conditions

A summary is provided in **Table 9B-6** of the average, fifth percentile, and ninety-fifth percentile of important water quality parameters throughout the treatment train.

Table 9B-6: Average Percentile of Water Quality Parameters Throughout the Treatment Train for Direct Potable Reuse

Parameter	Ozone Influent	BAC Influent	MF/UF Influent	Reverse Osmosis Influent	UV/AOP Influent
	Avg. (5^{th} / 95^{th})	Avg. (5^{th} / 95^{th})	Avg. (5^{th} / 95^{th})	Avg. (5^{th} / 95^{th})	Avg. (5^{th} / 95^{th})
Total organic carbon (mg/L)	7.4 (7.0 / 8.9)	7.7 (7.1 / 8.6)	4.8 (4.3 / 5.1)	5.2 (3.7 / 7.0)	0.31 (0.30 / 0.34)
Turbidity (NTU)	--	0.21 (0.15 / 0.41)	0.16 (0.12 / 0.30)	0.03 (0.02 / 0.04)	--
Nitrite (mg/L as N)	0.06 (0.01 / 0.20)	0.0053 (<0.0049 / 0.0072)	--	0.013 (<0.0049 / 0.031)	0.005[a] (<0.0049 / <0.0049)
Ammonia (mg/L as N)	0.27 (0.04 / 1.5)	0.40 (0.04 / 2.5)	--	1.1 (0.7 / 1.5)	0.39 (0.14 / 0.65)
Total suspended solids (mg/L)	<2.5	<2.5	<2.5	<2.5	<2.5
Ultraviolet absorbance (cm^{-1})	0.17 (0.16 / 0.18)	0.07 (0.06 / 0.09)	0.053 (0.045 / 0.059)	0.058 (0.051 / 0.066)	0.018 (0.005 / 0.061)
Iron (µg/L)	89 (65 / 121)	--	--	57 (28 / 84)	51[a] (<50 / <50)
Manganese (µg/L)	75.7 (46.3 / 94.2)	--	--	31 (0.3 / 89)	0.15 (0.04 / 0.84)
pH	7.0 (6.9 / 7.2)	--	--	7.2 (6.9 / 7.5)	6.9 (5.7 / 8.1)
Alkalinity (mg/L)	120 (109 / 138)	--	--	123 (103 / 142)	13 (10 / 23)

[a] Only a single data point was detected above the method detection limit; as a result, the ninety-fifth percentile value is lower than the average value.

mg/L = Milligram per liter. NTU = Nephelometric turbidity unit. N = Nitrogen. cm = Centimeter. µg/L = Microgram per liter. BAC = Biological activated carbon. MF = Microfiltration. UF = Ultrafiltration. UV = Ultraviolet light disinfection. AOP = Advanced oxidation process.

9B-3 Reference

Pecson, B., S. Triolo, R.S. Trussell, and Arc-Alternatives (2016). *San Diego Pilot Plant Reliability Analysis and Discussion*. Slide presentation given on January 29, 2016, to the Expert Panel on the Development of Water Recycling Criteria for Indirect Potable Reuse through Surface Water Augmentation and the Feasibility of Developing Criteria for Direct Potable Reuse.

APPENDIX 11A: PROJECTS UNDER THE WATEREUSE DIRECT POTABLE REUSE RESEARCH INITIATIVE

Through the WateReuse Direct Potable Reuse Research Initiative, the Water Environment & Reuse Foundation (formerly, the WateReuse Research Foundation) has invested over $20 million in research projects since 2011 to assist the Expert Panel in completing its state-mandated charge of investigating the feasibility of developing uniform water recycling criteria for direct potable reuse. These projects involve investigating different aspects of the technical feasibility of implementing direct potable reuse, such as the reliability of treatment trains, microbial and chemical water quality, monitoring, and operations. A list of these projects is provided in **Table 11A-1**.

Table 11A-1: List of Research Projects Supported by the Water Environment & Reuse Foundation through the WateReuse Direct Potable Reuse Research Initiative

