EPA/600/R-03/111
September 2003

Managing Urban Watershed Pathogen Contamination

by

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Notice

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Abstract

This document is written as a resource for state and local watershed managers who have the responsibility of managing pathogen contamination in urban watersheds. In addition it can be an information source for members of the public interested in watershed mitigation efforts aimed at reducing microbial contamination. It is written to support specific steps of the total maximum daily load (TMDL) process for meeting water quality standards in urban watersheds. The information provided can also support watershed evaluations conducted when disease outbreaks occur in the absence of standards violations. The document discusses the regulation of waterborne pathogens (Chapter 1), detection methods (Chapter 2), and combined sewer overflow control technologies and stormwater best management practices (Chapter 3). The table below identifies the steps of the TMDL process supported by each of the chapters.

The intent is to supplement the information included in the EPA document *Protocol for Developing Pathogen TMDLs*, Office of Water, January 2001, EPA 841-R-00-002 guidance. This document was developed using information collected through extensive literature reviews by researchers in the Urban Watershed Management Branch (UWMB) of EPA's National Risk Management Research Laboratory. The final document will be an official EPA report available through the UWMB Internet site http://www.epa.gov/ednnrmrl/.

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Hugh W. McKinnon, Director
National Risk Management Research Laboratory

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Acknowledgments

This report is a compilation of literature, reviewed, analyzed, and submitted by the U.S. EPA's National Risk Management Research Laboratory, Water Supply and Water Resources Division, Urban Watershed Management Branch (UWMB), Edison, NJ. This report contributes to EPA's long term goal to provide the tools to restore and protect aquatic systems and to forecast the ecological, economic, and human health outcomes of alternative solutions. The report preparation commenced in September 2002, and the report was completed in September 2003. Annual performance requirements are fulfilled upon report completion.

Editor for this literature review is Joyce Perdek. Reviewers, who provided instrumental comments, include Cecil Lue-Hing – President of Environmental and Water Resources Institute, Sydney Munger and Charles Wisdom of Parametrix, Inc. (Kirkland, WA), Peter Swenson of U.S. EPA Region 5, and Stephen Schaub and Don Waye of U.S. EPA Office of Water. In addition several members of the UWMB provided valuable review, advice, and input. These reviewers are Michael Borst, Carolyn Esposito, Chi-Yuan Fan, Richard Field, Bethany Madge, Thomas O'Connor, Ariamalar Selvakumar, Daniel Sullivan, and Anthony Tafuri. Judy Norinsky of the Environmental Careers Organization served as technical editor. The authors also wish to thank fellow EPA researchers Mark Meckes, Frank Schaefer, and Joyce Simpson who offered their microbiological expertise to assist in preparation of this and other UWMB pathogen projects.

Chapter One

Regulating Waterborne Pathogens

1.1 Introduction

Pathogens, disease causing microorganisms, are a major concern for managers of water resources. Once in a water body, pathogens infect humans through contaminated fish and shellfish, skin contact, or ingestion of water. Protection from pathogen contamination is most important for waters designated for (1) recreation, (2) public water supplies, (3) aquifer protection, and (4) protection and propagation of fish, shellfish, and wildlife. These uses are rigorously dealt with in Section 303(c) of the Clean Water Act (CWA) (U.S. EPA, 2001a). Data on U.S. water bodies in violation of microbiological ambient water quality standards, established by the states, for the years 1999 and 2000 are presented in Table 1-1.

The Maximum Contaminant Level Goals (MCLGs) established under the Safe Drinking Water Act are zero for all pathogens. These goals conform to the position of the World Health Organization (WHO) (1993):

> "....there is **no tolerable** lower limit for pathogens, and water intended for consumption, for preparing food and drink, or for personal hygiene should thus contain no agents pathogenic for humans."

The WHO estimates that 13 million people die from waterborne infections each year. The majority of these deaths occur in developing countries. However, in the U.S. approximately 900,000 cases of illnesses and 900 deaths occur each year as a result of microbial contamination of drinking water (Warrington, 2001a).

A pathogen may be a bacterium, protozoan, virus, worm, or fungi. Generally, waterborne pathogens are in human and animal feces, and are deposited directly into water bodies or transported to water bodies by overland flow and/or subsurface water flow. Urban pathogens are transported by stormwater runoff, combined sewer and sanitary sewer overflows, and wastewater treatment plant effluents. Pathogenic microorganisms originate from many animal species in watersheds including wildlife, pets and companion animals, and agricultural animals. There is increasing interest in the potential for molecular fingerprinting methods, also known as microbial source tracking techniques, for identification of pathogen sources (Simpson *et al.*, 2002). The majority of large scale pathogenic waterborne outbreaks in the past have been attributed to human contamination or inadequacies at water treatment plants. The most current waterborne

Table 1-1. U.S. Microbial Water Quality Assessments Summary – 1999 and 2000
Rivers and Streams • 19% of U.S. river and stream miles assessed • 39% of assessed river and stream miles impaired • Pathogens (bacteria) are leading cause of impairment • Agriculture is the primary source of impairment
Ocean Shorelines • 6% of U.S. ocean shoreline miles assessed • 14% of assessed shoreline miles impaired • Pathogens (bacteria) are leading cause of impairment • Urban runoff/storm sewers are primary source of impairment
Great Lakes Shorelines • 92% of U.S. Great Lakes shoreline miles assessed • 78% of assessed shoreline miles impaired • Pathogens (bacteria) are third leading cause of impairment • Contaminated sediments are the primary source of impairment
Estuaries • 36% of U.S. estuarine square miles assessed • 51% of assessed estuaries square miles impaired • Pathogens (bacteria) are fourth leading cause of impairment • Municipal point sources are primary source of impairment
Lakes, Reservoirs, and Ponds • 43% of U.S. lake, pond and reservoir acres assessed • 45% of assessed lake acres impaired • Pathogen (bacteria) are not a leading cause of impairment • Agriculture is the primary source of impairment

U.S. EPA, 2002a

outbreaks upon contact with contaminated recreational water bodies are attributed to human fecal contamination or sewage (Levy *et al.*, 1998; Upton, 1999).

Rosen (2000) identified the following characteristics of waterborne pathogens of concern:

1. The organisms are shed into the environment in high numbers, or they are highly infectious to humans at low doses.
2. The organism can survive and remain infectious in the environment for long periods or they are highly resistant to water treatment.
3. Some types of bacterial pathogens can multiply outside of a host under favorable environmental conditions.

Identifying the microorganisms causing water quality standard violations or waterborne disease outbreaks is the first step in managing watershed microbial contamination. Emerging

pathogens are disease agents that were unknown or not associated with water 10 to 20 years ago. Many emerging pathogens are not new, but are only now associated with waterborne disease. These novel disease-bearing microbes are engendered by a complex mixture including social, political, economic, ecological, and technological factors, and are prone to arise among an immuno-compromised population. *Cryptosporidium parvum*, *Legionella*, and *E. coli* O157:H7 are preeminent waterborne emerging pathogens (Cliver, 2000; U.S. EPA, 2001b).

For U.S. water bodies not meeting state-established water quality standards for microbial contaminants, a Total Maximum Daily Load (TMDL) must be developed. A TMDL is defined as the maximum amount of a pollutant that a water body can receive and still meet the water quality standard, and an allocation of that amount to the pollutant's sources. Usually, the TMDL target level will be the numeric water quality criteria maximum for the microorganism for which the standard was exceeded. In some cases, when the water quality standard does not sufficiently reflect the use impairment, it is appropriate to develop and meet an alternative standard. Examples of use impairments include waterborne disease outbreaks, degraded fisheries, and restrictions on using the water body for the desired use of primary contact recreation. For these situations, U.S. EPA recommends using a supplemental microorganism to provide additional means for measuring attainment of designated or existing uses (U.S. EPA, 2001a).

This chapter provides information to support the first two steps of the seven step TMDL process below. The information is also useful to support investigations of waterborne disease outbreaks and management of water bodies not subject to the TMDL process.

TMDL Process

1. **Problem Identification**
2. **Identification of Water Quality Indicators and Target Values**
3. Source Assessment
4. Linkage Between Water Quality Targets and Pollutant Sources
5. Allocations
6. Follow-up Monitoring and Evaluation
7. Assembling the TMDL

The **problem identification** step's objective (U.S. EPA, 2001a) is to:

> **Identify background information and establish a strategy for specific 303(d) listed waters that will guide the overall TMDL development process. Summarize the pathogen-related impairment(s), geographic setting and scale, pollutant sources of concern, and other information needed to guide the overall TMDL development process and provide a preliminary assessment of the complexity of the TMDL (what approaches are justified and where resources should be focused).**

The **identification of water quality indicators and target values** objective (U.S. EPA, 2001a) is to:

Identify numeric or measurable indicators and target values that can be used to evaluate the TMDL and the restoration of water quality in the listed waterbody.

The information in this chapter on applicable numeric water quality standards, alternative standards to support designated use, and evaluation of indicator microorganisms as water quality criteria should be understood when undertaking problem identification in the TMDL process. Information on pathogens causing waterborne disease outbreaks is provided as background and may be most useful in situations where outbreaks occur.

1.2 Health Effects

This section discusses waterborne disease outbreaks and known waterborne pathogens. The link between wet weather flow and outbreaks, and the data on pathogen related outbreaks reported in the U.S. is presented. The following are detailed descriptions of bacteria, protozoa, viruses, helminth worms, and fungi.

1.2.1 Waterborne Disease Outbreaks

Discharges of stormwater runoff, combined sewer overflows (CSOs), and sanitary sewer overflows (SSOs) (all known as wet weather flows) to receiving waters create the potential for disease outbreaks. Through climate and epidemiological records, Rose *et al.* (2000) demonstrated a potential correlation between extreme precipitation events (the highest 20 percent of total intensity over a 20-year period) and waterborne disease outbreaks. The authors found that statistically significant relationships could be identified between these precipitation events and waterborne disease outbreaks due to contact with water from both surface and ground water sources, although the relationship was much stronger for surface water outbreaks.

Swimming in contaminated marine and fresh recreational waters may result in a broad spectrum of illnesses. Water bodies may be contaminated and continuously re-contaminated, particularly if heavily used by people. For most pathogens warmer waters are more of a risk and are pathogen reservoirs. Lack of flow and water stagnation allows pathogens to accumulate. Swimming-associated disease outbreaks in natural U.S. waters between 1986 and 2000 due to microorganisms are listed in Table 1-2. Exposure pathways of pathogens in recreational waters are dermal contact, ingestion and inhalation resulting in skin, ear, eye, gastrointestinal, and respiratory illnesses. Few studies other than those related to outbreaks have been conducted to determine the etiological agents related to swimming associated illnesses (WHO, 1999). One large-scale epidemiological study of swimmers in marine waters receiving stormwater runoff involved interviewing over 15,000 individuals (Haile *et al.*, 1999). Researchers reported higher risks of upper respiratory and gastrointestinal infections for swimmers who swam (1) near storm-drain outfalls, (2) in waters with high levels of single bacterial indicators and a low ratio of total to fecal coliforms, and (3) in waters where enteric (intestinal) viruses were detected. These

positive associations with adverse health effects indicate an increased risk of illness associated with swimming in ocean water subject to untreated urban stormwater runoff. More than 1% of the swimmers who swam in front of the outfalls were affected by fevers, chills, ear discharges, vomiting, and coughing. Some studies attempting to link health effects to pathogen sources yield inconclusive results. For example, seventeen *E. coli* O157:H7 cases led Perez Guzzi *et al.* (2000) to investigate potential contamination from CSOs on California's Mar del Plata beaches. Their investigation detected no *E. coli* O157:H7, although other strains of *E. coli* were detected in 75% of the samples. None of the 98 strains detected in the outfalls were the strains that were known to cause human illness.

Pathogens present in a watershed can enter the drinking water supply through stormwater runoff, combined and sanitary sewer overflows, and illicit sanitary wastewater cross connections into storm drains. Exposure pathways for pathogens in drinking water include ingestion, dermal contact, and inhalation. Failures in water treatment systems, including the inability of disinfection procedures to inactivate all pathogens, allow these microorganisms to remain in finished water. Table 1-3 summarizes the effectiveness of water treatment processes on waterborne pathogens. *Giardia* and *Cryptosporidium* caused the largest number of drinking water-associated cases and outbreaks reported to the Center for Disease Control (CDC) from 1986-2000 (Table 1-4 and Figures 1-1,1-2). Although the drinking water treatment system met state turbidity effluent requirements at all times immediately prior to and during the Milwaukee *Cryptosporidium* outbreak in 1993, an assessment of the problem by a U.S. EPA investigative team identified a potential link between high turbidity levels in the influent and the occurrence of *Cryptosporidium* (Fox and Lytle, 1996). The American Society for Microbiology (ASM) reports that outbreaks are associated with pathogen contamination of municipal water systems that operate according to government standards, like Milwaukee. This indicates current methodologies are unable to fully detect treatment system failures and water quality that will adversely affect public health (Warrington, 2001a).

Pathogen survival in aquatic environments affects their ability to cause illness. Many environmental stressors effect survival, most notably sunlight intensity. Intense ultraviolet sunlight over surface waters enhances bacterial die-off, therefore limiting serious bacterial impacts (Chamberlin *et. al.*, 1978). Bacteria in turbid waters and bottom sediments are not as susceptible to sunlight as surface water microorganisms, and therefore survive longer. Protozoa and viruses survive UV radiation better than bacteria (Johnson *et. al.*, 1997). Pathogen survival is also dependent on water temperature. Increased water temperature decreases the survival of bacteria in surface water. Reduced cell metabolism in cold water enhances bacteria survival (Terzieva *et al.*, 1991). Protozoa and viral survival is also increased in cold water (LeChevallier *et al.*, 1991; Wait *et al.*, 2000). Salinity (Johnson *et al.*, 1997), competition and predation (Rozen *et al.*, 2001), and nutrient supply (Gauthier *et al.*, 1989) are additional environmental factors influencing die-off. Microbial survival is dependent on a combination of the above factors. U.S. EPA compiled die-off rates of microbial indicators and pathogens in Table 6-1 of *Protocol for Developing Pathogen TMDLs* (U.S. EPA, 2001a).

Table 1-2. Outbreaks Associated with U.S. Natural Recreational Waters				
1986-2000				
Etiological Agent	**Cases#**	**% of Cases**	**Outbreaks***	**% of Outbreaks**
AGI**	1744	29.53	22	23.16
Shigella spp.	1618	27.40	20	21.05
Naegleria fowleri	16	0.27	16	16.84
E. coli O157:H7	336	5.69	12	12.63
Schistosoma spp.	203	3.44	7	7.37
Cryptosporidium parvum	649	10.99	4	4.21
Norwalk-like	257	4.35	4	4.21
Giardia lamblia	83	1.41	4	4.21
Leptospira	389	6.59	3	3.16
E. coli O121:H19	11	0.19	1	1.05
unknown	4	0.07	1	1.05
Adenovirus 3	595	10.08	1	1.05
TOTAL	5905	100	95	100

A case is defined as a disease occurrence from an etiological agent.

* An outbreak is defined as 1) greater than or equal to 2 persons experiencing a similar illness after contacting the recreational water and 2) epidemiologic evidence that implicates the water as the probable source of the illness.

** Acute gastrointestinal illness of unknown etiology.

Barwick et al., 2000; CDC and U.S. EPA, 1993; Herwaldt et al., 1992; Kramer et al., 1996; Lee et al., 2002; Levine et al., 1990; and Levy et al., 1998.

Table 1-3. Water Treatment Effectiveness on Pathogens	
Pathogen Type	**Water Treatment and Effectiveness**
Bacteria	Normal disinfection procedures using chlorine are sufficient to kill bacteria
Protozoa	Multi-barrier approach including conventional physical processes of sedimentation, coagulation and filtration can remove 99% or better of most protozoa. Chemical disinfection effectiveness is minimal.
Viruses	Conventional physicochemical processes of sedimentation, coagulation, filtration and chlorination effectively removes better than 99.99% of enteric viruses. The exception is the Norwalk Virus which is resistant to chlorine disinfection and relies on physical processes.
Helminths	Conventional physicochemical processes of sedimentation, coagulation, filtration and chlorination effectively eliminate helminths.
Fungus	Sub-micron filtration removes fungi. Fungi are immune to normal levels of water chlorination but are inactivated by UV or destroyed by ozone.

AWWA, 1999.

Table 1-4. Outbreaks Associated with Drinking Water from U.S. Surface Sources 1986-2000				
Etiological Agent	**Cases#**	**% of Cases**	**Outbreaks***	**% of Outbreaks**
Campylobacter	250	0.06	1	2.08
Cryptosporidium parvum	419130	95.89	5	10.42
Cyanobacteria-like	21	0.00	1	2.08
Giardia lamblia	3424	0.78	20	41.67
Shigella sonnei	1800	0.41	1	2.08
Ca. Jejuni	102	0.02	1	2.08
E. coli O157:H7	38	0.01	3	6.25
SRSV	148	0.03	1	2.08
AGI**	12169	2.78	15	31.25
TOTAL	437082	100	48	100

A case is defined as a disease occurrence from an etiological agent.
* An outbreak is defined as 1) greater than or equal to 2 persons experiencing a similar illness
** Acute gastrointestinal illness of unknown etiology.
Barwick et al., 2000; CDC and U.S. EPA, 1993; Herwaldt et al., 1992; Kramer et al., 1996; Lee et al., 2002; Levine et al., 1990; and Levy et al., 1998.

Figure 1-1. Microbial Pathogens Attributed to Cases of Illness from Exposure to U.S. Surface Drinking Water Sources, 1986-2000. Total Number of Cases = 437,082.

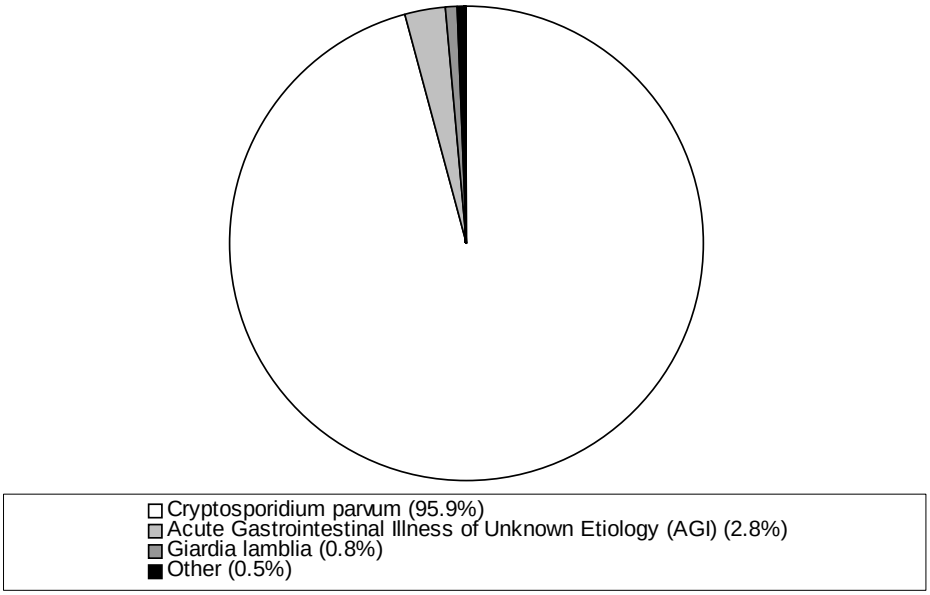
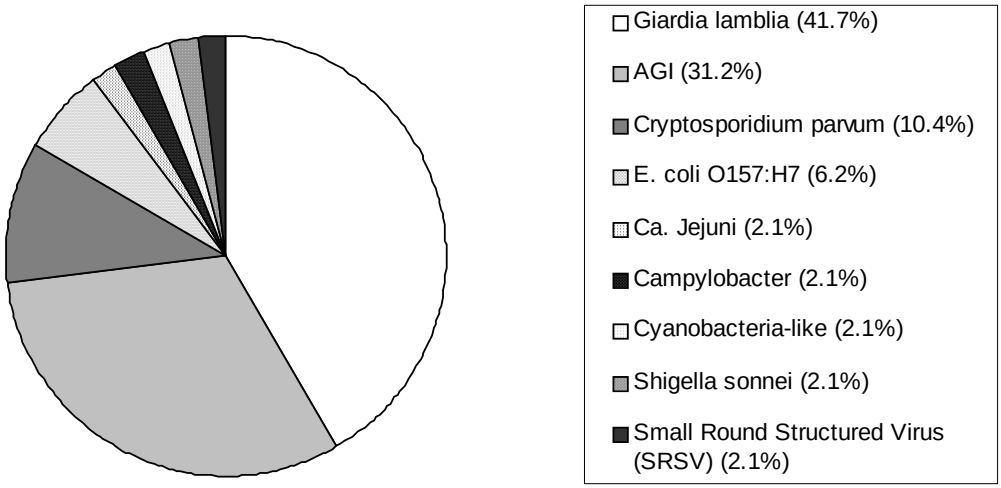


Figure 1-2. Microbial Pathogens Attributed to Outbreaks of Illness from Exposure to U.S. Surface Drinking Water Sources, 1986-2000.
Total 48 Outbreaks.



Barwick *et al.*, 2000; CDC and U.S. EPA, 1993; Herwaldt *et al.,* 1992; Kramer *et al.*, 1996; Lee *et al.*, 2002;Levine *et al.*, 1990; and Levy *et al.*, 1998

1.2.2 Pathogenic Bacteria of Concern

Bacteria are unicellular microorganisms that exist as either free living organisms or as parasites. Bacteria play a fundamental role in the decomposition and stabilization of organic matter in nature and in biological sewage treatment processes. Bacteria range in size from 0.4 to 14 micrometers or microns (µm) in length and 0.2 to 1.2 µm in width. Many types of enteric pathogenic bacteria occur in water supplies and in wastewater. The U.S. EPA (2000a; 2002a) assessed bacteria as one of the leading causes of impairments to surface waters. With increasing demands on water resources, the potential for contamination of surface and groundwater by pathogenic enteric bacteria is expected to rise resulting in an increase in waterborne disease outbreaks. Gastrointestinal illness, i.e., diarrhea, nausea, and cramps, is a common symptom of infections caused by enteric waterborne bacteria. Some pathogens spread through the body from the intestinal mucosa and cause systemic infections known as enteric fevers. One example of this is typhoid fever. Chlorine disinfection is highly effective for most bacteria (AWWA, 1999).