Project No.	Project Title	Principal Investigator(s)
WRRF-11-01	Monitoring for Reliability and Process Control of Potable Reuse Applications	Ian Pepper, University of Arizona
WRRF-11-02	Equivalency of Advanced Treatment Trains for Potable Reuse	R. Rhodes Trussell, Trussell Technologies, Inc.
WRRF-11-05	Demonstrating the Benefits of Engineered Direct Potable Reuse versus Unintentional Indirect Potable Reuse Systems	Glen Boyd, The Cadmus Group, Inc.
WRRF-11-10	Risk Reduction Principles for Direct Potable Reuse	Andrew Salveson, Carollo Engineers
WRRF-12-06	Guidelines for Engineered Storage for Direct Potable Reuse	Andrew Salveson, Carollo Engineers
WRRF-12-07	Methods for Integrity Testing of Nanofiltration and Reverse Osmosis Membranes	Joseph Jacangelo, MWH
WRRF-13-02	Model Public Communication Plan for Advancing Direct Potable Reuse Acceptance	Mark Millan, Data Instincts
WRRF-13-03	Critical Control Point Assessment to Quantify Robustness and Reliability of Multiple Treatment Barriers of Direct Potable Reuse Scheme	Troy Walker, Hazen & Sawyer
WRRF-13-12	Evaluation of Source Water Control Options and the Impact of Selected Strategies on Direct Potable Reuse	Alan Rimer, Black & Veatch
WRRF-13-13	Development of Operation and Maintenance Plan and Training and Certification Framework for Direct Potable Reuse Systems	Troy Walker, Hazen & Sawyer
WRF4508	Assessment of Techniques to Evaluate and Demonstrate the Safety of Water from Direct Potable Reuse Treatment Facilities	Channah Rock, University of Arizona
WRF4536	Blending Requirements for Water from Direct Potable Reuse Treatment Facilities	Andrew Salveson, Carollo Engineers
WRRF-14-01	Integrated Management of Sensor Data for Real Time Decision Making and Response	Jeff Neeman, Black & Veatch
WRRF-14-02	Establishing Additional Log Reduction Credits for Wastewater Treatment Plants	Zia Bukhari, American Water

Project No.	Project Title	Principal Investigator(s)
WRRF-14-03	Develop Methodology of Comprehensive (Fiscal/Triple Bottom Line) Analysis of Alternative Water Supply Projects Compared to Direct Potable Reuse	Ben Stanford, Hazen & Sawyer
WRRF-14-08	Economics of Direct Potable Reuse	Robert Raucher, Stratus Consulting
WRRF-14-10	Enhanced Pathogen and Pollutant Monitoring of the Colorado River Municipal Water District Raw Water Production Facility at Big Spring, Texas	Eva Steinle-Darling, Carollo Engineers
WRRF-14-12	Demonstrating Redundancy and Monitoring to Achieve Reliable Potable Reuse	R. Shane Trussell, Trussell Technologies, Inc.
WRRF-14-13	From Sewershed to Tap: Resiliency of Treatment Processes for Direct Potable Reuse	Sharon Waller, Sustainable Systems, LLC.
WRRF-14-14	Framework for Public Health Monitoring: Workshop	Jeffrey Soller, Soller Environmental, LLC
WRRF-14-15	Application of Bioanalytical Tools to Assess Biological Responses Associated with Water at Direct Potable Reuse Facilities	To Be Determined
WRRF-14-16	Operational, Monitoring, and Response Data from Unit Processes in Full-Scale Water Treatment, Indirect Potable Reuse, and Direct Potable Reuse	Andrew Salveson, Carollo Engineers
WRRF-14-17	White Paper on the Application of Molecular Methods for Pathogens for Potable Reuse	Krista Wigginton, University of Michigan
WRRF-14-18	Ensuring Stable Microbial Water Quality in Direct Potable Reuse Distribution Systems	WRRF Workshop
WRRF-14-19	Predicting Reverse Osmosis Removal of Toxicologically Relevant Unique Organics	Kerry Howe, University of New Mexico
WRRF-14-20	Developing Direct Potable Reuse Guidelines	Jeffrey Mosher, National Water Research Institute
WRRF-15-01	Direct Potable Reuse Research Compilation: Synthesis of Findings from Direct Potable Reuse Initiative Projects	Jeffrey Mosher, National Water Research Institute
WRRF-15-02	Creating a Roadmap for Bioassay Implementation in Reuse Waters: A Cross Disciplinary Workshop	To Be Determined
WRRF-15-04	Characterization and Treatability of Total Organic Carbon from Direct Potable Reuse Processes Compared to Surface Water Supplies	Larry Schimmoller, CH2M
WRRF-15-05	Developing Curriculum and Content for Direct Potable Reuse Operator Training	Ben Stanford, Hazen & Sawyer
WRRF-15-07	Molecular Methods for Measuring Pathogen Viability/Infectivity	To Be Determined
WRRF-15-10	Optimization of Ozone/Biologically Activated Carbon Treatment Processes for Potable Reuse Applications	Zia Bukhari, American Water
WRRF-15-11	Demonstration of High Quality Drinking Water Production Using Multi-Stage Ozone-Biological Filtration: A Comparison of Direct Potable Reuse with Existing Indirect Potable Reuse Practice	Kati Bell, CDM Smith, and Denise Funk, Gwinnett County Department of Water Resources
WRRF-15-13	N-Nitrosodimethylamine (NDMA) Precursor Control Strategies for Direct Potable Reuse	Roshanak Aflaki, Los Angeles Sanitation