Enteric bacteria tend to die off faster than strains indigenous to surface and groundwaters because they are unable to compete successfully with natural microflora for low nutrient concentrations (Sinclair and Alexander, 1984). However, some bacteria are able to adapt to low nutrient concentrations by transforming to a viable but nonculturable (VBNC) state (Wang and Doyle, 1998; Huq and Colwell, 1996). VBNC bacteria maintain metabolic activity and infectiousness, but do not grow and multiply on culture plates, making them difficult to detect with conventional methods. Enteric pathogenic bacteria transmitted by water and wastewater include *Campylobacter, E.coli* O157:H7, *Leptospira*, *Salmonella*, *Shigella*, *Vibrio cholerae*, and *Yersinia entercolitica*. *Legionella pneumophilia*, while not enteric, is a pathogenic bacteria distributed in the aquatic environment. Waterborne pathogenic bacteria of concern and their associated diseases are presented in Table 1-5.

1.2.2.1 *Campylobacter*

Campylobacters of concern to the water industry are the “thermophilic” group. They cause a variety of diseases in humans, principally acute diarrhea preceded by flu-like illness. *Campylobacter* enteritis is principally a zoonotic disease, communicated from lower animals to man under natural conditions. These bacteria are harbored in the intestines of domestic and wild animals, particularly birds. Indirect transmission by contaminated water and food is the most common infection mode. *Campylobacter* bacteria are killed by cooking procedures. *Campylobacter* is now recognized as the cause of a common enteric bacterial infection in the U.S. Over 21 cases for each 100,000 persons in the U.S. population (approximately 57,000 cases) are diagnosed each year (MMWR, 1999). Campylobacters are not found in water in the absence of *E. coli* (AWWA, 1999).

Table 1-5. Waterborne Bacteria of Concern to Human Health and Their Associated Diseases			
Bacteria	**Source**	**Disease**	**Effects**
Campylobacter	Bird feces	Diarrhea	Acute diarrhea
Escherichia coli O157:H7 (enteropathogenic)	Cattle feces	Gastroenteritis	Vomiting, diarrhea
Legionella pneumophilia	Aquatic environments	Legionellosis	Acute respiratory illness
Leptospira (150 spp.)	Urine of dogs, livestock, wild animals	Leptospirosis	Jaundice, fever (Weil's disease)
Salmonella typhi	Domestic and wild animal feces	Typhoid fever	High fever, diarrhea, ulceration of small intestine
Salmonella (~ 1700 spp.)	Domestic and wild animal feces	Salmonellosis	Diarrhea, dehydration
Shigella (4 spp.)	Human feces	Shigellosis	Bacillary dysentery
Vibrio cholerae	Asymptomatic human feces	Cholera	Extremely heavy diarrhea, dehydration
Yersinia entercolitica	Animal feces	Yersinosis	Diarrhea

Metcalf and Eddy, 1991.

1.2.2.2 *E. Coli* O157:H7

E.coli O157:H7 is a pathogenic strain of *Escherichia coli* belonging to the group enterohemorrhagic *E. coli*. Human infection causes severe diarrhea and abdominal cramps. In young children (under five years old) and the elderly, complications leading to life threatening kidney failure can result (U.S. EPA, 2002b). The reservoir of this pathogen is primarily cattle. This specific strain is an emerging cause of waterborne and foodborne illness. Fecally contaminated water has been linked to recreational and drinking water outbreaks. An estimated 73,000 cases of infection and 61 deaths occur in the U.S. annually (CDC, 2001a). *E. coli* O157:H7 was the responsible agent in the Cabool, MO disease outbreak that killed four people, hospitalized 32 and caused diarrhea and other problems in 243 people (Geldreich *et al*., 1992). It is believed that breaks in drinking water mains resulted in low water pressure that allowed contamination from nearby SSOs to enter the drinking water system. In 1999 this pathogen was also responsible for the disease outbreak at a Washington County, NY fair due to contaminated drinking water. Of the 781 people identified with illnesses related to this outbreak, 127 cases of *E. coli* O157:H7 were confirmed by culture (Safefood News, 2000).

U.S. public water systems must notify homeowners if the water is unsafe. Private well owners should have their well tested periodically. Typically the well is tested for total coliform. If the test is positive, the water is then tested for *E. coli*. If the *E. coli* is positive, the water should not be consumed for drinking. U.S. EPA does not believe it is necessary for an owner of a private well to test specifically for *E. coli* O157:H7 under normal circumstances because the test is expensive and many labs do not have the expertise to perform this test (AWWA, 1999).

1.2.2.3 *Legionella pneumophilia*

Legionella is ubiquitous in the environment. The disease, legionellosis, is a severe respiratory illness characterized by pneumonia. It is found typically in surface waters at concentrations of 10^4 - 10^5 per liter and is now recognized as part of the natural environment (Fliermans *et al*., 1981). It has also proliferated in artificial environments such as cooling towers, evaporative condensers, whirlpools, and hot water tanks. These environments act as amplifiers or disseminators of *legionella pneumophilia*. In the U.S., 17,000 to 23,000 cases a year are estimated. The largest outbreak occurred in Philadelphia, PA in 1976, where 220 cases and 34 deaths were reported, and the source is unknown. Most outbreaks since 1976 have been linked with hospital water distribution systems (AWWA, 1999).

1.2.2.4 *Leptospira*

Leptospira are spiral shaped bacteria. The induced disease, Leptospirosis or "Weil's disease," first described in 1886, produces fever, headache, chills, malaise, vomiting, and occasionally meningitis. This bacteria is transmitted through the urine of dogs, livestock, and wild animals, and can contaminate natural water bodies, which then serve as sources of the infection. Dogs are the major source for human infections. A vaccine is available for dogs but not for humans. Between 100 and 200 documented cases per year occur in the U.S. (CDC, 2001a). In the summer of 1998, 110 athletes competing in a triathlon in Illinois were diagnosed with leptospirosis, and 23 needed hospital care. The outbreak was traced to Lake Springfield (MMID, 1999).

1.2.2.5 *Salmonella*

Salmonella is a group of over 1,700 types of bacteria. There are three distinguishable forms of salmonellosis, including gastroenteritis, enteric fever, and septicemia (characterized by chills, fever, anorexia or loss of appetite) in humans. Gastroenteritis is characterized by diarrhea, fever and abdominal fever. Enteric fever caused by *Salmonella typhi* is prolonged, lasting from 7 to 14 days. *Salmonella* septicemia is characterized by chills, fever, anorexia, and viable bacteria circulating in the blood known as bacteremia. Domestic and wild animals, and humans are possible sources of *Salmonella*. Waterborne outbreaks of salmonellosis are normally classified as acute gastrointestinal illness of unknown etiology. These outbreaks in the U.S. are associated with poor quality source water and inadequate treatment and/or contamination of distribution systems. In the U.S., over 40,000 cases of salmonellosis are reported each year, with the incidence being about 17 cases per 100,000 people (CDC, 2000; CDC, 2001b). The largest

known waterborne incidence of this disease occurred in 1965 in Riverside, CA and affected 18,000 people. The water supply was blamed, but the source of contamination was never determined (AWWA, 1999).

1.2.2.6 *Shigella*

Shigella is a genus of bacteria that causes sudden and severe gastroenteritis in humans, known as shigellosis. Infected humans are the only significant reservoir. Waterborne outbreaks result from fecal contamination of nonchlorinated private and noncommunity water supplies. Septic tank contamination of wells, or cross-connections between wastewater and potable water lines are commonly implicated in drinking water outbreaks. Recreational exposure to fecally contaminated swimming areas is also prevalent (AWWA, 1999). Approximately 25,000 confirmed cases of shigellosis from all sources are reported in the U.S. each year. However, many cases go undiagnosed, and 450,000 cases are estimated annually (Baer *et al.*, 1999).

1.2.2.7 *Vibrio cholerae*

Over 130 groups of *Vibrio cholerae* have been studied. This bacteria is responsible for the illness cholera, which produces acute diarrhea, dehydration, vomiting, shock, and possibly death. Cholera is typically spread by poor sanitation. The most important reservoirs are asymptomatic human carriers and diseased people who shed this bacteria in their feces. Sporadic cases occur when shellfish are harvested and eaten raw from fecally polluted waters. The excellent sanitation facilities in the U.S. are responsible for the near eradication of epidemic cholera here. Cholera was reported in South America from 1991 to 1995, where it grew to epidemic levels (1,099,882 cases and 10,453 deaths). Since this outbreak, most cases of cholera in the U.S. have occurred among persons traveling from cholera-affected areas (CDC, 1995; U.S. FDA, 2003a). *Vibrio* cholerae can also be present naturally in the environment, and natural waters can be a source of this bacteria. This presence in the environment has been demonstrated in the U.S. and Australia, where toxigenic strains survived in aquatic environments for years in the total absence of fecal contamination (AWWA, 1999). Of particular concern is their presence in warm, shallow, Gulf Coast waters, where oysters, as filter-feeders, concentrate these *Vibrio spp.* organisms in their tissues (Hopkins *et al.*, 1997).

1.2.2.8 *Yersinia entercolitica*

Yersinia entercolitica is a facultative anaerobe (lives under either aerobic or anaerobic conditions). This bacteria causes gastroenteritis, usually in children under seven years old, characterized by fever, diarrhea, abdominal cramps, and sometimes vomiting. It is mainly recognized as a foodborne pathogen, but may be found in sewage and polluted waters, and can enter drinking water via pollution from these sources. Essentially, it is found where one might encounter coliform organisms. However, *Yersinia entercolitica* is able to survive for longer periods of time in aquatic environments (survival has been shown to grow at low temperatures and survive for 18 months at 4°C) than fecal coliform. Therefore, this organism can be present when the coliform indicator organisms are not (AWWA, 1997).

1.2.3 Pathogenic Protozoa of Concern

Protozoa are one-celled animals varying in size from 2 to 100 µm. They live in many animals and survive in cysts (protective shells) when outside of an organism. Protozoa reproduce rapidly inside a host organism; therefore, ingestion of only a few by a human causes disease. Once in water, protozoa can survive for several weeks, even longer if frozen in ice. The waterborne pathogenic protozoans of greatest concern in countries with temperate climates are C*ryptosporidium* and *Giardia.* Oocysts of *Cryptosporidium* and cysts of *Giardia* occur in surface water, where their concentration is related to the level of fecal pollution or human waste present. Oocysts and cysts are both very persistent in water and are very resistant to disinfectants commonly used in drinking water treatment. In industrialized countries, outbreaks of cryptosporidiosis and giardiasis are due to oocysts and cysts entering the drinking water because of treatment failure, contamination of the source water, and/or leakage into the distribution system (WHO, 1993).

Recently there is a growing concern regarding *Cyclospora,* especially in nonindustrialized countries. *Entamoeba histolytica* and *Naegleria fowleri* are additional water-transmitted intestinal parasites of concern worldwide due to their serious consequences. Table 1-6 lists waterborne pathogenic protozoa of concern and their associated diseases.

1.2.3.1 *Cryptosporidium*

Cryptosporidium induces the disease cryptosporidiosis, which is capable of producing unpleasant gastric and diarrheal illness (Rose, 1997). The parasite's transmittable stage is a 4 to 6 µm diameter spherical shaped oocyst which contains a hardy thick wall. The oocyst is spread through the feces of infected humans and animals, including mammals, birds, reptiles, and fish. *Cryptosporidium* is frequently waterborne in nature and infections have occurred through contact with contaminated drinking water supplies, as well as zoonosis (animal person contact), contaminated food, contaminated swimming pools, and other recreational waters. Oocysts may be present in animal slurry spread on farmland as fertilizer. Consequently, runoff from rain carries oocysts into streams, lakes, and other reservoirs. Sewage is another source. The infective dose varies from less than 30 oocysts to as many as one million oocysts. There are six species of *Cryptosporidium*, but only one species, C*ryptosporidium parvum*, found in animals, is known to infect humans. Both known *Cryptosporidium parvum* genotypes can cause infections in human beings. Genotype 1 has (so far) been found almost exclusively in humans, and is more virulent than Genotype 2, which is found in a wide variety of animals, including humans (Xiao *et al*., 2001).

Table 1-6. Waterborne Protozoans of Concern to Human Health and Their Associated Diseases			
Protozoan	**Source**	**Disease**	**Effects**
Cryptosporidium	Human, animal, and bird feces	Cryptosporidiosis	Diarrhea, death in susceptible populations
Cyclospora	Human feces	Cyclosporiasis	Diarrhea
Entamoeba histolytica	Human feces	Amebiasis (amoebic dysentery)	Prolonged diarrhea with bleeding, abscesses of the liver and small intestine
Giardia lamblia	Human, animal, and bird feces	Giardiasis	Mild to severe diarrhea, nausea, indigestion
Naegleria fowleri	Bird and aquatic mammal feces	Meningoence-phalitis (PAM)	Inflammation of brain and meninges

Fout, 2002; Metcalf and Eddy, 1991

States *et al.* (1997) found *Cryptosporidium* in treated sewage and CSO from an area incorporating dairy farms. In an investigation of CSO in urban areas, Arnone *et al.* (2003) reported essentially no *Cryptosporidium* in the two cities and three outfalls investigated. The largest recorded outbreak of cryptosporidiosis occurred in Milwaukee in 1993, where an estimated 403,000 people were infected, and approximately 50 to 100 area residents with compromised immune systems died prematurely (Blair, 1994; Hoxie *et al.*, 1996). Another significant cryptosporidiosis outbreak occurred in Las Vegas in 1994, and infected 78 people, most of whom had human immunodeficiency virus (HIV) infections (Roefer *et al.*, 1996). At present nothing other than the body's defense system can treat cryptosporidiosis. *Cryptosporidium*, therefore, poses some alarming public health problems, particularly for people with weakened immune systems, especially acquired immunodeficiency syndrome (AIDS) patients. These patients are prone to severe and protracted diarrhea which can persist for months with considerable weight loss and mortality (Gerba *et al.*, 1996; Rose, 1997).

A well-operated drinking water plant can physically remove only 99% of oocysts from infected raw waters. Traditional processes such as coagulation, clarification, and filtration remain the best defense against this parasite entering the water supplies. Encystment can protect protozoa from drinking water disinfection efforts (Frey *et al.*, 1998). U.S. EPA regulations addressing this contaminant in drinking water supplies are discussed in U.S. EPA (2001c) and Chapter 3.

1.2.3.2 *Cyclospora*

Cyclospora, species *Cyclospora cayetanensis*, are 8 to 10 µm in size. Disease symptoms mimic those caused by cryptosporidiosis, including mild nausea, anorexia, abdominal cramping, and diarrhea. Humans are the only natural host. Noninfectious *Cyclospora* oocysts are passed in the feces of infected individuals. The unsporulated oocysts are usually transmitted via water and require 7 to 15 days to sporulate and become infectious. Consumption of untreated water has led to infection. During the spring of 1996 approximately 850 cases of cyclosporiasis were confirmed in the U.S. and Canada. The infection lasts up to seven weeks. Symptoms typically mimic those of cryptosporidiosis (AWWA, 1999).

1.2.3.3 *Giardia lamblia*

Giardia lamblia, also known as *Giardia duodenalis* and *Giardia intestinalis*, is the most common cause of protozoa infection in humans. Sometimes referred to as "beaver fever," "hiker's disease," or "camper's disease," *Giardia* infection, or giardiasis, causes abdominal cramps, diarrhea, and bloating. *Giardia* is found in humans, dogs, cats, pigs, sheep, beavers, and many other domestic animals, as well as birds. Humans are usually infected by one particular species of the many that exist, *Giardia lamblia*, which also causes infections in domestic and wild animals. There are six strains of *Giardia lamblia*. The strain type is not consistently associated with disease severity. Different individuals show various degrees of symptoms when infected with the same strain (U.S. FDA, 2003b). The infection is transmitted by tiny spores or egg-like cells called cysts measuring 9 to 12 µm in length. Watershed runoff and untreated and treated sewage transport *Giardia* to lakes, rivers and other receiving water bodies. There is an increase in *Giardia* infections during and after heavy rainfalls. Due to its thick wall, the *Giardia* cyst can survive weeks or months in fresh water, although it is less hardy than the *Cryptosporidium* oocyst (Rosen, 2000).

There have been over 20 outbreaks of waterborne *Giardia* in the U.S. from recreational and surface drinking water contact between 1986 and 2000 (Barwick *et al.*, 2000; CDC and U.S. EPA, 1993; Herwaldt *et al.*, 1992; Kramer *et al.*, 1996; Lee *et al.*, 2002; Levine et al., 1990; and Levy *et al.*, 1998). The infective dose for *Giardia* cysts may be between 10 and one million viable cysts depending on the immune system of the host. Giardiasis can be treated with drugs, including metronidazola, furazolidone, trinidazole, and paromomycin. Therefore, giardiasis is not regarded as a fatal disease. *Giardia* infection occurs due to its reproduction in the digestive system and attachment to the small intestine. After ingestion, the cyst passes through the stomach to the duodenum where it hatches and produces two trophozoites, feeding configuration of the parasite. The trophozoites measure 12 to 18 µm in length and adhere to the surface of the mucous membranes of the small intestine. The trophozoites damage the membrane and inhibit adsorption of nutrients that cause the disease giardiasis. The trophozoites then form cysts as they pass along the small intestine and eventually pass out with the feces (Rosen, 2000). Many individuals are asymptomatically affected by *Giardia*, as demonstrated by a CDC study of a population who consumed water heavily contaminated with *Giardia* due to malfunction in the

water disinfection system. Only 11 percent of the exposed population developed symptoms even though 46 percent had the organism in their stools (Rockwell, 2002).

Giardia cysts are removed fairly readily by conventional drinking water treatment processes, such as coagulation, settlement, and rapid filtration. A well operated treatment plant utilizing coagulation, clarification, and filtration should remove 99.9% of *Giardia* from the water. Disinfection with chlorine is ineffective due to the cyst's thick wall. Current research indicates that irradiation with ultraviolet (UV) light is the most promising form of *Giardia* disinfection or inactivation (U.S. EPA, 2001c).

1.2.3.4 *Entamoeba histolytica*

Entamoeba histolytica causes the disease known as amoebiasis, characterized by dysentary, chronic colitis, and liver abscess. Infected humans, particularly asymptomatic carriers, are the only reservoirs of significance. Waterborne outbreaks in the U.S. are rarely documented. The most dramatic outbreak in the U.S. was the 1933 Chicago World's Fair outbreak caused by contaminated drinking water, infecting 1,400 individuals and causing 58 deaths (Warrington, 2001b). An estimated 40 million people worldwide develop this disease annually, and the mortality is estimated at 40,000.

1.2.3.5 *Naegleria fowleri*

Naegleria fowleri causes an acute rapid occurring disease of the central nervous system primary amebic meningoencephalitis (PAM). This disease is characterized by severe headache, fever, and coma leading to death within 3 to 10 days after the onset of symptoms. Birds and aquatic mammals such as beavers, otters, and muskrats are reservoirs for this pathogen. *Naegleria fowleri* is found free in the environment, specifically soils, freshwater, and sewage. It enters the body through the nasal passage and travels along the nerves to the meninges. It comprises both nonpathogenic and pathogenic strains (Geldreich, 1996).

1.2.4 Pathogenic Viruses of Concern

Viruses are a group of infectious agents that require a host to survive. They use the host cell's reproductive mechanism to replicate. After replication, and subsequent death of the host cell, viral particles are spread to neighboring cells. This results in infection to the individual. Viruses are the smallest and most basic life form, ranging in size from 0.02 to 0.09 μm. The virus protein or lipoprotein covering enables it to survive for long periods and adhere to surfaces (AWWA, 1999). Table 1-7 lists the viruses of concern to human health via exposure to water and their associated diseases (Fout, 2002; Metcalf and Eddy, 1991).

The viruses most significantly affecting water quality and human health are enteric viruses which are found in the gastrointestinal tract of infected individuals. These viruses are excreted in the feces of infected people and may directly or indirectly contaminate water intended

Table 1-7. Waterborne Viruses of Concern to Human Health and Their Associated Diseases			
Virus	**Source**	**Disease**	**Effects**
Adenovirus (48 serotypes; types 40 and 41 are of primary concern)	Humans	Respiratory disease, gastroenteritis	Acute respiratory disease, pneumonia, conjunctivitis, gastroenteritis
Astroviruses	Humans	Gastroenteritis	Vomiting, diarrhea
Calicivirus (e.g., Norwalk, Norwalk-like and Sapporo, Sapporo-like viruses)[2]	Humans	Gastroenteritis	Vomiting, diarrhea
Enterovirus (66 types, e.g., polio, echo, encephalitis, conjunctivitis, and Coxsackie viruses)	Humans	Gastroenteritis, heart anomalies, meningitis	Respiratory illness, polio, common cold
Hepatitis A	Humans	Infectious hepatitis	Jaundice, fever
Hepatitis E[1]	Humans, pigs	Infectious hepatitis	Jaundice, fever
Reovirus	Humans	Gastroenteritis	Vomiting, diarrhea
Rotavirus	Humans	Gastroenteritis	Vomiting, diarrhea

[1] Hepatitis E is an emerging virus that has caused large outbreaks of infectious hepatitis outside the U.S.
[2] Norovirus and Sapovirus are the new genus names for the Norwalk-like and Sapporo-like viruses.
Fout, 2002; Metcalf and Eddy, 1991

for drinking. Enteric viruses multiply only within living cells. They take over a living cell and use the cell's reproductive mechanism to replicate. Most waterborne virus disease outbreaks in the U.S. are caused by sewage contamination of untreated or inadequately treated private and semipublic water supplies. Conventional physicochemical water treatment processes of coagulation-flocculation and filtration remove up to 99% of most enteric viruses. Disinfection of water with free chlorine, chlorine dioxide, ozone, and UV light radiation can achieve 99.9% enteric virus inactivation. Norwalk virus is the exception; this virus is very resistant to chlorine and other disinfection measures (AWWA, 1999).

The predominant enteric viruses of concern are enteroviruses, rotaviruses, hepatitis A and E, caliciviruses, adenoviruses, reoviruses, and astroviruses. Each consists of subgroups totaling more than 140 different enteric viruses known to cause numerous illnesses that include diarrhea, fever, hepatitis, paralysis, meningitis, and heart disease. Some viral infections are asymptomatic (AWWA, 1999).

1.2.4.1 Adenoviruses

Human adenoviruses may cause acute respiratory disease, pneumonia, epidemic conjunctivitis, and acute gastroenteritis in children. Human adenovirus is not pathogenic to animals, and animal adenovirus is only pathogenic to the species of origin. Hurst *et al*. (1989) found that adenoviruses are transmitted through recreational and drinking water. Jiang *et al*. (2001) found adenoviruses at beach locations in southern California, with concentrations ranging from 880 to 7,500 plaque-forming units (PFU) per liter of water.

1.2.4.2 Astroviruses

Astroviruses have a unique star-shaped surface when viewed by a negative-stain electron microscopy. These viruses produce symptoms similar to those caused by rotaviruses, including vomiting, diarrhea, and mild dehydration. There have been no reports on infectivity for animals and there are no known reservoirs for these viruses. Astroviruses are primarily transmitted by the fecal-oral route (AWWA, 1999). Immunity to astrovirus infection is not well understood. Young children and the institutionalized elderly are usually the populations that develop symptomatic infection. This suggests that the antibody is acquired late in childhood and provides protection through adult life until the elder years when this protection is diminished.

1.2.4.3 Caliciviruses

Norwalk and Norwalk-like viruses are caliciviruses, also known as small round-structured viruses (SRSV). Norwalk virus, the prototype SRSV was first isolated in 1972 at an elementary school in Norwalk, Ohio. The genus name for the Norwalk-like virus is now called *Norovirus*. Another calicivirus is the Sapporo-like virus, of which the genus name is now *Sapovirus*. These viruses produce vomiting in children and diarrhea in adults. In the U.S., 40% of the outbreaks of gastroenteritis in adults are attributed to these two viruses. Humans are the only reservoir for caliciviruses. Norwalk and Norwalk-like viruses are transmitted by ingestion of fecally contaminated material. Infections have been associated with ingestion of surface water contaminated by fecal material, ingestion of groundwater contaminated by septic drainage, and swimming in sewage-contaminated waters. Outbreaks also occur following consumption of shellfish harvested from waters contaminated with human sewage. Oysters, clams, and other shellfish filter virus particles from contaminated water and accumulate them in their tissues (AWWA, 1999).

1.2.4.4 Enteroviruses

Enteroviruses cause a wide variety of illnesses, ranging from polio to the common cold. Non-polio enteroviruses are second only to the rhinoviruses, which cause the common cold, and are the most common viral infectious agent affecting humans. Infected persons who become ill develop respiratory flu-like symptoms. Less commonly, some people develop viral meningitis. Humans are the only natural hosts for these viruses. Enterovirus infection is prevalent upon exposure to human fecal contamination in a variety of sources, including groundwater, marine

waters, shellfish, crops irrigated with sewage, and where spray irrigation of sewage is practiced. The enteroviruses cause an estimated 10-15 million symptomatic infections each year in the U.S. and many more asymptomatic infections (AWWA, 1999). Noble and Fuhrman (2001) found enteroviruses in 32% of beach waters sampled from the Santa Monica Bay, CA suggesting the potential for enterovirus infection through recreational contact.

1.2.4.5 Hepatitis A and Hepatitis E

Hepatitis A (HAV) causes the disease known as "infectious hepatitis," which is an acute inflammation of the liver. Hepatitis E (HEV) also causes infectious hepatitis and is nearly indistinguishable from HAV. Humans are the main reservoir for infectious hepatitis and shed the virus in their feces. Pigs are also a reservoir for HEV. Direct and indirect person-to-person contact are the primary HAV exposure mechanisms. Fecally contaminated drinking and bathing water, and shellfish harvested from fecally contaminated waters serve as reservoirs and transmission pathways. In the U.S., 20,000 to 30,000 cases of the HAV cases are reported annually. HEV is rare in the U.S. although it is widespread in other parts of the world (AWWA, 1999). Although mortality from infections caused by these viruses is comparatively low, the disease may be severe and incapacitating. Case fatality rates of 20-40% are on record for HEV infections in pregnant women (Grabow, 1997). Vaccines are only available for HAV, and no meaningful treatment is available for any of the hepatitis viruses, making disease control dependent solely on preventing transmission. In Oocee, FL SSOs periodically flooded a mobile home park during heavy rains and caused occasional outbreaks of hepatitis A from November 1988 to April 1989; 39 cases were identified among residents and 100 cases were linked to food handlers living in the park. The initial reports by public health officials attributed the outbreaks to poor personal hygiene rather than to the SSOs. It took four years for officials to determine the connection between the SSOs and the outbreaks (Vonstille *et al.*, 1993).

1.2.4.6 Reoviruses

Reovirus infections are mostly subclinical or very mild. These viruses are found in the respiratory and enteric tracts. They lack a direct association with a specific human disease. Reports associate reoviruses with a host of different diseases such as juvenile onset diabetes, fever, rash, respiratory disease, pneumonia, eye infections, and meningitis. Reoviruses are ubiquitous in nature and are commonly found in sewage and fecally polluted waters. Their main source is human excreta.They are the most commonly isolated viruses from water and are easily recognized (AWWA, 1999).

1.2.4.7 Rotaviruses

Rotavirus is responsible for 3.5 million cases of diarrhea and 125 deaths per year in the U.S. Humans and animals are the primary reservoirs for rotaviruses. Infections occur mostly in infants and children under two years old. Rotaviruses are predominately transmitted by the fecal-oral route. Most rotavirus infections occur in the winter in temperate climate. Rotavirus is responsible for 30 to 50 percent of U.S. hospitalizations for diarrhea in children under five years

old. Immunocompromised patients and the elderly are also susceptible to this virus. This virus is believed to be the cause of gastroenteric waterborne illnesses, and has been detected in freshwater and sewage. Associated attacks have been documented. One such outbreak occurred among users of the Vail, CO community water system where a very high adult attack rate of 43.8% was recorded (AWWA, 1999).

1.2.5 Pathogenic Helminth Worms

Helminth worms refer to those of the cestode (tapeworm), trematode (fluke), and nematode (roundworm) groups. There are many waterborne helminth worms that are pathogens. Although these worms are multicellular with complex reproduction systems and life cycles, helminths are more completely understood than many other group of pathogens. Many of them require invasion of a host which results in illness, damage, and sometimes the death of the host to complete its life cycle. These are known as parasites. Some helminths normally live and replicate in the natural environment or in other species. These infect humans when conditions are right for causing disease, but do not complete their life cycle in the individual. They are not parasites but opportunistic pathogens that can spread from individual to individual and from individuals to water. Over one billion people worldwide are infected annually with intestinal helminths. In the U.S., intestinal helminth disease has been largely eliminated due to improved sanitation. Table 1-8 lists the waterborne pathogenic helminth worms of concern and their associated diseases (Warrington, 2001c). This information on helminths is for informational purposes only since helminth infection is not prevalent in the U.S.

1.2.5.1 Nematodes

Nematodes (roundworms) are elongated, unsegmented, cylindrical worms, distinguished by both sexes. *Ascaris lumbricoides* is the largest nematode, reaching 35-cm long in adult females and 30-cm long adult males. These worms are the most common human helminth infection, afflicting over 800 million people annually worldwide. The highest prevalence is in tropical and subtropical regions, especially in areas with inadequate sanitation, but infections are also reported in rural areas of the southeast U.S. *Ascaris lumbricoides* causes parasitism in the human intestine, known as ascariasis. *Ancylostoma duodenale* and *Necator americanus* are hookworms that infect over 350 million humans worldwide each year. Iron deficient anemia accompanied by cardiac complications are the most common symptoms of this disease known as ancylostomiasis. *Trichuris trichiura* is the third most common roundworm found in humans. Distribution is worldwide, with infections more frequent in tropical areas and environments with poor sanitation systems. The disease known as trichuriasis is most frequently asymptomatic. Heavy infections, especially in children, can cause gastrointestinal problems such as abdominal pain, diarrhea, rectal prolapse and possible growth retardation (Warrington, 2001c).

Table 1-8. Waterborne Helminth of Concern to Human Health and Their Associated Diseases			
Helminth	**Source**	**Disease**	**Effect**
Ascaris lumbricoides	Human feces	Ascariasis	Asymptomatic, respiratory problems
Ancylostoma duodenale	Human Feces	Ancylostomiasis	Anemia
Necator americanus	Human feces	hookworm	Anemia
Trichuris trichiura	Human feces	Trichuriasis	Gastrointestinal problems
Taenia solium	Pigs	Taeniasis	Intestinal disturbance
Diphyllobothrium latum	Fish	Diphyllobothriasis	Anemia, diarrhea
Schistosoma haematobium	Snails, human feces	Schistosomiasis	Diarrhea, lesions, cystitis
Schistosoma intercalatum	Snails, human feces	Schistosomiasis	Diarrhea, lesions, cystitis
Schistosoma japonicum	Snails, human feces	Schistosomiasis	Diarrhea, lesions, cystitis
Trichobilhartzia spp.	Snails, waterfowl, aquatic animals	Swimmer's itch	Open sores and lesions in skin

Warrington, 2001c; WHO, 1999

1.2.5.2 Cestodes

Cestodes (tapeworms) are flat segmented worms that are hermaphroditic (having both male and female reproductive organs). These parasites are found in the gut and acquired by ingesting contaminated food and water. Intermediate hosts (cattle, pig, fish) ingest these waterborne parasites. Infections are passed to humans who eat the meat of the intermediate hosts. The head or scolex attaches to the wall of the gut, and segments up to 25 meters long called proglottids are attached behind the head. The proglottids are full of eggs and as new ones are produced, the old one, containing up to 1,000,000 eggs, detach and are shed with the feces. Annually *Diphyllobothrium latum* (fish tapeworm) infects 10,000,000 people, and *Taenia solium* (pork tapeworm) infects 6,500,000 people worldwide (Warrington, 2001c).

1.2.5.3 Trematodes

Trematodes (flukes) have complex life cycles, usually involving a snail and some other intermediate host such as fish, crustaceans and sheep. Flukes are unsegmented, flat, leaf-shaped worms having a variety of organ systems. Most flukes are hermaphroditic. They attach to the host by means of an oral sucker and a ventral sucker. Flukes, as adults, infect either the portal

blood vessels, intestines, liver, or lungs of humans. *Schistosoma haematobium*, *Schistosoma japonicum*, and *Schistosoma intercalatum* penetrate the skin of humans and cause schistosomiasis. This disease worldwide affects approximately 195,000,000 people annually and is responsible for 15,000 deaths. Swimmer's itch is a skin rash caused by parasites of birds and mammals. In the U.S., the species is normally *Trichobilharzia spp*. and is reported in areas along the migratory bird flyways where avian hosts are common. Waterfowl, mainly ducks and geese, are the hosts of the schistosomes that cause schistosomiasis (Warrington, 2001c).

1.2.6 Pathogenic Fungi

Fungi, including yeasts and filamentous species or molds, are ubiquitously distributed heterotrophic (requiring complex organic compounds for metabolic synthesis) organisms found in lakes, ponds, streams, estuaries, marine environments, wastewaters, rural and urban stormwater runoff, and aquatic sediments. Normal healthy individuals rarely suffer from waterborne fungal diseases; it is the immunocompromised individuals that are at risk of fatal fungal infections. Fungi are not generally problems in drinking water. They may present problems when water is used for bathing and recreational activities (Warrington, 2001d).

Fungi are aerobic, multicellular, nonphotosynthetic organisms having organized nuclei, usually rigid walls, and lack chlorophyll. The presence of fungi in stream water represents soil runoff because nearly all zoopathogenic fungi exist saprobically (feeding on dead or decaying material) with soil as their natural reservoir. Fungi pathogenic to humans are found in pools and beaches and in accompanying washing facilities. Table 1-9 lists the waterborne pathogenic fungi of concern and their associated diseases. Along with bacteria, fungi are the main organisms responsible for the decomposition of carbon on earth. Without the presence of fungi to break down organic matter, the carbon cycle (the process by which carbon is exchanged between organisms and the environment) would cease to exist and organic matter would start to accumulate. Aquatic species include fungi that are transiently present in water, terrestrial fungi that disperse in water, and species that function entirely within water. Unpolluted stream water has a large number of species representing true aquatic fungi (species possessing flagellated zoospores and gametes), aquatic Hyphomycetes, and soil fungi. Moderately polluted waters may carry cells of all three types, but with fewer true aquatic fungi and aquatic Hyphomycetes, and soil fungi are more numerous. Heavily polluted water has large numbers of soil yeast-like fungi (Clesceri *et al.,* 1998).

The association between fungal densities and organic loading implies that fungi may be useful indicators of pollution. However, no single species of fungi has been identified as important in this role. Fungi are found in potable water and on the inner surface of distribution system pipes. They either survive water treatment or they enter the system after treatment. Having survived treatment fungal spores can remain viable for extended periods. For instance, pathogenic spores of *Histoplasma capsulatium* remain highly infective to mice after 400 days (Clesceri *et al.,* 1998).

Table 1-9. Waterborne Fungi of Concern to Human Health and Their Associated Diseases			
Fungus	**Source**	**Disease**	**Effects**
Aspergillus fumigatus	Soil, decaying organic matter	Pulmonary aspergillosis	Inflammation of bronchi and lungs
Candida albicans	Raw wastewater	Candidiasis	Infection of moist cutaneous areas of body
Geotrichum candidum	Sewage, soil	Geotrichosis	Infection of mouth, respiratory tract
Histoplasma capsulatum	Bird droppings	Histoplasmosis	Respiratory infections
Pseudallescheria boydii	Sewage, soil	Eumycotic mycetoma	Infection of the cutaneous and subcutaneous tissues
Rhinocladiella mansonii	Soil, plants, water	Chromomycosis	Skin lesions

Clesceri *et al.*, 1998

Aspergillus fumigatus, an agent of an inflammatory and destructive disease of the bronchi and lungs known as pulmonary aspergillosisi, has been found almost everywhere on every conceivable type of substrate, especially soil and decaying organic debris. *Candida albicans,* found in raw wastewater, wastewater treatment plant effluents, or contaminated water, is a parasitic fungus that can infect the mouth, skin, intestines, and vagina. *Geotrichum candidum* is found worldwide in sewage, soil, and water as well as in plants, cereals, and dairy products. It is responsible for geotrichosis, an infection of the mouth, respiratory tract and digestive tract.

Histoplasma capsulatium is a thermally dimorphic fungus found in nature. Soil contaminated with bird droppings is the common natural habitat for *Histoplasma capsulatium.* The spectrum of the disease varies from an acute pulmonary infection to a chronic pulmonary disease. *Pseudallescheria boydii* is a causal agent of the slow, destructive infection of cutaneous and subcutaneous tissues recognized as eumycotic mycetomas. It is found in soil, sewage, contaminated water, and the manure of farm animals. *Rhinocladiella mansonii*, also known as *Exophiala mansonii*, is a saprophyte found in soil, plants, water, and decaying wood material. It is responsible for the disease chromomycosis, or skin lesions. (Clesceri *et al.*, 1998).

1.3 Microbial Water Quality Standards

Section 303(d) of the CWA requires each state to develop a list of impaired waters. Impairment is determined relative to state water quality standards for a given water body. Section 303(d) requires states to develop pollutant-specific TMDLs for each impaired water body. The objective of establishing and implementing the TMDL is to achieve the water quality target for that pollutant class. Generally, the target established is the state's water quality standard, which is based on U.S. EPA's recommended water quality criteria. There are exceptions, however, as explained in the *Protocol for Developing Pathogen TMDLs* (U.S. EPA, 2001a):

> "In some cases, the water body of concern has a numeric water quality standard that might not appropriately or sufficiently reflect the use impairment, and the use of a supplementary indicator or set of indicators might provide additional means for measuring attainment of designated or existing uses."

Examples of use impairments include waterborne disease outbreaks, degraded fisheries, and restrictions on using water body for the desired use of primary contact recreation. In these cases, U.S. EPA (2001a) recommends using a supplementary microbial indicator or pathogen that reflects the problem affecting the designated use and establishing the target receiving-water concentration and TMDL accordingly. The sections below describe the water quality legislation relevant to selecting the water quality target.

1.3.1 Clean Water Act

Section 303(c) of the CWA requires states to adopt water quality standards that take into account the designated uses for the water body. Standards must be set to support those designated uses and be based on U.S. EPA's recommended water quality criteria developed pursuant to Section 304(a) (U.S. EPA, 2000a).

Section 305(b) of the CWA requires all states and jurisdictions to:

1. assess the health of their waters and the extent to which their waters support water quality standards
2. identify the pollutants and sources contributing to water quality impairments
3. analyze the economic and social costs and benefits of achieving the goals of the CWA
4. submit reports every two years to the U.S. EPA describing water conditions

The CWA Section 305(b) further requires U.S. EPA to summarize reports from the states and other jurisdictions and convey this information to Congress biennially, currently the National Water Quality Inventory 2000 Report (U.S. EPA, 2002a). The assessments reported under Section 305(b) are used to identify and prioritize water quality problems within states. This report is developed from impaired water bodies identified in accordance with Section 303(d).

Section 303(d) of the CWA identifies waters that do not or are not expected to meet water quality standards after implementation of water pollution controls.

Once the 303(d) list is prepared, states develop TMDLs. The U.S. EPA and state water programs are currently working on sequencing water quality monitoring to determine appropriate water quality standards to support the full range of water quality management (U.S. EPA, 2000a). The sequence of activities consists of:

1. Characterizing waters for the 305(b) assessment
2. Using the subset of waters identified as not supporting water quality standards to develop 303(d) lists
3. Identifying source contributions
4. Developing TMDLs
5. Implementing source controls
6. Performing follow up monitoring to evaluate the effectiveness of source controls and to track trends in water quality improvements

1.3.1.1 TMDL Description and Definition

TMDLs are developed for a variety of pollutants, such as: (1) oxygen depleting substances, (2) nutrients, (3) sedimentation and siltation, (4) bacteria and pathogens, (5) toxic organic chemicals and metals, (6) pH, (7) habitat and hydrologic modification, (8) suspended solids, (9) noxious aquatic plants, (10) oil and grease, and (11) salinity and mineralization (U.S. EPA, 2000a). A maximum pollutant amount, TMDL, is required for each water body that cannot be improved by simply enforcing the minimum required source treatment. A TMDL sets a pollution cap. The cap is a formula representing the maximum amount of a pollutant (pathogen in the case of this document) that a water body can receive and still meet water quality standards. The sum of the allowable contributing point and nonpoint sources must not exceed this cap. A TMDL is the sum of the individual wasteload allocations for point sources and load allocations for nonpoint sources and natural background with a margin of safety (CWA section 303(d)(1)(c)). The TMDL, expressed in terms of mass (or organism counts for microorganisms) per time, can be described generically by the following equation:

$$TMDL = LC = \sum WLA + \sum LA + MOS \qquad \text{(U.S. EPA, 2001a)}$$

where:
- LC = loading capacity, the greatest loading a water body can receive without exceeding water quality standards
- WLA = wasteload allocation, the portion of the TMDL allocated to existing or future point sources
- LA = load allocation, the portion of the TMDL allocated to existing or future nonpoint sources and natural background
- MOS = margin of safety which is provided implicitly through analytical assumptions or explicitly by reserving a portion of loading capacity

TMDLs are developed to meet applicable water quality standards. Standards may be expressed by numeric water quality targets, narrative criteria for designated uses such as drinking water, recreation, fish and wildlife habitat. The numeric target may be equivalent to a numeric water quality standard as found in U.S. EPA's *Bacterial Water Quality Standards Status Report* (U.S. EPA, 1998a), or it may represent a quantitative interpretation of a narrative standard. U.S. EPA's water quality criteria provide guidance for the amount of pathogen degradation a water body can accommodate while still supporting the specific uses.

Understanding when a water body is most vulnerable to pathogen contamination is critical to developing load reduction scenarios that will result in attainment of water quality standards. When an impairment is the result of contributions from sewage treatment plants and industrial point sources, it is usually most pronounced at low flows. This is because point source contributions are relatively constant over time. When stream flow is low, these point source discharges constitute a relatively large proportion of the total stream flow. If an impairment is more pronounced at higher flows, the pollutant is associated with wet weather, i.e., stormwater runoff, combined sewer overflows, and some sanitary sewer overflows (U.S. EPA, 2000b).

As stated in the introduction, the seven components of the TMDL development process are:

1. Problem identification
2. Identification of water quality indicators and targets
3. Source assessment
4. Linkage between water quality targets and sources
5. Allocations
6. Follow-up monitoring and evaluation
7. Assembling the TMDL

These seven components, discussed in detail in the document *Protocol for Developing Pathogen TMDLs* (U.S. EPA, 2001a), provide a guidance and framework for the TMDL development process. TMDL calculations and allocations are a legally required components of the TMDL package submittal.

1.3.1.2 Stormwater, Combined Sewer Overflow and Sanitary Sewer Overflow Regulations

1.3.1.2.1 Stormwater

Stormwater runoff is generated from land and impervious areas during rainfall and snow events. These runoffs often contain pollutants, including pathogens, that adversely affect water quality. Polluted stormwater runoff is a leading cause of impairment to nearly 40 percent of water bodies in the U.S. that do not meet water quality standards (U.S. EPA, 2002c). Urbanization drastically alters the stormwater quality and quantity through hydraulic modifications. These modifications include catchbasins, inlets, curb and gutter, gutter and

downspouts, storm sewers, ditches, lined channels, culverts, and pavement. Stormwater travel time is reduced and flow velocity is increased as compared to the original natural conditions (Field and Sullivan, 2003). Studies show a linear relationship when the runoff volume is regressed against watershed imperviousness (Schueler, 1987).

Most stormwater discharges are considered point sources and require a National Pollutant Discharge Elimination System (NPDES) permit. U.S. EPA developed Phase I of the NPDES stormwater program in 1990 in response to the 1987 amendments to the Clean Water Act. Phase I requires operators of medium and large municipal separate storm sewer systems (MS4s) to (1) obtain a NPDES permit, (2) develop a stormwater management program to prevent pollutants from being washed by stormwater into the MS4, then discharged from the MS4 into local water bodies. A medium MS4 is a system that is located in an area with a population between 100,000 and 249,999. A large MS4 is a system that is located in an area with a population of 250,000 or more. In addition, Phase I requires a NPDES permit for stormwater discharges from construction areas that disturb five acres or greater of land. The Phase II Final Rule was signed by the U.S. EPA Administrator on October 29, 1999. Phase II requires NPDES permit coverage to (1) stormwater discharges from certain regulated small MS4 (communities less than 100,000, primarily those located in urbanized areas), and (2) small construction areas disturbing between 1 and 5 acres of land. Best management practices (BMPs) are the primary method to control stormwater discharges (U.S. EPA, 2002c). Use of BMPs for microbial contaminants is discussed in Chapter 3.

1.3.1.2.2 Combined Sewer Overflow

Combined sewer systems (CSSs) convey sanitary wastewater and stormwater through a single pipe to a publicity owned treatment works for treatment prior to discharge to surface waters. The U.S. EPA 2001 Report to Congress (U.S. EPA, 2001d) reports that CSSs are found in 32 states (including the District of Columbia). CSSs are concentrated in older communities in the Northeast and Great Lakes regions. This report documents 772 CSO communities with a total of 9,471 CSOs that are identified and regulated by 859 NPDES permits. Approximately 30 percent of the CSS communities have populations greater than 75,000, and approximately 30 percent have total service populations of less than 10,000. The annual CSO discharge is estimated at 1,269 billion gallons per year. CSO receiving water are distributed to 43 percent rivers, 38 percent streams, five percent oceans, estuaries and bays, and two percent other waters (ditches, canals, unclassified waters). CSOs are a source of impairment for 12 percent of assessed estuaries (in square miles) and two percent of assessed lakes (in shore miles). Overflows occur during moderate or heavy rainfall when capacity is exceeded. CSOs deposit water with varying concentrations of sanitary wastewater onto public areas, potentially resulting in a range of adverse health effects (Colford *et al.*, 1999).

1.3.1.2.3 Sanitary Sewer Overflow

SSOs are discharges of raw sewage from municipal sanitary sewer systems. Most SSOs are associated with wet weather conditions, when sanitary systems receive stormwater in-flow or

infiltrating groundwater through cracks. The SSO may occur during extreme hydrologic events in many separate sanitary systems, even though systems are intended to collect and contain all the sewage that flows into them. U.S. EPA estimates 40,000 SSOs annually (U.S. EPA, 2001e). Discharges to waters of the U.S. from municipal sanitary sewer systems are prohibited, unless authorized by an NPDES permit. There are approximately 19,000 municipal sanitary sewer collection systems in the U.S. The U.S. EPA proposed SSO Rule clarifies and expands requirements for these collection systems, with the premise of reducing SSOs (U.S. EPA, 2002d).

1.3.2 Safe Drinking Water Act

In the U.S., both the 1986 and 1996 Amendments to the Safe Drinking Water Act (SDWA) focused attention on source water protection and its role in protecting public water supplies. Developed to support SDWA implementation, U.S. EPA's Surface Water Treatment Rule (SWTR) (U.S. EPA, 1989), Interim Enhanced SWTR (IESWTR) (U.S. EPA, 1998b) and Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR) (U.S. EPA, 2002e) are designed to prevent waterborne diseases caused by viruses, bacteria, and the protozoans *Giardia lamblia* and *Cryptosporidium*, which are present in varying concentrations in most surface waters. These rules set unenforceable maximum contaminant level goals (MCLGs) of zero for pathogens in treated drinking water because exposure to them at any level poses a health risk. Rather than establishing a maximum contaminant level (MCL) for these contaminants in drinking water, U.S. EPA opted instead to impose a treatment requirement. Utilities using surface water must filter and disinfect the water to provide at least 99% removal/inactivation of *Cryptosporidium*, 99.9% of *Giardia*, and 99.99% of viruses. Unfiltered public water systems must have watershed control programs to reduce the sources and limit the migration of these pathogens into raw waters. The U.S. EPA has established a MCL for total coliform detection at no more than 5.0% of samples per month (for water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive per month). Every sample that has total coliform must be shown to contain no fecal coliform (U.S. EPA, 2003a).

In November 2001, the U.S. EPA issued the pre-proposal draft of the National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (U.S. EPA, 2001f). The purposes of the LT2ESWTR are to improve control of microbial pathogens (specifically *Cryptosporidium*) in drinking water and to address risk trade-offs with disinfection byproducts. The LT2ESWTR provisions are:

1. source water monitoring for *Cryptosporidium* with reduced monitoring requirements for small systems
2. additional *Cryptosporidium* treatment for filtered systems based on source water *Cryptosporidium* concentrations
3. inactivation of *Cryptosporidium* by all unfiltered systems
4. disinfecting, profiling and bench marking to assure continual levels of microbial protection while public water systems take the necessary steps to comply with new disinfection byproduct standards

5. covering, treating, or implementing a risk management plan for uncovered finished water reservoirs
6. criteria for a number of treatment and management options to meet additional *Cryptosporidium* treatment requirements

1.3.3 State Standards

The CWA allows states, tribes, and other jurisdictions to develop their own water quality standards to protect their waters. At a minimum, they include the swimmable and fishable goals of the CWA. States must submit their standards for U.S. EPA approval. Monitoring data are compared to the standards for water quality assessment and decisions on whether to list waters as impaired under the CWA Section 303(d). Water quality standards have three critical elements (U.S. EPA, 2002a):

1. Standards should state designated uses that water quality should support, such as recreation, aquatic life, fish consumption, drinking water supply, industry, agriculture, and navigation. Each use has unique set of water quality criteria that must be met for the use to be realized.

2. State water quality criteria are both numeric and narrative. Numeric criteria are thresholds required to support a beneficial use. Narrative criteria describe conditions that must be maintained to support a designated use.

3. States provide an antidegradation statement intended to prevent waters currently in degraded condition from further deteriorating, and minimizing deterioration of high quality water.

The U.S. EPA is actively promoting its goal of ensuring that all states and tribes update their bathing beach standards. After state standards are set, states assess their waters to determine the degree to which these standards are met. The U.S. EPA *Bacterial Water Quality Standards Status Report* (U.S. EPA, 1998a) is an overview of the bacterial water quality standards that have been adopted by states for their marine and fresh recreational waters. The *U.S. EPA Bacterial Water Quality Standards For Recreational Waters, Freshwater and Marine Waters* (U.S. EPA, 2003b) is an update of the 1998 status report. The 1998 and 2003 reports indicate that many states have adopted *E. coli* and enterococci standards; however, many of these states are still regulating according to fecal coliforms requirements while building databases for *E. coli* and enterococci monitoring data.

1.3.4 Other Applicable Standards

1.3.4.1 Coastal Zone Act Reauthorization Amendments (CZARA)

In 1990, Congress passed legislation to protect America's coasts from runoff pollution. It created the Coastal Nonpoint Program, also known as Section 6217 of the CZARA of 1990.

Section 6217 requires the participating U.S. coastal states and territories to establish effective programs to control and prevent polluted runoff into coastal waters. Section 6217 is the first national program to tackle, in a comprehensive and enforceable fashion, the problem of coastal nonpoint pollution. Currently, every eligible coastal state participates in the Coastal Zone Program with the exception of Illinois (U.S. EPA, 2003c).

1.3.4.2 Beaches Environmental Assessment, Closure, and Health (BEACH) Program

The U.S. EPA announced on May 23, 1997 the Beaches Environmental Assessment, Closure, and Health (BEACH) Program (U.S. EPA, 2003d). The BEACH program goal is to reduce the risk of infection to users of the nation's recreational waters. High levels of pathogens in recreational waters can increase human exposure through inhalation, ingestion, and body contact. Scientific studies document the presence of disease-carrying bacteria, viruses, and other pathogens present in local beach water, primarily from sewage and stormwater. The BEACH Program focuses on the following areas to meet the program goals of improving public health and environmental protection programs and providing the public with information about the quality of their beach water:

1. Strengthening the beach standards and testing
2. Providing faster laboratory test methods
3. Predicting pollution
4. Investing in health and methods research
5. Informing the public

Congress subsequently passed the BEACH Act in October 2000 that authorizes U.S. EPA to award grants to eligible states, tribes, and territories to develop and implement beach water quality monitoring programs at coastal and Great Lakes recreational waters near beaches. These grants further support the development and implementation of programs to inform the public about the risk of exposure to disease-causing microorganisms in the waters at the nation's beaches. Nearly $10 million in grants were awarded on April 4, 2003 to 35 eligible states (U.S. EPA, 2003d).

In addition to the BEACH Program initiatives, U.S. EPA is involved with the following activities in other programs to make its waters cleaner and safer for swimming:

1. Assist communities to build and properly operate their sewage treatment plants
2. Work to end sewage overflows in communities with outdated sewer systems
3. Implement a national stormwater program to reduce urban runoff
4. Adapt the CZARA
5. Improve sewage disposal from recreational vessels

National implementation of strong, consistent beach programs will provide the public with important information about the quality of their beach water and allow the public to make

decisions on when and where to swim. The U.S. EPA operates a Website called "Beach Watch" that provides information about the water quality at our nation's beaches, local protection programs, and other beach related programs. The "Beach Watch" Website is updated as new information becomes available, and is available at http://www.epa.gov/waterscience/beaches/ (U.S. EPA, 2003d).

1.4 Evaluation of Pathogen Indicators [1]

This section addresses the complexities associated with using indicators to evaluate water quality for microbiological contamination. Measuring microbial indicators is less expensive, easier, and more common than measuring pathogens directly. Generally, standards set are based on the presence or concentrations of bacterial indicator organisms and the designated use of the water body. In many cases, the sources for the increased indicator microorganism levels are known and establishing the corresponding TMDL is appropriate. However, as stated earlier, there are exceptions, which include cases where numeric water quality standards do not exist and there is identifiable impairment of designated uses (U.S. EPA, 2001a). A target value that reflects attainment of the designated uses should be selected and the TMDL developed to meet it. If there are existing standards that do not adequately address the designated use, a supplementary indicator should be used along with the existing standard. The supplementary indicator would provide additional means for measuring attainment of designated or existing uses.

Indicator organisms and monitoring programs are limited in their ability to predict pathogen presence and health risks. Watershed managers need to understand the complexities associated with using indicators in order to protect public health. Therefore, they may need to conduct monitoring and management measures in addition to those required by the TMDL process. For example, in the event that standards are not met and the pollution source is not evident, watershed evaluations may be necessary to identify causes of elevated indicator concentrations and pathogens present. Alternatively, evaluations would also be warranted when outbreaks occur in the absence of standards violations. The sections below provide background on the indicators used as water quality standards in the U.S.

1.4.1 Use of Indicators

Bacterial indicators were originally adopted to alert public health officials to the presence of human fecal contamination in drinking water supplies. Because of the time, labor, expense, complexity, and analytical limitations associated with directly analyzing for a variety of specific pathogens, bacterial indicator use has remained the mainstay of microbial water quality monitoring for decades. A good indicator organism is present when the pathogens of concern

[1] *Much of this section is excerpted from Monitoring Pathogens in the Watershed: Indicator Organisms and Detection Methods, submitted for peer-reviewed publication by M.E. Tuccillo and J.M. Perdek, and Investigating Watershed Microbial Pathogen Contamination to Manage Public Health Risks, submitted for peer-reviewed publication by J.M. Perdek, M.E. Tuccillo, and S.M. Wankel*.

are present and is easy and inexpensive to detect. It must also occur in much greater numbers than the pathogens and should be at least as resistant to adverse environmental conditions as the pathogens (Armon and Kott, 1996). Indicators are used as the basis for water quality criteria developed to support designated uses, such as primary contact recreation and drinking water supply.

Current U.S. microbial water quality standards are based on criteria developed between 20 and 40 years ago. Based on the Federal Water Pollution Control Administration's (now U.S. EPA) technical advisory report on water quality criteria (Federal Water Pollution Control Administration, 1968), the U.S. EPA recommended that states adopt as a bathing water quality standard fecal coliforms not to exceed 200 organisms/100 mL (U.S. EPA, 1976). There was concern, however, that insufficient data existed to support this decision (National Research Council, 1972). The U.S. EPA conducted research between 1972 and 1979 to reexamine the question of health effects related to swimming in sanitary wastewater-polluted waters. Central to this program, several epidemiological-microbiological studies concluded that fecal coliforms density showed little correlation with swimmer gastrointestinal illness (Cabelli, 1983; Dufour, 1984). Dufour (1984) reported a high correlation between gastrointestinal illness in fresh waters and both *Enterococcus* and *E. coli* concentrations, while Cabelli (1983) reported a high correlation between *Enterococcus* concentrations and gastrointestinal illness in marine waters. Based on this research, the U.S. EPA (1986) revised its recreational water quality criteria to the indicators and concentrations shown in Table 1-10. Fresh water criteria are 33/100 mL for *Enterococcus* or 126/100 mL for *E. coli*. The marine water criterion for *Enterococcus* is 35/100 mL. While most experts agree that *E. coli* and *Enterococcus* are superior indicators than fecal coliform, fecal coliform is still widely used because of its historic use. The information in Table 1-10 also portrays differences in microbial recreational water quality standards in countries around the world. These differences reflect not only the differences in indicator suitability for various geographical areas, water types, and pathogen sources, but also the diversity of opinions as to the most appropriate indicator.

Present approaches to regulating and monitoring recreational waters for pathogen contamination in the U.S. and worldwide suffer from limitations. To examine the issue, experts representing U.S. EPA and the WHO met in November 1998 in Annapolis, MD. The outcome of this meeting, known as the "Annapolis Protocol," is an improved approach for regulating recreational waters that better reflects health risk and yields an enhanced scope for effective management intervention (WHO, 1999). The protocol consists of a classification scheme that provides for assigning a level of risk to a beach area and indicates the management and monitoring actions likely to be appropriate. Risk is determined using a combination of

Table 1-10. Primary Contact Recreational Water Quality Criteria for Microorganisms

Country	*Enterococcus* / 100mL	*E. coli* / 100mL	Total Coliform / 100mL	Fecal Coliform / 100mL
U.S. Marine Water [1]	35	—	—	—
U.S. Fresh Water [1]	33	126	—	—
Australia	—	—	—	300
Brazil [2], Peru [3]	—	—	80%<5000	80%<1000
Canada [4]	350/L	2,000/L	—	—
Colombia, Ecuador	—	—	1000	200
Cuba [1]	—	—	1000	200, 90%<400
European Union [5]	90%<100	—	95%<10,000 [6] 80%<500 [7]	95%< 2,000 [6] 80%<100 [7]
France [8]	—	—	<2000	<500
Hong Kong (marine) [3]	—	180	—	—
India	—	—	500	—
Israel [9]	—	—	—	1000 [6] 100 [7]
Japan	—	—	1000	—
Mexico	—	—	80%<1000 [10] 100%<10,000	—
Poland	—	<1000	—	—
Uruguay	—	—	—	<500 [11], <1000 [12]
Yugoslavia	—	—	2000	—

[1] Geometric mean of at least 5 samples equally spaced over a 30-day period.
[2] "Satisfactory" waters, samples obtained in each of the preceding five weeks.
[3] Geometric mean of 5 most recent concentrations.
[4] Geometric mean of at least 5 samples, taken during a period not to exceed 30 days. When experience has shown that greater than 90 percent of fecal coliforms are *E. coli*, either fecal coliforms or *E. coli* may be measured.
[5] The European Union also has a *Salmonella* requirement of 0/L, a fecal streptococcus guideline of 100/100mL, and an enterovirus requirement of 0/10L.
[6] Mandatory [7] Guideline
[8] France also has a fecal streptococci requirement of <100/100 mL.
[9] Israel uses World Health Organization Guidelines. [10] At least 5 samples per month.
[11] Geometric mean of at least 5 samples. [12] Not to be exceeded in at least 5 samples.

Corbett *et al.*, 1993; Council of European Communities, 2000; Fattal *et al.*, 1987; Federal-Provincial Working Group, 1992; Govt. of India MOEF, 2000, Ho and Tam, 1998; Salas, 1998 (in WHO 1999); and U.S. EPA, 1986.

microbiological indicator monitoring data and an inspection-based assessment of the susceptibility of an area to direct influence from human fecal contamination.

1.4.2 Relationships between Indicators and Illness

The most common microbiological indicator of pathogens is the presence of coliform bacteria, which are commonly found in the enteric tracts of humans and other warm-blooded animals. Testing for these organisms is relatively fast, easy, and inexpensive. If these bacteria are detected in sufficiently high concentrations, then there is a high probability of contamination of human fecal matter, which may contain pathogens. Coliform bacteria are members of the Enterobacteriaceae family and include species of *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia*. Fecal coliforms, a subset of total coliforms, are defined by their ability to grow at elevated temperature (44.5^{o}C). They are associated with the enteric tracts of warm-blooded animals, whereas total coliforms can include bacteria from cold-blooded animals and soil organisms. Fecal coliforms include the familiar *Escherichia coli.* Although most *E. coli* (and most coliforms) are not harmful, some strains, including *E. coli* O157:H7, are pathogenic as discussed in Section 1.2.2.2.

Studies evaluating the use of coliform bacteria as indicators of fecal contamination have shown mixed results (Table 1-11). Some researchers have reported favorably on coliform testing, often in conjunction with other microorganisms, as an indicator of pathogens. Epidemiological research in the United Kingdom concluded that the European Union's recreational water testing requirements, which include total coliforms, fecal coliforms, and other organisms, adequately protect the health of swimmers in coastal waters (Pike, 1994). Several other investigators (Corbett *et al.*, 1993; Ferley *et al.*, 1989; Haile *et al.*, 1999; Seyfried *et al.*, 1985) have reported correlations between fecal coliforms concentrations and incidence of general morbidity or total illness.

Numerous studies, however, have found that fecal coliforms or total coliforms concentrations do not correlate well with illness (Calderon *et al.*, 1991; Cheung *et al.*, 1990; Fattal *et al.*, 1987; Kay *et al.*, 1994; Kueh *et al.*, 1995; and McBride *et al.*, 1998). As stated above, the U.S. EPA epidemiological-microbiological studies conducted in the 1970s concluded that fecal coliform densities showed little or no correlation with gastrointestinal illness among swimmers (Cabelli, 1983; Dufour, 1984). Although fecal coliforms are primarily associated with the enteric tracts of warm-blooded animals, Dufour (1984) suggests that many bacteria in the environment fit the description of fecal coliforms but do not come from gastrointestinal sources. Thus, these bacteria are of questionable use as fecal indicators.

The relationships between coliform bacteria and pathogenic microorganism densities are also problematic. Studies have found poor or no correlations between coliform densities and pathogenic bacteria or enteric viruses in environmental waters (Metcalf *et al.*, 1995; Morinigo *et al.*, 1992; Olivieri *et al.*, 1977). Griffin *et al.* (1999) found enteroviruses in canals of the Florida Keys although none of the sites studied violated state water quality standards for total coliforms or fecal coliforms. Coliform bacteria are poor indicators for the pathogenic protozoa

Table 1-11. Key Points of Epidemiological Studies

Author	Country	Water/ Discharge Types	Indicator Best Correlated with Swimming-Associated Gastrointestinal Illness	Indicator Best Correlated with Other Swimming-Associated Illnesses
Cabelli, 1983	U.S.	Marine/Sewage	*Enterococcus*; EC to a lesser extent	N/A
Fattal *et al.*, 1987	Israel	Marine/Raw Sewage	*Enterococcus*	N/A
McBride *et al.*, 1998	New Zealand	Marine/Treated	*Enterococcus*	N/A
Cheung *et al.*, 1990	Hong Kong	Marine/Sewage and Stormwater	EC (r=0.73) and *Enterococcus* (r=0.60)	N/A
Kueh *et al.*, 1995	Hong Kong	Marine/Sewage and Stormwater	Turbidity, *Clostridium perfringens*, *Aeromonas* spp., *Vibrio cholerae* (non-01)	none found
Kay *et al.*, 1994	United Kingdom	Marine/Various	FS	N/A
Corbett *et al.*, 1993	Australia	Marine/Primary treated	None found	FC for cough, ear symptoms, eye symptoms, fever
Haile *et al.*, 1999	U.S.	Marine/ Stormwater	FC, *Enterococcus*, EC, Viruses, Distance from Storm Drain	FC for skin and respiratory symptoms, enterococcus for skin symptoms, EC for eye, ear and skin symptoms, Viruses for fever, chills, eye, and respiratory symptoms
Pike, 1994	Great Britain	Marine	TC and enteroviruses; FS in cohort study	none found
Dufour, 1994	U.S.	Fresh/Sewage	EC and *Enterococcus*	N/A
Ferley *et al.*, 1989	France	Fresh/Untreated Sewage	FS	FC for general morbidity; FC, *Aeromonas*, and *Pseudomonas aeruginosa* for skin diseases
Seyfried *et al.*, 1985	Canada	Fresh/Not Stated	None found at 0.05 level of significance; FS at 0.069 level of significance	Total staphylococcus (strongest), FC and FS (weakest) for total illness; total staphylococcus for eye and skin illnesses
Calderon *et al.*, 1991	U.S.	Fresh/Animal Nonpoint Source	Staphylococci; bather density	N/A

FC - fecal coliform, FS - fecal streptococcus, EC - *E. coli*, TC - total coliform

Cryptosporidium parvum and *Giardia lamblia*. According to Craun *et al.* (1997), coliforms have been detected during most waterborne outbreaks caused by bacteria and viruses, but during relatively few outbreaks caused by protozoa. Drinking water disease outbreaks, most notably from *Cryptosporidium* and *Giardia*, have occurred in water systems that did not violate the U.S. EPA-issued MCL for total coliforms (Craun *et al.*, 1997). Rose *et al.* (1991) found no associations between either *Cryptosporidium* or *Giardia* and the total coliform or fecal coliform indicator in drinking water sources. Because coliform bacteria are killed more easily by disinfection than viruses or protozoa, coliform absence in evaluating disinfection does not guarantee the absence of health risk (Toranzos and McFeters, 1997).

Several epidemiological studies support the use of *E. coli* and the *Enterococcus* group as indicators of fecal contamination. *Enterococcus*, a subgroup of fecal streptococci, is frequently found in the human digestive tract. They are tolerant of a wide range of environmental conditions and are easy to culture. These characteristics render the *Enterococcus* group a promising indicator. As stated above, research in the U.S. has demonstrated high correlations between *Enterococcus* densities and gastrointestinal illness among swimmers in fresh (Dufour, 1984) and marine (Cabelli, 1983) waters. Epidemiological research in Israel and New Zealand demonstrated strong relationships between *Enterococcus* densities and the incidence of illness among swimmers in marine waters receiving raw sewage (Fattal *et al.*, 1987) and treated sewage discharges (McBride *et al.*, 1998). Other investigators have also reported positive relationships between the incidence of gastrointestinal illness among marine water swimmers and *Enterococcus* densities (Cheung *et al.*, 1990; Haile *et al.*, 1999). Dufour (1984) found a high correlation between *E. coli* and illness in fresh waters.

For cases of nonhuman pollution sources, i.e., runoff from rural areas containing animal waste, using *E. coli* or *Enterococcus* may not be the best approach. In a study of swimmers in a rural pond receiving animal fecal wastes, Calderon *et al*. (1991) found swimmer illness to be correlated with numbers of swimmers and staphylococci densities. They concluded that illnesses appeared to be caused by swimmer-to-swimmer transmission.

The primary focus of many of the epidemiological studies was gastrointestinal illness although a few studies examined other health effects (Corbett *et al*, 1993; Ferley *et al*., 1989; Haile *et al*., 1999; Pike, 1994; Seyfried *et al*., 1985). Fecal coliforms, S*taphylococcus Aeromonas*, *Pseudomonas aeruginosa*, and viruses were found to be related to a variety of symptoms as shown in Table 1-11. The studies were conducted in North America, Australia, and Europe in marine and fresh waters with a variety of discharge types. Fecal coliform was related to respiratory, ear, eye, and skin illnesses. It was also related to fever and total illness. *E. coli* was associated with eye, ear, and skin symptoms. Total staphylococcus was related to eye, skin, and total illness. Viruses were related to fever, chills, eye, and respiratory symptoms. *Aeromonas* and *pseudomonas aeruginosa* were related to skin diseases. While these studies provide general information on relationships between indicators and various ailments, a great deal of additional research is needed to determine the appropriate microorganisms that should be measured for assessing risk of nonenteric illness. The organisms are likely to vary for fresh and marine waters, for different regions of the world, and for different pathogen sources.

While the use of indicators has these limitations, the use of direct pathogen analysis has limitations as well as expressed by Pontius and Clancy (2000):

> "Current testing methods cannot determine with certainty whether *Cryptosporidium* detected in drinking water is alive or whether it can infect humans. In addition, the current method often requires several days to get results, by which time the tested water has already been used by the public and is no longer in the community's water pipes. ...Analytical method limitations prevent using *Cryptosporidium* monitoring data to accurately assess risk or even to set an acceptable level of risk for *Cryptosporidium* in drinking water. Water utilities must be vigilant in applying source water protection and appropriate treatment to protect customers against this organism."

This scenario can be applied to many pathogens besides *Cryptosporidium*. Microbial indicators are not always indicative of pathogens. Since we are ultimately concerned with pathogens, and not indicators, the relative safe number or infectious dose is often zero. Pathogens are capable of reproducing to large numbers if only one is originally present. The way to provide a safe number is to set treatment standards to remove or kill all pathogens that are present (Warrington, 2001a). It therefore is imperative for the development of new water quality monitoring methods that can identify fecal pathogen contamination, specifically human fecal contamination (Calderon *et al*., 1991). This would provide a greater assessment of the potential for waterborne disease to occur. Artificial neural networks are modeling tools that have the potential for predicting peak microbial concentrations and for identifying land use-associated fecal pollution sources and relative ages of runoff (Brion and Lingireddy, 2003). Using a ratio of atypical to total coliform colonies (AC/TC) from a membrane filtration analysis of a receiving water sample, identification of fecal pollution sources has been 90% accurate. Presently, there are many practices to minimize the exposure to pathogens, such as prudent outfall placement and maximizing inflow reduction. Chapter 3 discusses management and control of pathogens aimed at reducing pathogen contribution to receiving waters.

1.5 Conclusions

For water bodies regulated under the CWA and not meeting standards for microbial contaminants, a TMDL or approach for bringing the water body into compliance must be developed. All legislation and regulations applicable to the water body need to be considered throughout the TMDL process. These include the SDWA, CZARA, NPDES permits, and BEACH program. In most cases, the TMDL will be established for the microbial indicator involved in the violations of the state ambient water quality standard. However, in some cases, it is appropriate to develop and meet an alternative narrative standard that addresses the designated use impairment. These could include situations where a pathogen source is identified as the cause of waterborne disease outbreaks.

Waterborne disease outbreaks can be caused by pathogenic bacteria, protozoa, viruses, helminth worms, and fungi. Worms and fungi have not been identified in the waterborne disease outbreak data compiled by U.S. EPA and the CDC. These reports identify *Cryptosporidium*

parvum, Giardia lamblia, E. coli O157:H7, and *Shigella* as causative agents for significant numbers of outbreaks and illnesses from exposure to contaminated recreational waters and drinking waters from surface sources. In addition, many waterborne disease outbreaks and cases go unreported, and there are outbreaks where the pathogen responsible is not identified.

Indicator organisms and monitoring programs are limited in their ability to predict pathogen presence and health risks. However, because of their ability to provide a general indication of the presence of human fecal material and their low cost and complexity, microbial indicators have remained the primary means for assessing microbiological contamination in water. Fecal coliform has historically been the microbiological indicator of choice, but its presence does not always correlate well with the incidence of disease. Coliforms are being replaced by the more specific indicators *Enterococcus* and *E. coli* for fresh waters, and *Enterococcus* for marine waters (U.S. EPA, 2003b). The acceptance of these indicators is gradually occurring. Once a large database is established for *Enterococcus* and *E. coli*, these organisms will be used by the states with conviction and confidence. Most of the investigations concerning relationships between total coliform bacteria and pathogenic microorganisms in environmental waters found poor or no correlations. Of the 13 epidemiological research studies from around the world reviewed for this publication, *Enterococcus* density appears to be the indicator most strongly correlated with gastrointestinal illness among bathers in recreational waters. *E. coli* is also related to gastrointestinal illness in a number of studies. Both of these organisms were found to be related to enteric illness more frequently than fecal coliforms. Five of the studies reviewed investigated non enteric effects in addition to gastrointestinal. Fecal coliforms, staphylococcus, *Aeromonas*, *Pseudomonas aeruginosa*, and viruses were found to be related to respiratory, eye, and skin symptoms as well as fevers. Climate, water type, and pollution sources (i.e., sewage or stormwater runoff) are all factors affecting the ability of an indicator to be a predictor of pathogenic pollution and illness.

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Chapter Two

Detection Methods and Alternate Indicator Organisms [1]

2.1 Introduction

Public demand and regulatory requirements compel monitoring for pathogen risks. Such monitoring requires feasible and accurate detection methods for appropriately selected microbes. Water quality monitoring in the U.S. is most frequently conducted for bacterial indicators using the standard membrane filtration or multiple tube fermentation/most probable number methods. U.S. EPA requires that a Total Maximum Daily Load (TMDL) be developed for water bodies violating standards, which are determined using the monitoring results. The TMDL is generally developed for the microorganism responsible for the violation. There are exceptions, however, such as when there are waterborne disease outbreaks. In these instances, other detection methods may need to be employed to identify causative agents and determine their presence and concentrations in a watershed.

Microorganisms responsible for waterborne disease outbreaks are identified through clinical testing of individuals who seek medical care for their illness. Illnesses are classified as waterborne disease outbreaks when more than one individual is found to be infected with the same microbe believed to be from a common source of drinking or recreational water. Environmental officials assigned to investigate and manage the pollution responsible may use microbial source tracking and pathogen detection methods to investigate possible sources and determine the extent of contamination.

This chapter presents information on detection methods for bacteria, viruses, and protozoa, summarized in Tables 2-1, 2-2, and 2-3, respectively. In the section on bacteria, detection methods for both indicators and pathogens are discussed, as well as alternatives to the traditional indicator organisms and an overview of selected methods for microbial source tracking. Although helminths and fungi are discussed in Chapter 1, their methods were not reviewed for this chapter due to the high unlikelihood that these organisms will be encountered in urban watersheds in the U.S. Information about pathogenic fungi is available in *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.*, 1998), hereafter referred to as *Standard Methods*. A method for helminth ova is presented in the U.S. EPA document *Control of Pathogens and Vector Attraction in Sewage Sludge* (U.S. EPA, 1999) available at http://www.epa.gov/ORD/NRMRL/Pubs/1999/625R92013.pdf.

[1] *Much of this chapter is excerpted from Monitoring Pathogens in the Watershed: Indicator Organisms and Detection Methods, submitted for peer-reviewed publication by M.E. Tuccillo and J.M. Perdek, and Investigating Watershed Microbial Pathogen Contamination to Manage Public Health Risks, submitted for peer-reviewed publication by J.M. Perdek, M.E. Tuccillo, and S.M. Wankel*.

2.2 Detection Methods

2.2.1 Bacteria

2.2.1.1 Cultural and Enzyme-Based Methods

Cultural methods, or those that grow bacteria in a prepared medium, have been used for indicator bacteria detection and enumeration for over a century (Pyle *et al.*, 1995). Membrane filtration methods are well established and routinely used. The details of these methods are described in *Standard Methods*. The water sample is filtered, the filters are incubated on a growth medium for a specific time and temperature, and the resulting colonies are enumerated. The membrane filtration incubation period is 24 hours for fecal coliforms and total coliforms, but for other bacteria can take longer; *Staphylococcus aureus* and *enterococcus* cultures must be incubated for 48 hours, and *Pseudomonas* cultures should be incubated for 72 hours. An improved U.S. Environmental Protection Agency (EPA) method for *enterococcus* using a modified type of agar (mEI) requires only 24 hours of incubation (U.S. EPA, 1997). The membrane filtration methods require confirmation tests, which entail further effort and additional incubation time.

Multiple-tube fermentation/most probable number methods for coliform bacteria are based on the ability of the organisms to ferment lactose. Tubes with growth medium are inoculated with a series of undiluted and diluted samples, with several tubes inoculated per dilution. Following incubation at the specified temperatures, the numbers of tubes demonstrating a positive response are recorded and a statistical estimate of the bacterial density is determined. Most probable number methods take 48 hours for coliform incubation, plus an additional 24-hour confirmation test. *Enterococcus* and fecal streptococcus are incubated for 24-48 hours with an additional 24 hours for confirmation (Clesceri *et al*., 1998). Fecal coliforms, however, can be analyzed in 24 hours by the A-1 broth 1-step method (*Standard Methods* # 9221E.2).

Methods that rely on counting the colonies that form during incubation, including membrane filtration, tend to underestimate bacterial numbers (Sartory *et al*., 1999). This phenomenon affects analyses for both indicators and pathogens. This may be due to clumping, particle association, cell injury, and the viable-but-nonculturable (VBNC) state of the bacteria. In the VBNC state, cells may maintain viability and metabolic activity, but fail to grow and multiply on culture plates. Huq and Colwell (1996) reviewed this topic with special attention to *Vibrio cholerae*, although this condition applies to *Aeromonas*, *Shigella*, *Staphylococcus*, and *Campylobacter*, among others. Such underestimation of bacterial counts presents the obvious danger of giving rise to misleading reports.

Substrate hydrolysis by a specific enzyme with colorimetric endpoints forms the basis of several detection methods. In substrate hydrolysis, the hydrolysis reaction between an enzyme in the bacteria and the substrate results in a color change that is used to determine the analytical results. Cultural methods for *E. coli* are based on detecting the action of the enzyme β-

glucuronidase upon the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) (Sartory *et al*., 1999; Shadix *et al*., 1991). The product fluoresces blue under long wavelength ultraviolet (UV) light, indicating the presence of *E. coli*. The *E. coli* technique in *Standard Methods* requires additional incubation of coliform-positive membrane filtration samples to test for MUG utilization by β-glucuronidase. The U.S. EPA method using membrane-Thermotolerant *E. coli* (mTEC) agar for *E. coli* analysis (U.S. EPA, 1985) relies upon detection of the enzyme urease; a modified mTEC method relies upon β-glucuronidase. Substrate hydrolysis by β-galactosidase is used for detection of thermotolerant coliforms. The rapid method tested by Robertson *et al.* (1998) uses only a 6-hour incubation to test for β-glucuronidase for *E. coli* and β-galactosidase for thermotolerant coliforms.

There are rapid alternatives to membrane filtration methods based on enzyme substrate utilization by coliform bacteria and *enterococci*. IDEXX Laboratories (Westbrook, ME) produces a series of widely used EPA-approved products. Their Colilert® Quantitray™ , which uses their patented Defined Substrate Technology, is an easy-to-use commercial most probable number method designed for simultaneously determining the presence of total coliforms and *E. coli* in 24 hours (Edberg *et al.,* 1989; Townsend *et al*., 1996). Total coliforms are detected by the action of β-galactosidase, and *E. coli* detection is based on the action of β-glucuronidase. Coliforms produce a yellow product and *E. coli* produces a product that fluoresces yellow. Colilert-18® permits detection of these organisms in only 18 hours. Colilert® has been shown by some researchers to be as sensitive as Multiple Tube Fermentation (MTF) and membrane filtration (Eckner, 1998; Fricker *et al*., 1997; Edberg *et al*., 1990). Francy and Darner (2000) used recreational water to compare Colilert to the U.S. EPA-recommended mTEC method (U.S. EPA, 1985), a β-glucuronidase-based membrane filtration technique. The authors found statistically significant differences between the methods, but note that their test area was small and further work is needed. The expression of β-glucuronidase can, however, be suppressed by environmental stress (Sartory *et al*., 1999; Edberg *et al*., 1990), raising the possibility of underestimating bacterial densities. Furthermore, *E. coli* O157:H7 does not possess this enzyme, so a separate test for *E. coli* O157:H7 would be needed if it is suspected.

Similar to Colilert®, IDEXX Laboratories' enzyme-based Enterolert® method is designed to provide a most probable number method in 24 hours for *enterococcus* in water. The hydrolyzation product of the substrate fluoresces blue. Abbott *et al.* (1998) found a positive correlation between Enterolert® and membrane filtration in marine waters in New Zealand. Budnick *et al.* (1996) and Eckner (1998) reported equal or better sensitivity and specificity with Enterolert compared to membrane filtration in recreational waters.

Because indicator bacteria are used as a basis for public health decisions in dynamic aquatic environments such as beaches, long analysis times are problematic because levels of *E. coli* and thermotolerant coliforms fluctuate. Fortunately, there are rapid method alternatives to the commonly used cultural methods can speed decision-making about protective measures such as beach closings. The 18-hour incubation time for Colilert and the 6-hour incubation used in the method of Robertson *et al.* (1998) are two examples of incubation methods that require less time. In addition to rapid cultural methods, other classes of detection methods, such as immunological and genetic techniques, offer possibilities for faster analysis times.

2.2.1.2 Immunological Methods

A group of immunological detection methods for microorganisms is based on the use of antibodies, which bind with antigens on the organism's surface. A limiting factor with all immunological techniques is the specificity of the antibody used. Ideally an antibody should bind only with a single antigen, thereby targeting only the organism of concern. Monoclonal antibodies (Mabs) are clonally derived from a single antibody-producing cell. This means that they are exceptionally pure and highly specific in their action.

Some immunological methods are applicable for efficient bacterial detection methods. In immunofluorescence (IF), the antibodies are tagged with a dye that fluoresces under UV light; enumeration can be accomplished by epifluorescent microscopy. Sartory and Watkins (1999) note that there is promise for a limited cultural period (4-6 hours) coupled with detection either by substrate light emission or immunological techniques for same-day results. In their review of rapid methods, McFeters *et al.* (1999) cite examples of the staining of bacteria with fluorescent antibodies performed directly on membrane filters. This avoids steps such as sample concentration and fixation on glass slides.

Because pathogenic *E. coli* O157:H7 does not produce β-glucuronidase, the *E. coli* procedures in *Standard Methods* will not detect it without additional steps. Immunological techniques may be useful in situations where this pathogen is suspected. The rapid *E. coli* O157: H7 methods of Pyle *et al.* (1995, 1999) involve incubation with a dye that indicates viability, followed by fluorescent antibody staining and enumeration by epifluorescent microscopy or laser scanning cytometry (the study and measurement of cells). Kfir *et al.* (1993), however, caution against problems of specificity with the use of monoclonal antibodies as a rapid tool for detecting fecal bacteria in water, and in particular *E. coli*.

Commercially available instruments such as Chem Scan® can detect and enumerate fluorescent bacteria (McFeters *et al.*, 1999), further facilitating rapid detection methods. Commercial sensors continue to be developed and were reviewed by Ivnitski *et al.* (1999). Some are immunologically based; others rely on enzyme detection or nucleic acid detection. A rapid immunological technique for *E. coli* O157 and *Salmonella typhimurium* (Yu and Bruno, 1996) uses a commercial sensor and shows promise as a screening tool, identifying samples that should be further analyzed. These simplified, commercial screening tools provide additional options for situations where easy, rapid screening is desired.

A process called enzyme-linked-immunoabsorbent assay (ELISA) tags an antibody with an enzyme. After incubation, an enzyme substrate is added, and the formation of a pigmented product is indicative of the amount of enzyme present in the sample and, therefore, the amount of microorganism in the sample (Bitton, 1980). Advantages of ELISA are that it is robust, versatile and simple to perform (Kfir *et al.*, 1993). As with any immunoassay, limitations are related to the specificity of the antibody used. Various easy-to-use commercial ELISA kits are available, such as the Wellcolex kits (Murex Biotech Dartford, United Kingdom). Developed mostly for clinical or food applications, these techniques may be useful for water quality testing when simple techniques are desired. Limited trials with wastewater have, however, raised the

possibility of cross reactions with competing organisms in the samples (Meckes, 2001). Further testing of these kits with environmental waters is needed.

2.2.1.3 Genetic Methods (Gene Probes and PCR)

Development of genetic methods has provided new sensitive options for pathogen detection. Gene probes are nucleotide sequences that pair with corresponding sequences in the sample through a process called hybridization (Hurst *et al*., 1989). The probes make good detection tools and can be labeled with a radioisotope, an enzyme, or a fluorescent chromogene to permit detection. Although genetic methods require more sophisticated equipment and techniques than cultural methods, there are commercially available gene probe kits that only require typical microbiological laboratory equipment and are easy to use. For example, Gene-Trak (Hopkinton, MA) produces gene probe assays for several organisms including *E. coli* and *Salmonella.* The kits, which are geared primarily toward food or clinical applications, have a colorimetric endpoint and come with a photometer. Rice *et al.* (1995) found that the probe performed well with pure cultures, but failed to detect seven of thirteen positive cultures in creek and river samples, possibly due to low bacterial densities in the natural waters. The authors note that further research is needed to improve the performance of the method with environmental samples, possibly through increased enrichment or larger sample aliquots.

The polymerase chain reaction (PCR) has greatly improved the ability to detect low densities of pathogens in environmental samples. PCR produces many copies of a target section of a microorganism's deoxyribonucleic acid (DNA). With the large number of copies produced by PCR, the target DNA can be detected using gene probes or gel electrophoresis (Toze, 1999). Gel electrophoresis is a process used to impart an electric current to DNA fragments in a gel of specific density. Different size fragments move at different rates and can be visualized as a series of bands in the gel. The use of PCR offers several advantages, including specificity, sensitivity, rapidity, accuracy, and the capacity to detect small amounts of target nucleic acid in a sample. PCR-based methods can be used both to rapidly identify bacteria that have been isolated and for direct pathogen detection in environmental samples (Toze, 1999).

Several researchers have published protocols for PCR-based detection of *E. coli* in water (Fricker *et al*., 1999; Kong *et al*., 1999b; Tsen *et al*., 1998). The method of Fricker *et al.* (1999) is especially quick, identifying *E. coli* from membrane filters within two hours. Tsen *et al.* (1998) use an 8-hour pre-culture step, and claim detection of 1 cfu per 100 mL. By combining PCR and radiolabeled gene probes, Bej *et al.* (1990) developed a sensitive and specific method for *E. coli*, *Salmonella* and *Shigella spp.* A PCR method for *Salmonella spp.* published by Way *et al.* (1993) can also detect other coliform bacteria (e.g., *Shigella*, *E. coli* and *Citrobacter*), rendering the technique very useful for environmental samples. Palmer *et al.* (1993) found PCR to be sensitive and specific for *Legionella* in sewage treatment plant influent and in ocean receiving waters. A method for detecting *Aeromonas* in seawater (Kong *et al.*, 1999a) may be useful for monitoring because of the prevalence of *Aeromonas spp*. in the aquatic environment.

There are, nevertheless, several disadvantages to PCR-based methods. They require specialized equipment and skilled technicians (Toze, 1999). The results of PCR alone do not

provide a means for quantification; they indicate presence or absence of the target genetic material. Furthermore, PCR alone does not directly provide information about the viability or infectiousness of the organisms because DNA may persist in the environment (Alvarez *et al*., 1993; Gantzer *et al*., 1999; Kopecka *et al*., 1993; Metcalf *et al*., 1995; Sobsey *et al*., 1998a). These techniques are still at the research stage and are beyond the capabilities of most state and local municipalities for routine analyses. However, they may eventually become a viable option for routine pathogen analysis and may be especially useful for studies characterizing the identities and sources of pathogens within a watershed.

2.2.2 Viruses

Current routine monitoring strategies do not test for viruses; they rely on indicator bacteria. Various viruses (e.g., rotavirus, adenovirus, hepatitis A virus and Norwalk-like viruses) are important agents of illness in sewage-polluted waters (Metcalf *et al.*, 1995). There are clearly cases where virus identification is needed, such as in investigations of outbreaks or in research studies. In cases where direct detection of viruses is needed, a variety of methods exists and new methods continue to be developed.

2.2.2.1 Sample Concentration

Because of the low concentrations of viruses in environmental samples, methods used to detect enteric viruses require an initial concentration step to make them detectable. For environmental waters this is typically accomplished by sorption of viruses onto a filter. According to Schwab *et al.* (1993), hundreds to thousands of liters of water may need to be filtered through a special filter cartridge to achieve sufficient virus concentration for detection. A yarn fiber filtration cartridge or a cartridge with pleated sheets of filter material are particularly useful because of field portability. After filtration, the viruses are generally recovered from the filter into about 1L of eluant. *Standard Methods* describes techniques for virus concentration by adsorption to and elution from microporous filters. Beef extract is one of the most common eluants (DeLeon and Sobsey, 1991; Schwab *et al.*, 1993). A secondary concentration step may be needed, such as ultrafiltration or flocculation. In their review of filtration and elution methods, DeLeon and Sobsey (1991) caution that humic and fulvic substances in water may interfere with virus sorption onto filters. They also point out that adsorption/elution efficiencies vary for different viruses; for some the recoveries are low.

Table 2-1. Summary of Detection Methods for Bacteria			
Cultural and Enzyme-Based			
Method	**Duration**	**Results Provided**	**Capabilities Needed**
Membrane Filtration	24 hours or longer depending on bacteria + 24-hour confirmation	Enumeration, Presence-Absence	General Microbiology Laboratory
Multiple Tube Fermentation/Most Probable Number (MTF/MPN)	24 hours or longer depending on bacteria + 24-hour confirmation	Enumeration, Presence-Absence	General Microbiology Laboratory
Substrate Hydrolysis – Colorimetric	6 to > 24 hours depending on method and organism	Presence-Absence	General Microbiology Laboratory
Defined Substrate Technology	*E. coli* and Total Coliform – 24 hours; *Enterococcus* – 24 hours	Enumeration	General Microbiology Laboratory
Immunological			
Immunofluorescence (IF)	< 24 hours	Enumeration by epifluorescent microscopy	Specialized Microbiology Lab.
Commercially Available Instruments	< 24 hours	Enumeration	General Microbiology Laboratory
Enzyme-Linked-Immunoabsorbent Assay (ELISA)	Varies	Enumeration	Kits available for clinical and food applications; more research for environmental app. needed
Genetic			
Gene Probes	Time varies	Presence-Absence, Enumeration in research stage	Kits available for clinical and food applications; more research for environmental app. needed
PCR	< 24 hours	Presence-Absence, Enumeration in research stage	Specialized Microbiology Lab.; techniques still in research stage

Table 2-2. Summary of Detection Methods for Viruses			
Method	**Duration**	**Results Provided**	**Capabilities Needed**
Cultural			
Cultural Assay	Varies, on the order of days	Presence-Absence, enumeration; indicates viability	General Microbiology Laboratory
Immunological			
Immunological	Varies	Enumeration by epifluorescent microscopy; Does not indicate viability	Specialized Microbiology Lab.
Immunological: ELISA	Varies	Presence-Absence; Enumeration	More research needed for environmental app.
Genetic			
Gene Probes	Varies	Presence-Absence by radioisotope or enzyme	Specialized Microbiology Lab.; More research for environmental app. needed
PCR	< 24 hours	Presence-Absence	Specialized Microbiology Lab.; techniques still in research stage

2.2.2.2 Cultural Assay

Several assay techniques are available for virus detection in the concentrated sample. Detection methods given in *Standard Methods* rely on the infection and destruction of host cells by the virus (cytopathic effects). In the plaque assay method, for example, a viral suspension is placed on a monolayer of cells, and areas of cell destruction due to infection (plaques) are enumerated and expressed as plaque-forming units (PFU). An advantage of cell culture is that it indicates viability. There are, however, disadvantages. Cell culture assays such as the plaque assay method require different cell lines for detection of different viruses. Although most enteric viruses can be cultured, some viruses, such as Norwalk virus, hepatitis A and E, calciviruses, rotaviruses and astroviruses either do not grow or grow slowly in cell culture assays (DeLeon and Sobsey, 1991; Metcalf *et al*., 1995). Thus, cell cultures cannot be used to detect several important pathogenic viruses.

2.2.2.3 Immunological Techniques

Immunological techniques are useful in virus detection. The viruses may be in suspensions, trapped on filters, or in cell cultures (Hurst *et al*., 1989). When they are trapped on filters, without some form of cell culture, the assay cannot indicate infectivity. As with the bacterial techniques mentioned earlier, use of a fluorescently-tagged antibody permits enumeration by epifluorescent microscopy. Oragui *et al.* (1989) have used immunofluorescence for detection of rotaviruses in wastewaters. Similarly, radioimmunoassay uses an antibody tagged with a radioactive isotope to bind to the viral antigen, and detection is accomplished by measuring the radioactivity of the antibody-antigen complex.

Variants of the enzyme-linked assay can detect viral antigens trapped on a filter or associated with infected cells (for viruses that can be cultured). ELISA has been used to detect Hepatitis A virus in tap water (Schnattinger, 1985). Nasser and Metcalf (1987) and Nasser *et al.* (1993) developed an amplified ELISA (A-ELISA) method for virus detection that has greater sensitivity than ordinary ELISA, as well as good specificity, speed, and low cost. Nasser *et al.* (1994) used A-ELISA to indicate the presence of viable poliovirus in water. According to Kfir and Genthe (1995), commercial clinical ELISA kits have been used for environmental waters and are available for some viruses, including rotaviruses and adenoviruses.

2.2.2.4 Gene Probes

Viruses may be detected by the use of gene probes. As with the immunological methods, the target material may be present in a solution, trapped on a filter, or present in infected cells. Detection may be accomplished via a radioisotope or enzyme attached to the gene probe. An effective method must specify a target nucleic acid sequence that is specific to the organism of concern. As with other assays, prior amplification by cell culture indicates that the viruses are infective. Hurst *et al.* (1989) note that hybridization is more sensitive and faster than plaque assays or immunofluorescence. According to Gerba *et al.* (1989), hybridization is much more sensitive than ELISA methods, and gene probes have been developed for the major groups of enteric viruses. Gene probes have been used for the detection of hepatitis A virus and other enteroviruses in drinking water samples that were negative by radioimmunoassay and that required weeks of propagation in cell cultures to be detectable by immunoassays (Shieh *et al*., 1991). Other examples of studies using gene probes include the detection of rotavirus in fresh and estuarine waters (Nasser *et al.*, 1991), enteric viruses in raw and treated waters (Genthe *et al.*, 1995), and poliovirus in sewage-contaminated groundwater (Margolin *et al.*, 1990). Margolin *et al.* (1993) found excellent agreement between cell culture and gene probe methods for a variety of environmental water samples. As noted earlier, however, genetic techniques require sophisticated equipment and techniques. The research studies show promise for efficient viral detection, but easy-to-use kits are not readily available.

2.2.2.5 PCR-based Methods

The polymerase chain reaction is particularly useful for virus detection because it amplifies the low quantities of viral genetic material present in environmental samples. The use

of PCR for detecting viruses offers many advantages over the traditional methods, including lower detection limits, increased range of viruses detectable, specificity, and shorter processing time (Toze, 1999). As with other methods, water samples may need to be filtered or otherwise concentrated first. Reverse transcriptase, a compound that catalyzes the formulation of DNA using RNA as a template (RT-PCR), is used when a virus' genetic material is RNA. The RT-PCR methods can detect less than 10 PFU of a virus in a filter eluate sample in less than two days.

Standard sample concentration procedures can pose problems for PCR. Humic acids, which cause interference, can be concentrated along with the viruses. Proteins and salts in beef extract eluant can also interfere with molecular methods (Schwab *et al*., 1993). It is, therefore, necessary to separate the viruses and their DNA from such impurities (Kopecka *et al*., 1993). The inhibitory problems in some samples have been avoided by using immunologic-based methods to capture viruses for subsequent PCR amplification (Metcalf *et al*., 1995; Schwab *et al*., 1996; Toze, 1999).

Polymerase chain reaction-based techniques have been used successfully for detection of viruses in various types of environmental samples, often with relatively short analysis times. Methods have been developed for astroviruses (Marx *et al.*, 1998), enteroviruses (Gilgen *et al.*, 1995; Griffin *et al.*, 1999; Vantarakis and Papapetropoulou; 1998, 1999), rotaviruses (Soule *et al*., 2000), and adenoviruses (Vantarakis and Papapetropoulou, 1998, 1999) in a variety of environmental waters. In a comparison of three detection methods for enteroviruses in activated sludge and sewage waters, Kopecka *et al.* (1993) found PCR to be vastly more sensitive than cell culture methods and direct hybridization. A number of RT-PCR methods offering various advantages have been devised. These include a triple RT-PCR method for the simultaneous detection of hepatitis A virus, poliovirus, and rotavirus (Tsai *et al*., 1994), an assay for enteroviruses with a tissue culture state to indicate infectivity (Fricker *et al*., 1999), and a relatively rapid method using RT-PCR, followed by hybridization and a form of ELISA (Greening *et al*., 1999).

2.2.3 Cryptosporidium and Giardia

2.2.3.1 Immunofluorescence

As with viruses, identification of *Cryptosporidium parvum* oocysts in water is not routine, limiting our ability to assess the public health threat from *Cryptosporidium* (Rose, 1997). The public health impacts of this organism are discussed in detail in Chapter 1. The detection procedure for *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts described in *Standard Methods* is an immunofluorescence (IF) procedure. To prepare the sample, hundreds of liters of water are passed through a filter cartridge. Cysts and oocysts are recovered from the cartridge, concentrated, and filtered onto a membrane. In addition to the epifluorescent microscopy phase, contrast microscopy is used for confirmation of the internal structures of the organisms. The newest U.S. EPA-recognized IF method for *Cryptosporidium* and *Giardia* (U.S. EPA, 2001) is a more streamlined method that entails filtration of only 10 L of water, uses well

slides instead of membrane filters, and uses differential interference contrast (DIC) microscopy for confirmation.

The IF procedures have low recoveries, are costly and time-consuming, and cannot indicate viability (Slifko *et al*., 1997). The most recent edition of *Standard Methods* acknowledges these limitations, but does not provide an updated method, noting that methods research is evolving rapidly. Allen *et al.* (2000) note that IF techniques have a high rate of both false positives and false negatives, rendering monitoring results highly suspect.

Two methodologies address the problem of viability. Jarmey-Swan *et al.* (2000) improved upon IF for *Giardia* cysts by staining with fluorescein diacetate prior to antibody staining. The combination of the two stains allows identification of viable cysts via microscope. Slifko *et al.* (1997, 1999) have developed and statistically standardized a detection method based on cell culture technology combined with an IF assay. The technique, called the Foci Detection Method (FDM), can be used to detect concentrations as low as 10 oocysts per sample. This method has good promise of being a specific test for *Cryptosporidium parvum*, but it has not yet been tested with all *Cryptosporidium* species.

Table 2-3. Summary of Detection Methods for *Cryptosporidium* and *Giardia*

Method	Duration	Results Provided	Capabilities Needed
Immunological			
Immunofluorescence	72-96 hours	Enumeration by epifluorescent and contrast microscopy; Does not indicate viability	Specialized Microbiology Lab.
Genetic			
Gene Probes	Time varies	Presence-Absence	Specialized Microbiology Lab.; more research for environmental app. needed
PCR	< 24 hours	Presence-Absence; does not indicate viability	Specialized Microbiology Lab.; techniques still in research stage

2.2.3.2 Gene Probes and PCR-Based Methods

While immunofluorescence remains the primary approach for *Giardia* and *Cryptosporidium* analyses, work is continually underway to devise improved techniques that may replace the current methods. Rose (1997) notes that PCR, ELISA, cultural, immunomagnetic separation (IMS), and colorimetric methods are not yet sufficiently developed for routine use. Below is an overview of methods employed in research studies; these may point the way for future routine detection options.

As an alternative to the antibody approaches, gene probes have been used with fluorescent staining of *Cryptosporidium parvum* oocysts in water (Vesey *et al*., 1998). Prescott *et al.* (1999) describe the use of gene probes for the detection of *Cryptosporidium parvum*. The method has good specificity and determines viability.

Studies using PCR for detection of *Cryptosporidium* and *Giardia* (Rochelle *et al*., 1997; Stinear *et al*., 1996; Ware *et al*., 1995) have shown that PCR has excellent sensitivity. Furthermore, simultaneous detection of *Cryptosporidium* and *Giardia* is possible. Wiedenmann *et al.* (1998) provide a thorough review of PCR for the detection of *Cryptosporidium parvum*. As with viruses, methods are available for separation of cysts and oocysts from substances that can inhibit PCR. For example, a technique called the Xtra Bind Capture System has been used to facilitate the concentration of *Cryptosporidium* from water prior to RT-PCR (Kozwich *et al.*, 2000). In this method, potential inhibiting contaminants were removed and PCR amplification was performed without needing to elute the oocysts from the capture material. The authors completed the analysis within only three hours. Other rapid and sensitive PCR methods combine immunomagnetic (magnetic beads with antibodies) separation of *Cryptosporidium* oocysts, followed by PCR for amplification and hybridization for detection (Hallier-Soulier and Guillot, 1999; U.S. EPA 2001). Champliaud *et al.* (1998), however, note difficulties differentiating between *Cryptosporidium parvum* and other nonpathogenic *Cryptosporidium* species using PCR. Furthermore, as with viruses, PCR alone cannot indicate protozoan viability. An alternative is to use messenger RNA (mRNA) for the PCR. The mRNA tends to have a short half life and therefore should not be present to be recovered from dead organisms (Wiedenmann *et al.*, 1998).

2.3 Alternative Indicator Organisms

2.3.1 Clostridium perfringens

Clostridium perfringens is a hardy, spore-forming bacterium that has potential use as an indicator of pathogenic bacteria, viruses, and protozoa. In wastewater treatment and disinfection evaluations, *C. perfringens* was found to be more disinfection-resistant than fecal coliform and *enterococcus*, and was a good indicator of the inactivation of *Cryptosporidium parvum* oocysts (Sobsey *et al.*, 1998b). It was also found to be a good indicator for human enteric viruses, *Cryptosporidium,* and *Giardia* in treated drinking water and river water (Payment and Franco, 1993). Research by Kueh *et al.* (1995) demonstrated correlations between gastrointestinal symptoms and concentrations of *Clostridium perfringens*. In marine waters it has been found to correlate with *Salmonella spp*. (Morinigo *et al.*, 1992) and *Giardia* and *Aeromonas* densities

(Ferguson *et al.*, 1996). *C. perfringens* has several desirable characteristics, including its presence in human feces but not bird droppings, and the superiority of spore survival to human pathogen survival. Furthermore, it can be easily and reliably enumerated using a membrane filter method.

2.3.2 Bacteriophages

Bacteriophages, viruses that infect bacteria, show promise as water quality indicators. Almost all bacteria known today have one or a group of specific bacteriophages that infect them. Coliphages are bacteriophages specific to coliform bacteria. As with *C. perfringens,* coliphages were found to be more resistant to disinfection than *E. coli,* fecal coliform and *enterococcus* in evaluations of wastewater treatment and chlorine disinfection (Farrah *et al.*, 1993; Sobsey *et al.*, 1998b).

Bacteriophages that infect through the bacterium's pili are called F+ (male-specific) phages, and bacteriophages that infect through the bacterium's membrane are called somatic phages. Studies have found F+ bacteriophages to be effective indicators of enteric virus concentrations in fresh waters (Havelaar *et al.*, 1993; Nasser and Oman, 1999). Lucena *et al.* (1996) suggested using phages of Bacteriodes fragilis, *C. perfringens,* and sometimes enteroviruses as indicators of persistent fecal pollution in marine sediments. In an urban estuarine study, however, F+ RNA bacteriophages did not correlate well with the pathogens measured (Ferguson *et al.*, 1996). Serrano *et al.* (1998) found that F+ RNA phages had low correlations with microbiological parameters in coastal waters, but that coliphages had statistically significant correlations with microbiological parameters. More evaluations are needed before a consensus will be reached regarding the selection and use of bacteriophages as indicators in various types of receiving waters.

2.4 Microbial Source Tracking

Attempts to reduce loads and prevent outbreaks via watershed management can be aided by accurate determination of the sources of microbial contamination. Microbial source tracking (MST) techniques can help give an indication of whether the sources of indicators or pathogens are human, wildlife, or agricultural. Categories of MST techniques include, among others, phenotypic and genetic methods, and may or may not require the development of a library of known samples for comparison with unknown samples. Drawbacks for MST methods include uncertainty in the spatial and temporal stabilities and variabilities of target characteristics. Ease of use and costs are also important in determining whether a method can be widely applied. While a summary is provided here, a critical review conducted by fellow EPA researchers (Simpson *et al.*, 2002) can be reviewed for more detailed information.

2.4.1 Antibiotic Resistance Analysis

Antibiotic resistance analysis (ARA) is a phenotypic method that takes advantage of the exposure of bacterial sources to different antibiotics and the resulting patterns of resistance that

develop. To determine a multiple antibiotic resistance (MAR) profile, a bacterial isolate is exposed to a suite of antibiotics. The antibiotics to which the isolate is resistant define the MAR profile, which acts as a fingerprint. First, a database of MAR profiles is acquired for samples of known sources in a given region. MAR profiles of unknown samples can then be compared to the database to determine their probable sources.

Wiggins (1996) analyzed 1,435 fecal streptococci isolates from animal and human sources for their resistance to five antibiotics. He then used discriminant analysis of the resulting patterns to classify the known isolates with a high rate of correct classification (92% of human isolates). Parveen *et al.* (1997) used MAR profiles to investigate *E. coli* sources within Apalachicola Bay and were able to identify MAR profile differences between point and nonpoint sources. Hagedorn *et al.* (1999) used antibiotic resistance in fecal streptococci to identify sources of nonpoint fecal pollution. Antibiotic resistance patterns have also been used in subtropical surface waters (Harwood *et al.*, 2000) and industrially perturbed stream waters (McArthur and Tuckfield, 2000). The analytical techniques for obtaining an antibiotic resistance profile are easy to perform. Antibiotic resistance patterns are, however, region-specific and compiling a MAR database of known sources is labor intensive. Furthermore, the MAR profiles of bacterial populations may shift with time. This approach may be best used in small watersheds with demonstrated nonpoint source problems and a limited number of potential sources (Simpson *et al.*, 2002).

2.4.2 Molecular Methods

The advance of molecular-based methods in recent years has aided source identification through the use of genetic markers. More commonly applied to microbial indicators because of their prevalence in the environment, these molecular-based MST methods are an active area of research and development. The review prepared by Simpson *et al.* (2002) describes the state of development of a number of techniques as well as their advantages and drawbacks. The genetic methods described in the review include ribotyping, length heterogeneity-PCR (LH-PCR), repetitive PCR (REP-PCR), denaturing gradient gel electrophoresis (DGGE), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (FLP). Although not yet ready for routine use, genetic methods are being tested in research studies. For example, a library-dependent PFGE was used to identify coliform sources in Northern Virginia's Four Mile Run Watershed (Simmons *et al.*, 2000). The study concluded that nonhuman species (waterfowl, raccoon, dog, deer, and Norway rat) were the primary *E. coli* sources in the urban stream. Human sources contributed only 18% of the *E. coli* (NVRC, 2002).

Because of the lack of a therapeutic cure or drug therapy for cryptosporidiosis, MST techniques for *Crytosporidium parvum* oocysts are particularly appealing. The Centers for Disease Control (CDC) has evaluated a molecular species- and strain-specific method for analyzing *Cryptosporidium* parasites in environmental samples (Royer *et al.*, 2002; Xiao *et al.*, 2000; Xiao *et al*., 2001). The method is a nested PCR-restriction fragment length polymorphism technique. It produces numerous copies of a targeted DNA sequence, uses an enzyme to break it into fragments and uses gel electrophoresis and staining to separate and visualize the fragments. Numerous *Cryptosporidium* species have been examined using this method. It has been tested

on stream water, surface water, and wastewater, and is claimed to be able to differentiate between potential sources such as humans, cattle, pets, and wildlife.

In storm stream flow in a mostly undeveloped and forested portion of the New York City watershed, the procedure identified no genotypes from humans or farm animals, indicating the genotypes were likely from wildlife. In raw surface water collected less than a mile downstream of a large commercial cattle operation and a wastewater treatment plant, the method confirmed the presence of *C. parvum* human and bovine genotypes. In Milwaukee, wastewater containing pretreated effluent from a large cattle slaughterhouse was found to contain several genotypes that were known to be associated with humans, bovines, dogs, cattle, and rodents. The method used by CDC to identify *Cryptosporidium* sources shows promise, but needs further development technologically and is as yet too expensive for routine monitoring (Xiao *et al.*, 2002; Royer *et al.*, 2002).

2.5 Conclusions

Speed, reasonable cost, accuracy, and the level of difficulty in performing the techniques remain considerations in the selection and execution of microbiological analyses for water quality. For analysis of total coliform, fecal coliform, *enterococcus*, and *E. coli*, membrane filtration methods are well established and straightforward to perform without specialized equipment. Disadvantages include length of analysis times and potential underestimation. Rapid commercial enzyme-based methods such as Colilert® and Enterolert® show promise for easy screening. This is especially useful in situations where water quality can change rapidly, requiring frequent testing. Users should initially test rapid methods against the traditional membrane filtration or most probable number techniques in order to check their technique and understand any limitations of the methods. Because *E. coli* O157:H7 lacks the enzyme β-glucuronidase, a separate test, such as an immunological method, is needed if its presence is suspected. Commercial gene probe kits are available for some bacteria such as *E. coli* and *Salmonella*. Commercial ELISA kits can also be purchased. These have been developed for food and clinical applications; their use for environmental samples can be explored.

Immunofluorescence and ELISA methods are currently available options for detection of nonculturable viruses and bacteria as well as *Cryptosporidium*, *Giardia*, and *E. coli* O157:H7. Commercially prepared ELISA kits are available for some viruses. Although not as sensitive as PCR-based techniques, immunological methods permit quantification. Allen *et al.* (2000) have warned, however, of limitations of the IF methods for *Cryptosporidium* and *Giardia*, including poor recoveries and inability to determine viability. Poor recoveries are an issue for viruses as well because elution efficiencies from filters can be low. Recovery may be less of an issue in the detection of bacteria, especially indicator bacteria, because they do not need to be retained and eluted from a filter for concentration. However, recovery and enumeration of pathogenic bacteria remains an issue when concentrations are low and exposure is high.

Problems with low viral and protozoan concentrations are being overcome by the high sensitivities of nucleic acid techniques, which include gene probes for detection and PCR for amplification of small amounts of a pathogen's DNA or RNA. The large number of research

studies using PCR in the detection of pathogens illustrates the versatility and promise of these methods. In particular, the ability to detect low concentrations is beneficial because of the low infectious doses of protozoa and viruses. PCR also permits detection of nonculturable viruses and viable but nonculturable bacteria. These methods are still at the research stage and they are not widely available, although they may be in the future. A major drawback to PCR-based methods is the inability to indicate viability; results should be considered evidence of recent contamination and should not necessarily imply risk. Expensive and specialized analytical needs are another drawback.

Although the ability to detect low concentrations of pathogens offers advantages in pathogen monitoring, results must be interpreted with care. The calculation of pathogen density from the analysis of a water sample is based on the assumption that the pathogens are distributed evenly in the water body being sampled. If this assumption is not true, then the absence of microorganisms in a sample may not mean that the organism is absent in the water. On the other hand, detection of a pathogen may give rise to an erroneously high estimate of pathogen density (Allen *et al.*, 2000). Furthermore, pathogen contamination may be transient and easily missed. Ongoing background sampling is important for establishing the normal microbiological conditions of a watershed; sampling should also be conducted when a disturbance such as a storm increases the likelihood of pathogen presence.

Detection methods are continually evolving, but direct routine monitoring for pathogens is not feasible at this time. Indicator use is far from ideal, but it still represents the most viable option for a basic level of water quality monitoring. Unfortunately, indicator bacteria make poor proxies for viruses and protozoa because their survival characteristics are different from those of viruses and protozoa. Potential incorporation of *C. perfringens* and bacteriophages into monitoring strategies may improve the representativeness of the indicator organisms. Because organisms such as *Aeromonas*, an opportunistic pathogen, and some fecal coliform have nonhuman sources, looking only for human-based fecal contamination does not cover all risk factors. MST techniques can allow watershed managers to determine whether the sources of indicator or pathogens are human, wildlife, or from domesticated animals. ARA is currently the easiest to execute, but in time genetic methods may play an increasing role in tracking down the microbiological sources of water quality impairments.

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Chapter Three

Management and Control of Pathogens

3.1 Introduction

This chapter presents technical information supporting the Total Maximum Daily Load (TMDL) process for pathogens, specifically Step 5 – Allocations (U.S. EPA, 2001). The **allocations** step's objective (U.S. EPA, 2001) is to:

> **Using total assimilative capacity developed in the linkage component, develop recommendations for the allocation of loads among the various point and nonpoint sources, while accounting for uncertainties in the analyses (i.e., margin of safety) and, in some cases, a reserve for future loadings.**

The information provided will assist watershed managers in determining the capabilities of control technologies, i.e., disinfection, and best management practices (BMPs) for reducing microbial concentrations in point and nonpoint sources.

Following is the definition of point sources as presented in the Clean Water Act (CWA), Section 502 (14):

> The term "point source" means any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged. This term does not include agricultural stormwater discharges and return flows from irrigated agriculture.

Wet weather flows (WWFs) regulated by the National Permit Discharge Elimination System (NPDES) program are considered point sources. These include combined sewer overflows (CSOs), stormwater associated with industrial activity, construction-related runoff, and discharges from municipal separate storm sewer systems (MS4s). MS4 stormwater types regulated through NPDES permits are described in Section 1.3.1.2. The CWA does not provide a detailed definition of nonpoint sources; these are defined by exclusion, i.e., anything not considered a point source in the CWA or EPA regulations. All nonpoint sources are caused by runoff of precipitation over or through the ground. Therefore, WWFs not covered through NPDES permits are nonpoint sources (U.S. EPA, 2003a).

As discussed in Chapter 1, there are detailed procedures and different approaches for determining recommended loads among the various point and nonpoint sources, while reserving a margin of safety and room for future loading increases. The TMDL definition is provided in Section 1.3.1.1. Development of a single waste load allocation (WLA) for all point sources of pathogens - publicly owned treatment works (POTW) or wastewater treatment plant (WWTP) effluents, and CSO, sanitary sewer overflow (SSO), and stormwater discharges - within one or more municipalities in a given urban watershed requires knowledge of treatment system capabilities and effective control strategies. Different approaches can be used to develop WLAs. One control strategy, a direct approach, is to calculate respective WLAs because treatment system capabilities and effective control strategies can be fully quantified. Another control strategy can be to sum up all the major sources of pathogen discharges. This approach provides the flexibility of adjusting the proportion of flow and loadings among any of the sources present, such as stormwater, CSO, SSO, and POTW or WWTP discharge locations, to maximize the treatment of sewage and load reductions. Point sources are generally discharged from a discrete point and are treated by control technologies and structural BMPs. However, there are discrete end-of-pipe or drainage channel conduit discharges that do not fit within the legal definition of a point source.[1]

Load allocations (LAs) consist of nonpoint sources and a natural background level of a given water body. WLAs and LAs pertaining to stormwater, CSO, and SSO occur intermittently as their origins are WWF events. Therefore, in establishing TMDLs, there needs to be a conversion of these intermittent loads into daily loads. Also, if there are known occurrences of untreated CSO and SSO discharges, these should be dealt with and accounted for independently.

LAs are established for nonpoint sources and, where necessary, may include implementation of BMPs and source reduction strategies. Discrete discharges and diffuse sources considered legally to be nonpoint sources can be managed using either control technologies or BMPs. Diffuse sources are generally managed through nonstructural BMPs. BMPs will be described in the latter part of this chapter (Section 3.3). In some cases, states have certain mandatory BMP requirements for specific land activities associated with large fecal indicator loads, such as confined animal operations or with flood control. Often, implementation of BMPs occurs through voluntary or incentive programs. Therefore, when establishing nonpoint source allocations within a TMDL, the documentation should include a reasonable assurance that the BMP(s) will be implemented and maintained and that the effectiveness of the BMP will be demonstrated. If pathogen loadings are to be reduced by a BMP, the TMDL strategy may require a long-term water quality monitoring program to demonstrate effectiveness of the BMP used.

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[1] *The reader should be aware that "point source" is a legal term, as defined on page 3-1. It is also commonly used to describe all discrete discharges.*

The effectiveness of BMPs for controlling stressors in general, and pathogens, in particular, has not been fully established. There are few references with quantified pathogen removals. There is a difference between a treatment technology and a BMP (see Table 3-1).

Table 3-1. Distinction between a Treatment Technology and a BMP for Pathogen Control

	Treatment Technology (Disinfection for Pathogens)	BMP
Source treated	discrete end-of-pipe or drainage channel conduit discharges	discrete end-of-pipe or drainage channel conduit discharges; diffuse sources
Effectiveness	known	uncertain; little data
Prediction of results	reasonably accurate	uncertain
Design	specific	specific
Improvement	to the level required	uncertain
Cost	known	known

While the effectiveness and pollutant load reduction by a given BMP may be just an estimate, the effectiveness of a given technology is usually known and treatment results can be predicted with reasonable certainty. Although some structural BMPs can perform like treatment technologies, any misjudgement of treatment effectiveness will either reduce its usefulness and/or increase costs (Field, 1996).

The common practice for managing stormwater has been the use of structural and nonstructural BMPs. BMPs can achieve significant environmental improvements, such as reduction of flow volume and removal of suspended solids by sedimentation and filtration. BMPs achieve different degrees of removal of toxic substances and nutrients associated with the removed flow and solids. Removal of pathogens through the use of BMPs can also be associated with reduced flow and removed solids. Disinfection using treatment technologies is feasible for stormwater that can be collected and confined, but it is seldom implemented.

The following are three examples of collecting and treating stormwater or dry weather urban runoff:

1. The city of New Orleans, LA evaluated a prototype disinfection facility for stormwater using sodium hypochlorite in the late 1960s and early 1970s (Pavia and Powell, 1968); but they did not implement the practice permanently.

2. Santa Monica's urban runoff recycling facility (SMURRF, 2000) is treating dry weather runoff and some wet weather runoff since December 2000. (http://Epwm.Santa-Monica.Org/Epwm/Smurrf.html).
3. Moonlight Beach urban runoff treatment facility in the City of Encinitas, CA has been treating dry season urban runoff since September 2002 (Rasmus and Weldon, 2003).

3.2 Disinfection Technologies for Control of Pathogens

3.2.1 Introduction

As long as satisfactory levels of suspended solids concentration and particle size are achieved upstream, disinfection technologies can achieve effective reduction of pathogen-contaminated concentrated sources such as:

1. POTW or WWTP effluent; sometimes referred to as secondary effluent
2. CSO, SSO, and stormwater discharges, all referred to as WWF because these discharges occur during wet weather events
3. Industrial wastewater discharges
4. Confined animal feeding operations (CAFOs)

While disinfection of WWTP effluent (or secondary effluent) and of industrial wastewater discharges is an established practice (U.S. EPA, 1986a), achieving disinfection of WWF is difficult. Because WWF is a significant contributor of microbial contamination to receiving waters, disinfection of WWF released as point sources is warranted.

As stated above, WWF point sources consist of CSO, SSO, and stormwater. Stormwater draining directly into a receiving water body, rather than through a sewerage system, also falls under the definition of WWF and may be considered to be either a point source or a nonpoint source. Human fecal contamination is the main concern for sanitary sewer systems. For stormwater systems, nonhuman-origin (other warm-blooded organisms) and human-origin fecal coliform microbial contamination from unauthorized sanitary sewage cross-connections are the concerns. In combined sewer and storm drainage systems, fecal contamination of both human- and non-human origin are of concern.

Since issuance of the National CSO Control Policy (U.S. EPA, 1994), which requires disinfection of CSO after primary clarification, the CSO became the most frequently disinfected component of WWF. Most WWF disinfection studies, with conventional and alternative technologies, have been conducted on CSO (U.S. EPA, 2002a). However, all components of WWF, such as SSO and stormwater, carry significant loads of fecal and pathogen contamination that would be reduced by disinfection.

Numerous factors need to be considered in discussing WWF disinfection:

1. Disinfection effectiveness as demonstrated by the pathogen reduction levels
2. The need for a high-rate disinfection process
3. The need for suspended solids removal prior to disinfection
4. A description of individual disinfection technologies in the diminishing order of their commercial availability for WWF treatment and their relative costs
5. A description of disinfection studies and implementation examples

3.2.2 WWF Disinfection Effectiveness

Disinfection effectiveness is conventionally judged by the reduction of microorganism densities, generally a bacteriological indicator. Disinfection technologies have been tested using a variety of bacterial and viral indicators and selected individual pathogenic organisms as well. Where available, this information is presented in the subsequent sections on individual disinfection technologies. Different indicators may respond very differently to the disinfection process. A study by the Massachusetts Water Resources Authority, Boston, MA compared *Enterococcus* to fecal coliform data in secondary treated effluent and in effluent from CSO facilities. The investigators found significant differences between how the indicators respond to treatment. Satisfactory reduction of fecal coliform was achieved with chlorination, but the reduction of *Enterococcus* was unsatisfactory (Rex, 2000).

Development of bacteriological indicators was necessitated by the fact that it is both impractical and expensive to isolate and measure specific pathogenic organisms in water. Use of the various indicators is discussed in Chapter 1 and summarized here. A group of enteric bacteria known as coliform are plentiful in human wastes and easy to measure. Therefore, bacteria of the total coliform group became the generally accepted indicator for fecal pollution, even though this group includes different genera that do not all originate from fecal wastes (e.g., *Citrobacter, Klebsiella*, and *Enterobacter*). An improvement over the total coliform indicator is the more selective fecal coliform indicator, since fecal contamination of human origin is known to cause diseases in humans. Fecal coliform selects primarily for *Klebsiella* and *Escherichia coli* (*E. coli*) bacteria. *E. coli* is the bacterium of interest because it is a consistent inhabitant of the intestinal tract of humans and other warm-blooded animals. However, the fecal coliform test is still not fully specific to enteric bacteria and human-enteric bacteria in particular (O'Shea and Field, 1992).

As discussed in Chapter 1, stormwater runoff can contain high densities of the non-human indicator bacteria, and epidemiological studies of recreational waters receiving stormwater runoff have found little correlation between fecal coliform indicator densities and swimming-related illnesses (U.S. EPA, 1984; Calderon *et al.*, 1991). In 1986, U.S. EPA recommended that states begin the transition process to the *E. coli* and *enterococci* indicators (U.S. EPA, 1986b). However, many states still retain the total and fecal coliform criteria. The most widely used bacteriological criterion in the U.S. is a maximum of 200 fecal coliform/100 mL in waters designated for swimming (Field, 1990). Because the fecal coliform indicator is the

most widely used, disinfection effectiveness is often reported as reduction of this indicator. Untreated WWF may contain densities of 1×10^5 to 1×10^7 fecal coliform/100 mL. Achieving hundreds (10^2) of fecal coliform/100 mL in treated WWF with the use of a given disinfection technology would indicate a very successful treatment. Achieving thousands (10^3) of fecal coliform/100 mL in treated WWF with the use of a given disinfection technology may still indicate an adequate treatment if there will be a significant dilution upon discharge of the treated effluent.

3.2.3 Requirement for a High-Rate Disinfection Process

Experience has shown that the long contact time required for conventional wastewater treatment is extremely costly for the treatment of WWFs due to their relatively high flow rates and intermittently occuring volumes. However, WWF disinfection can be achieved at shorter contact times. (U.S. EPA, 1979a; U.S. EPA, 1979b; Stinson *et al.*, 1998). This approach has been termed "high-rate disinfection." There is currently no clear definition as to what constitutes high-rate disinfection other than achieving the required bacterial reductions at detention times significantly less than 30 minutes, the standard contact time (U.S. EPA, 1993).

High-rate disinfection is accomplished by: (1) increased mixing intensity, (2) use of higher concentrations of disinfectant, (3) use of chemicals or irradiation with higher oxidizing rates or microorganism-kill potential, or (4) combinations of these (Field, 1990). The use of increased mixing with any disinfection technology provides better dispersion of the disinfectant and forces disinfectant contact with a greater number of microorganisms per unit time. The increased rate of collisions decreases the required contact time enabling high-rate disinfection (Glover, 1973). An effective disinfection process will have to provide the desired microbial deactivation very rapidly under the specific WWF conditions and carry an insignificant amount of disinfectant residual into the receiving water.

3.2.4 Requirement for Suspended Solids Removal

Effective use of any disinfection technology on WWF requires use of a treatment train, where its initial segment removes excess suspended solids and its final segment is the disinfection process. WWF disinfection requires some form of filtration or clarification/sedimentation prior to introduction of disinfecting chemicals (U.S. EPA, 1973). High levels of particulate matter in WWF can provide a "shielding effect" in which particles present in the medium protect the microbes either from disinfecting agent. (Sakamoto and Cairns, 1997).

Microbial aggregation and particle association are two phenomena that protect microbes and, thus, are major causes of decreased disinfection efficiency. Microorganisms have a tendency to clump together to form aggregates. While the organisms living on the outer layer of the aggregate can be easily disinfected, the microbes living inside are only partially (if at all) penetrated by the disinfectant or by UV light (Katzenelson *et al.*, 1976). Particle association can be represented by attachment of the microorganisms to the particle's surface and by microbial

occlusion within the particle. Microbes attached to the particle's surface are usually properly disinfected but microbes occluded or hidden within the particles may not be disinfected at all.

Studies have shown that pretreatment processes (e.g., filtration, sedimentation) can significantly reduce the effects of both aggregation and occlusion on disinfection efficiency. Johnson *et al.* (1983), for instance, tested both filtered and unfiltered secondary wastewater effluents that were subjected to UV disinfection in side-by-side UV reactors. The filtered effluent showed significantly better disinfection than the unfiltered medium. The study concluded that microbial protection by large particle occlusion is the major reason for increased disinfection efficiency after filtration. Therefore, particle count and size distribution are important indicators of the influent quality and its need for pretreatment. Particularly sensitive to suspended solids content is UV disinfection, which is significantly more effective at suspended solids contents of less than 150 ppm (U.S. EPA, 2002b). UV disinfection tested on CSO and SSO after compressed media filtration (Fuzzy Filter) showed improved performance (U.S. EPA, 2002c). In case of chemical disinfection, the lower suspended solids content in the treated effluent, the less chemical addition and shorter contact times are needed for effective disinfection.

3.2.5 WWF Disinfection Technologies

Alternatives to chlorination disinfection technologies, for example UV light irradiation, chlorine dioxide (ClO_2), and ozonation (O_3), generate significantly less toxic byproducts and residuals when compared to chlorine (Cl_2). However, only chlorination/dechlorination, as opposed to alternative technologies, is currently used for WWF disinfection. There have been several pilot studies on WWF with alternative technologies. The Water Environment Research Foundation (WERF) were sponsoring a study that evaluates the risks and benefits associated with various CSO disinfection technologies. A report on its results will be published by 2004. Disinfection technologies are discussed below in diminishing order of their commercial availability for WWF treatment.

3.2.5.1 Chlorination and Dechlorination

Disinfection by Cl_2 has proven to be effective, and has been used for wastewater disinfection in the U.S. since 1855 (White, 1999). Chlorine or its derivatives are the most commonly applied disinfectants in the U.S. (SAIC, 1998). Chlorine is readily available in several forms, inexpensive, and effective against bacteria, though not fully effective against viruses. Chlorine is ineffective in killing protozoa. The easiest way to increase Cl_2 effectiveness is to increase the Cl_2 dosage within the system. This, however, results in the additional generation of toxic, carcinogenic, and/or mutagenic byproducts, as well as a high residual concentration of Cl_2 in the receiving waters. In the last 20 years, disinfection by chlorination has come under scrutiny. Research studies, particularly for drinking water, have cited health risks with regard to Cl_2 and its byproducts. Excess of free Cl_2 can cause chlorinated hydrocarbon formation, i.e., chloroform and trihalomethanes (THMs), which are suspected carcinogens. Chlorine residuals discharged to natural waters may be harmful to aquatic life.

Disinfecting high volumes of WWF requires large quantities of Cl_2. Because of the high risk of gas leaks when transporting gaseous Cl_2, use of liquid Cl_2 in the form of calcium hypochlorite and sodium hypochlorite is preferred but more expensive. Liquid Cl_2, as sodium or calcium hypochlorite, is easier to handle, safe to store in onsite tanks, and immediately available for use. The effectiveness of liquid versus gaseous Cl_2 for disinfection of WWF has been investigated. In general, the studies confirmed that liquid Cl_2 is a better disinfectant for WWF, and WWF facilities are encouraged to changeover from gaseous to liquid Cl_2. When necessary, excess of free Cl_2 can be removed by using either gaseous sulfur dioxide or sodium bisulfite solution. This will eliminate further byproduct formation, but will neither eliminate nor reduce the already-formed harmful byproducts. Dechlorination also means the addition of another process, which raises the cost of disinfection. On the average, dechlorination will add about 30% to the total cost of disinfection. After dechlorination, there is an analytical challenge in measuring the required low residual level of Cl_2 and the associated monitoring of Cl_2 levels in receiving waters.

The chlorination/dechlorination pilot study at the 26th Ward WWTP testing facility in New York City, NY demonstrated that hypochlorite disinfection was a cost-effective technology for the upgraded Spring Creek facility because of the existing tanks at this facility. Dechlorination will be added at a later date. Improvements will be made to increase disinfectant flash mixing and to automate hypochlorite feed and control of the residual chlorine (U.S. EPA, 2002b). The study is described in greater detail in Section 3.2.6.1.

Chlorination/dechlorination of CSO was tested on over 40 wet-weather events at a full-scale Advanced Demonstration Facility (ADF) in Columbus, Georgia. This study is summarized under the 3.2.6.3 subsection of this Chapter. Detailed performance results and relative costs are presented in a report (Columbus Water Works, 2001).

3.2.5.2 Ultraviolet Light Irradiation

Since the early 1900s, UV light irradiation from mercury arcs has been recognized as an efficient disinfecting agent. At the germicidal wavelengths, within the range of 200 to 320 nanometers (nm) wavelength, UV light disinfects water by altering the genetic material in microbial cells, preventing reproduction. Peak effectiveness occurs near 253.7 nm, the wavelength of emission from a mercury arc lamp. UV irradiation has become an acceptable alternative to chlorination for wastewaters undergoing a secondary or tertiary treatment. Until recently, it has not been used for low-quality effluents such as WWF as an alternative to chlorination.

Certain parameters determine the UV dose required for effective disinfection. Understanding these parameters and their variability in WWF is very important for proper disinfection system design (Ashok *et al.* 1997). High variability in WWF flow rates influences UV disinfection effectiveness, because flow rate is a principal determinant of the dosage of UV light necessary for effective disinfection (Wojtenko *et al.*, 2001a). This is generally true for all WWF disinfectants, but UV disinfection is more affected by wastewater quality than chemical

disinfection technologies. High levels of suspended solids containing particles larger than 2 microns and minerals present in WWF also reduce UV light effectiveness. During disinfection, the negatively charged quartz sleeves surrounding the UV lamps foul by picking up positive ions (e.g., Ca, Mg, and Fe) from the water. Fouling materials decrease transmittance of UV light and thus its disinfection capability (Oliver and Gosgrove, 1975). Use of an in-place cleaning system can remove fouling materials from the quartz sleeves.

Using UV irradiation for disinfection eliminates many problems arising from chlorination, such as the need for chemicals and their associated transportation, handling, and storage, as well as the need for expensive dechlorination facilities. Eliminating large contact tanks and facility buildings significantly lowers capital and operating costs. UV light irradiation affects a wide range of microorganisms and does not generate known harmful secondary chemical byproducts (e.g., THMs). Based on investigations, UV light irradiation for CSO disinfection shows promise as an effective and safe alternative to chlorination. To inactivate the target microorganisms efficiently, UV light must penetrate the water. Therefore, the water to be disinfected must be as clear as possible.

High levels of particulate matter in WWF absorb a large amount of energy, significantly decreasing the amount of UV light available for disinfection. UV light can disinfect free-living microorganisms very effectively with a low dose of irradiation, but microbes are often adsorbed to surfaces of particles (e.g., soil, sediment) or embedded within solid materials (e.g., fecal material). Solid particles shield the microbes from the disinfecting agent. Adsorption of the microorganisms to inorganic surfaces does not affect disinfection efficiency as significantly as adsorption to organic matter. The presence of a surface like clay does not inhibit UV disinfection because it tends to scatter UV light rather than absorb it.

UV light irradiation is a physical procedure that does not alter the smell or chemical composition of water. UV disinfection for WWF requires some level of physical pretreatment (with or without chemicals) to make UV light more effective for WWF (Field, 1996). Pilot studies have shown that filtration prior to UV disinfection can minimize the effects of particle occlusion/association (Johnson *et al.*, 1983).

In a 1996 pilot study of high-rate CSO treatment technologies in the Metropolitan Toronto Area, Canada, UV disinfection was used to achieve an *E. coli* count of 200 cfu/100 mL in a CSO effluent treated by a vortex separator, marketed as the Storm King. UV collimated beam tests were undertaken on only two samples, and in both cases the vortex separator was operated at a surface load of 10 m/h, with a cationic polymer dosage of 8 mg/L. The residual total suspended solids (TSS) in vortex effluent samples averaged 42 mg/L and the interim target fecal coliform count had been achieved at a UV dosage of 30 mWs/cm^2, which was considered to be a feasible dosage for full-scale application. The cationic polymer coagulant was used to improve the solid/liquid separation efficiency and, thus, facilitate UV disinfection (Marsalek *et al.*, 1996).

UV testing on CSOs in the ADF study in Columbus, GA, was also done in a treatment train arrangement. UV was tested after both vortex and compressed media filtration and its performance was better on the filtered CSO than on the unfiltered CSO. This study is summarized under the 3.2.6.3 subsection of this chapter. Detailed performance results and relative costs are presented in a report (Columbus Water Works, 2001). UV testing after the use of compressed media filtration (Fuzzy Filter) was done on SSO-type wastewater at the Rockland County, NY sewer district testing facility. This study is summarized under the 3.2.6.3 subsection of this chapter (U.S. EPA, 2002c).

Investigations of UV light irradiation for CSO disinfection have shown this technology to have the potential to be an effective and safe alternative to chlorination, assuming the adequate removal of suspended solids prior to UV application. A CSO disinfection pilot study conducted at the 26th Ward WWTP testing facility in New York City that evaluated and compared UV light, O_3, ClO_2, and chlorination/dechlorination disinfection units showed that the UV light unit was the simplest unit to operate. This study is summarized under the 3.2.6.3 subsection of this chapter (U.S. EPA, 2002b).

It is evident from studies and implementation examples described under section 2.6.3, UV technology has been gaining acceptance for treatment of CSO.

3.2.5.3 Chlorine Dioxide

The use of ClO_2 for WWF disinfection has also been investigated as an alternative to chlorination. The lack of any significant reactions of ClO_2 with water is the main reason for its biocidal effectiveness over a wide pH range. Chlorine dioxide was found to provide excellent disinfection at a fraction of the Cl_2 dosage, making it cost effective and relatively safe. In addition to its high effectiveness over a wide pH range, the low reactivity of ClO_2 with ammonia and reduced formation of halogenated organic compounds are its major advantages over Cl_2. However, the presence of organic and inorganic impurities in water is a limitation of ClO_2 disinfection. The impurities create a large oxidation demand for ClO_2. These reactions take place together with disinfection (Katz *et al.*, 1994). In such a system, the effectiveness of the disinfecting agent is greatly reduced. Effective treatment of the wastewater by filtration and/or sedimentation is a precursor for successful ClO_2 disinfection (Stinson *et al.*, 1999). This is of great importance for CSO applications.

Chlorine dioxide is a very strong and effective wastewater disinfectant. It is not a chlorinating agent and does not lead directly to the formation of organochlorine compounds (Dernat and Pouillot, 1992). The major advantages of ClO_2 are: its disinfection effectiveness for Cl_2-resistant pathogens (e.g., viruses and protozoa) within a wider pH range, its high solubility in water, the production of stable and measurable residue, and no reactivity with ammonia to produce chloramines. Due to these advantages, ClO_2 was found to be an attractive candidate for WWF applications. Because ClO_2 is a more powerful disinfectant than Cl_2, lower levels of ClO_2 can be used resulting in lower levels of toxic byproducts to get the same level of inactivation.

For several decades, researchers have compared the respective disinfection efficiencies of ClO_2 and Cl_2. In potable water as well as in wastewater treatment applications, a number of researchers have found a significantly lower ClO_2 demand compared to that of Cl_2. In studies where equivalent amounts of each of the disinfectants were added to water with various levels of contamination, after 30 minutes of contact, Cl_2 was found to be largely consumed while ClO_2 remained mostly unreacted. This result indicates that ClO_2 reacts with fewer compounds than Cl_2. Due to the limited reactions of ClO_2 with organic compounds in water, more of the disinfectant remained available as a biocidal agent. Chlorine dioxide was found to be a stronger disinfectant than Cl_2 at shorter contact times and, in addition, was found effective against a greater number of different microorganisms (Moffa, 1975). Chlorine dioxide was also found to be a better disinfectant of bacteria and more effective than Cl_2 against viruses and protozoa (Aieta *et al.*, 1980).

The possibility of using a combination of ClO_2 and Cl_2 was investigated for municipal wastewater treatment by Katz *et al.* (1994). After adding both agents in equal amounts, improved disinfection efficiency was observed with all doses, and production of the byproducts, such as chlorite ion (ClO_2^-) and THMs, was greatly reduced. Chlorine dioxide used in combination with Cl_2 also resulted in a lower residual Cl_2 concentration. A bench-scale study was conducted by the U.S. EPA on high-rate disinfection using Cl_2 and ClO_2 and its findings were verified by two full-scale prototype treatment facilities for CSOs (Moffa, 1975). The concentration of residual ClO_2, increased while the concentration of toxic ClO_2^- decreased. This was explained by Katz *et al.* (1994) as being the result of an oxidation reaction between Cl_2 and ClO_2^- to produce ClO_2. When the combination of ClO_2 and Cl_2 is used, ClO_2 competes with Cl_2 for the oxidation of organic precursors to THM and chloroorganic compounds. Chlorine reduced the concentration of ClO_2^- by oxidizing it back to ClO_2. In this case, Cl_2, the cheaper disinfectant, increased the concentration of ClO_2, the more expensive disinfectant, thus lowering the cost of the disinfection process.

Despite the numerous advantages of ClO_2 disinfection, the necessity for ClO_2 generation onsite due to its instability is a major disadvantage. The most commonly used ClO_2 generation method is the reaction of $NaClO_2$ with acid (White, 1999). There are safety considerations associated with ClO_2 generation: instability of ClO_2 as a gas, storage and transport of its precursors (e.g., gaseous Cl_2, sodium chlorite $NaClO_2$) on site, and proper operation of the equipment. There is a serious problem with a delivery of gaseous Cl_2 as it is prohibited to be transported through most densely populated areas. There is a new process of ClO_2 generation that uses $NaClO_2$ in the presence of UV light (Stinson *et al.*, 1998). In this process the transport and handling of gaseous Cl_2 is totally eliminated but this process is still under development and is not commercially available. Other disadvantages of ClO_2 disinfection include lack of data available for full-scale application to WWF and the potential explosion hazard under certain conditions.

The potential advantages of using ClO_2 as a disinfectant greatly outweigh the possible disadvantages and inconvenience of onsite generation. When produced, handled, and used properly, ClO_2 is an effective and powerful disinfectant. The sequential addition of Cl_2 with

ClO_2 greatly enhances the disinfection process and is cost effective. Chlorine dioxide appears to have potential for becoming an effective Cl_2 alternative for WWF disinfection. Further investigations, however, are recommended to determine its effectiveness in a full-scale WWF application (U.S. EPA, 2002b).

Chlorine dioxide performed better than chlorination/dechlorination in the Columbus ADF study (Columbus Water Works, 2001) and in the New York City study (U.S. EPA, 2002b). Of particular interest was the second phase of the New York City study where a new process of ClO_2 generation using UV light, which avoids the need for gaseous Cl_2, was used as the source of ClO_2. While ClO_2 was superior in effectiveness and similar in cost to chlorination/dechlorination, the UV generation technology for ClO_2 needs further development. Currently, Cl_2 gas cannot be transported within New York City. Thus, because an effective Cl_2-gas-free process of ClO_2 generation has not been proven to be reliable, disinfection with ClO_2 cannot be considered for use within New York City, or any other metropolitan area, at this time. The Columbus ADF study is summarized under the 3.2.6.3 subsection of this chapter. Detailed performance results and relative costs are presented in a report (Columbus Water Works, 2001). The New York City study is summarized under the 3.2.6.3 subsection of this chapter. (U.S. EPA, 2002b).

3.2.5.4 Ozonation

Ozone's ability to inactivate microorganisms was already well known as early as 1886 (White, 1999). It is the strongest and fastest-acting oxidant of all the classical disinfecting agents used for water sanitation today. Ozone inactivates a wider range of microorganisms than Cl_2, has a relatively high disinfection-kill power, releases limited byproducts, has the ability to increase dissolved O_2 concentration, is non-reactive with ammonium, and has an excellent ability for removing undesirable odor and color. In addition to being a strong disinfectant, O_3 reacts with organic impurities (e.g., saturated hydrocarbons, amines, and aromatic compounds) destroying them in the process and forming such byproducts as acids, aldehydes, bromates, ketones, and peroxides. Studies evaluating ozonation byproducts are limited, and further investigation in this area is necessary.

Because O_3 is a very strong oxidant, it has the potential for being effective for low-quality wastewater and WWF disinfection. Organic and inorganic impurities, chemical oxygen demand (COD), pH, temperature, and suspended solids in waters have a significant impact on O_3 disinfection efficiency. The presence of water impurities is a major limiting factor of ozonation for CSO applications. As a strong oxidant, O_3 will react with many organic (e.g., aromatic and aliphatic compounds, pesticides, humic acids) and inorganic (e.g., sulfide, nitrogen, iron, manganese, cyanide) compounds producing reaction byproducts (U.S. EPA, 1986a). Reactions with impurities consume O_3, which is then no longer available as a disinfecting agent. As a result, wastewater with high levels of impurities requires a high dosage of O_3 and, thus, an increased O_3 demand, for disinfection to be successful. Although O_3 is a strong oxidant and a powerful disinfectant, its application for WWF disinfection has been very limited. As indicated

by White (1999), effective ozonation requires relatively good water quality; thus, filtration is recommended before the ozonation process.

Similar to every other disinfection process, ozonation is most effective for free-floating organisms. The presence of particles in water makes ozonation challenging. In addition to particle occlusion, microbial aggregation was also found to be a major factor negatively affecting ozonation. The rates of inactivation of aggregates were found to be much slower when compared to free organisms.

The equipment and operating costs associated with ozonation are relatively high. Due to its high instability, O_3 must be produced onsite and used within a short period of time. Skilled operators and constant attention are required. The necessity for onsite generation makes its application to the intermittent nature of WWF difficult.

In general, the ozonation process, if properly run, can be successful for disinfection of various water qualities (wastewater and drinking water). The CSO disinfection pilot study in New York City showed that there are some safety issues with O_3 generation and use, such as collection of off-gas and destruction of O_3, use of water-tight and gas-tight contactors, proper monitoring of the ventilation system, and use of corrosion-resistant construction materials (Stinson *et al.*, 1998; U.S. EPA, 2002b). Ozone instability is also a major factor contributing to the high cost of this technology. There are currently no WWF facilities using this technology in the U.S.

In the New York City study, the capital costs of O_3 generation were found to be the highest of all technologies that had been investigated concurrently. Costs of ozone disinfection were found to be dependent on the cost of electricity as well as the source of oxygen used as a feed (air vs. pure O_2). This study is summarized under the 3.2.6.3 subsection of this chapter. (U.S. EPA, 2002b).

3.2.6 Description of Disinfection Studies and Implementation Examples

3.2.6.1 Disinfection Pilot Study at the 26th Ward WWTP Testing Facility in New York City

This pilot study demonstrated alternatives to hypochlorite disinfection for application to the Spring Creek CSO storage facility and potentially to other CSO facilities. The pilot testing was divided into two phases. Phase I evaluated treatment performance of five technologies: UV, O_3, ClO_2, chlorination/dechlorination, and electron beam irradiation (E-Beam). Based on the results from Phase I, Phase II provided additional evaluation of technologies that had shown potential for CSO applications. These were UV, ClO_2, and chlorination/dechlorination.

Major findings

- With the exception of E-beam, the tested technologies achieved targeted bacterial reductions of 3 to 4 logs.

- Chlorination/dechlorination, ClO_2, and O_3 provided targeted levels of disinfection over the full range of wastewater quality tested.
- Chlorine dioxide was superior in effectiveness and similar in cost to chlorination/dechlorination. The new technology for ClO_2 generation that does not require use of chlorine gas needs further development.
- The upgraded Spring Creek facility will continue to use sodium hypochlorite for disinfection, with provisions to add dechlorination at a later date. Improvements will be made to increase disinfectant flash mixing and to automate hypochlorite feed and residual control.

Wastewater quality

Five disinfection technologies, UV, ClO_2, Cl_2, O_3, and E-Beam, were tested for their effectiveness in reducing bacteria levels in water representative of the CSO at the Spring Creek storage facility. Tests were conducted during wet and dry events. To achieve a four-log reduction of fecal coliform and a fecal coliform effluent concentration less than 1,000 colony forming units/100 mL (cfu/100 mL) required doses for UV, O_3, ClO_2, and Cl_2 of 60-80 mWs/cm^2, 24 mg/L, 8-10 mg/L, and 20-28 mg/L, respectively. The range of disinfectant doses for each technology reflects the variation in performance between Phase I (December through March) and Phase II (August through November). The variation in wastewater temperature between Phase I (mean of 11.6 ^oC) and Phase II (mean of 20.9 ^oC) had a significant impact on the performance of Cl_2 disinfection. The colder winter temperatures impede the formation of monochloramine, which has approximately 25 times less germicidal efficiency than free Cl_2.

Treatment Performance

Four bacteria indicators were used as a measure of the effectiveness of each of the disinfection technologies; namely total coliform, fecal coliform, *E. coli*, and *Enterococcus*. Kills of each of the indicators, in terms of log reduction and concentration, were related to dose for each of the disinfection technologies. Chlorination/dechlorination, ClO_2, and O_3 at the doses tested were able to provide the disinfection levels of the four-log reduction over the full range of wastewater quality tested. UV disinfection effectiveness tended to drop off at higher TSS concentrations (e.g., TSS greater than approximately 150 mg/L). This was attributed to lower effective penetration of UV due to harboring of bacteria in solids.

Fecal coliform and *E. coli* exhibited similar dose-response relationships. However, total coliform and *Enterococcus* generally required higher doses to achieve the same level of inactivation as that for fecal coliform and *E. coli.* This was observed in all technologies except for the E-beam, where the inactivation results were inconclusive.

The UV and ClO_2 technologies provided nearly complete reductions of bacteriophage, a bacterial virus and microbial indicator. However, the viral inactivation data for the ClO_2 system was limited to only two of the four runs due to operational problems. Of the valid data considered, the effluent concentrations of bacteriophage ranged from non-detect to 60 cfu/mL. Low influent concentrations of the seeded phage limited the maximum log reduction that could

be observed. The log reduction of bacteriophage ranged from 1.9 to 6.0. Because of the low concentrations of naturally occurring enteroviruses in the pilot influent, the UV disinfection could not be evaluated satisfactorily on the basis of the tissue culture infectivity assays, discussed in Chapter 2. However, based upon the reductions of the marginal concentrations found and upon the bacteriophage results, these technologies would inactivate most natural enteroviruses found in wastewater at concentrations on the order of 10^6 cfu/mL.

UV disinfection achieved 4-log bacteria reduction but at extremely high dosage levels owing to the impediments of poor water quality. UV effectiveness tended to be reduced by high TSS concentrations (e.g., greater than 150 mg/L). Additionally, UV effectiveness tended not to increase at doses greater than 75 mWs/cm^2, a phenomena known as "tailing-off."

Ozone disinfection can be accomplished only at high O_3 dosage levels. However, the O_3 pilot unit did not include a contactor design appropriate for the wastewater conditions tested. Thus, the required O_3 dosages may have been less if a more applicable O_3 dissolution/contactor system were provided. An O_3 disinfection system would require contact chambers other than the tankage that presently exists at Spring Creek.

Chlorine disinfection included dechlorination to eliminate residual Cl_2. Chlorination as well as dechlorination can be accomplished using the existing tanks at the Spring Creek Advanced Wastewater Pollution Control Plant (AWPCP). High-rate mixing can be added to the head end of the tanks. Chlorine dioxide disinfection can be accomplished at doses on the order of 30% of the required Cl_2 dose.

Chlorination/dechlorination and ClO_2 were determined to be the most cost effective technologies for application to Spring Creek. However, neither of the ClO_2 generation methods tested are currently feasible for use within New York City. The Cl_2 gas/solid sodium chlorite generation method is not feasible because of its use of Cl_2 gas, and the UV/sodium chlorite generation method is not feasible because of its developmental status as a prototype. The capital costs for UV and O_3 were significantly more expensive than chlorination/dechlorination or ClO_2. For other CSO facilities that do not have existing tanks for contact time, UV could be economically attractive.

In the case of ClO_2, there is no significant increase in disinfection performance beyond a contact time of three minutes. This is in contrast to the chlorination results, which show a greater dependence on contact time and required five minutes for comparable kills. The difference is attributed to ClO_2's greater bactericidal properties and solids penetration characteristics than those of chlorination. The results of this study confirm the optimum contact times for ClO_2 and chlorination/dechlorination of three and five minutes, respectively, originally determined in the Syracuse and Rochester studies (U.S. EPA, 1979a and 1979b). Chlorination/dechlorination and ClO_2 were determined to be the most cost-effective technologies for application at this facility. Further development of the UV-chlorite ClO_2 generator is required before reliable costs for this technology can be developed.

Disinfection Residuals and Toxicity

Selected disinfection effluent residuals and byproducts, namely ClO_2, chlorate, chlorite, total residual chlorine (TRC), volatile and semivolatile organics, haloacetic acids, were monitored to relate these residuals to disinfectant dose. UV disinfection had the distinct advantage of producing no byproducts. This is in contrast to Cl_2 and ClO_2, which produced increased levels of TRC, chlorate, chlorite and haloacetic acids in the effluent. The slightly increased haloacetic acid concentrations were considered insignificant. The increased TRC, chlorate and chlorite concentrations were directly related to increased Cl_2 and ClO_2 dose.

No additional toxicity was observed in the UV effluent as compared to the UV pilot influent. In contrast, there were occurrences where the ClO_2 effluent was considerably more toxic than the pilot influent. An attempt was made to correlate this toxicity with the specific disinfection byproducts, in particular TRC, chlorate and chlorite, but no correlation could be made. It is likely that the increased effluent toxicity is directly related to influent toxicity (i.e., influent water quality) or a synergistic effect of the disinfectant residuals, which could not be measured. Although the concentrations of TRC, chlorate and chlorite did not cause concern about effluent toxicity, this relationship should be revisited when establishing ClO_2 dose for specific sites.

Effluent TRC was generally below 0.1 mg/L following dechlorination as compared to a receiving water quality standard of 0.0075 mg/L. This TRC value of dechlorinated effluent reflects the practical quantitation limit of the process instrumentation used. Lower TRC values could not be quantified. Often, the dechlorinated effluent TRC instrumentation displayed a negative value indicating the presence of excess bisulfite. Residual Cl_2 was also monitored in the ClO_2 effluent. However, these TRC values include all oxidizing species of Cl_2 and the possible presence of free and combined Cl_2 could not be differentiated from ClO_2, ClO_2^- and ClO_3^-.

Chlorine Dioxide Generation

The method of generating ClO_2 must be considered when selecting the appropriate disinfection process. The Cl_2 gas/solid sodium chlorite generation method was tested during the Phase I and Phase II pilot studies. Although this pilot unit was reliable, the use of Cl_2 gas (either with Cl_2 cylinders or with on-site Cl_2 gas generation) in this process may limit its application in residential and urban areas, including New York City. The UV-sodium chlorite solution generation method was also tested during the Phase II pilot study. This method had the distinct advantage of not using or generating chlorine gas in the generation process. However, this technology is currently in the prototype stage of development and would need to be developed as a full-scale unit to be considered further. The UV-chlorite generator from the UVD, Inc., was a prototype unit.

Cost Comparison

During the Phase I pilot study, conceptual level cost projections were prepared for each disinfection technology for comparison purposes, with the goal of recommending a technology for implementation at the Spring Creek storage facility. The Phase II pilot study results served to verify the Phase I result; as such, the assumptions and approach used for the original cost comparison were applicable. Costs for each disinfection technology were prepared on a common flow basis and were prepared for a range of flow rates experienced at Spring Creek. See Table 3-2. This approach shows the sensitivity of cost to flow rate, and allows independent comparison of technology costs at similar flow rates. Equipment capital costs were developed for peak design flow conditions of 1,250 cubic feet per second (cfs) (800 million gallons per day (mgd)), 2,500 cfs (1,600 mgd), and 5,000 cfs (3,200 mgd) for a duration of 4 hours. (U.S. EPA, 2002b).

3.2.6.2 Continuous Deflection Separation, Fuzzy Filter and UV Treatment of SSO-Type Wastewaters: Pilot Study Results

This study evaluated three technologies for treatment of SSO and CSO overflows. These were the Continuous Deflection Separation (CDS) and Fuzzy Filter high-rate solids removal technologies, and UV high-rate disinfection. The study was conducted at the Rockland County Sewer District No.1, in Orangeburg, NY from August 1998 to January 2001.

Three different lamp systems were evaluated within the UV disinfection studies. These were:

- PCI Wedeco UV Technology (now Wedeco Ideal Horizons). This system represents newer low-pressure lamp UV systems, which takes advantage of the high power conversion efficiency of the low-pressure lamps, while getting higher UV outputs.

- Aquionics UV Technology. This system utilizes medium-pressure lamps. These are less efficient than conventional lamps but their total UV output is higher resulting in a lower number of lamps to achieve the required light intensity.

- Generic Medium-Pressure, Open-Channel System. The channel was designed to operate lamps at two different spacings: 4- and 6-inch.

The overall objective of the study was to evaluate high-rate solids removal technologies on SSO and CSO type wastewaters, and the subsequent UV disinfection of the treated wastewaters. The results are given below.

Table 3-2. Cost Projection of Disinfection to be Implemented at the Spring Creek Facility												
	Conceptual Level Facility Disinfection Costs ($)											
	Chlorination/Dechlorination			Chlorine Dioxide			Ozone			UV		
Peak Design Flow (cfs)	1,250	2,500	5,000	1,250	2,500	5,000	1,250	2,500	5,000	1,250	2,500	5,000
Capital Costs	912,000	1,045,000	1,219,000	695,000	1,159,000	1,932,000	19,221,000	24560,000	30,539,000	48,052,000	67,272,000	87,774,000
Annualized Capital Costs	93,000	107,000	124,000	70,000	119,000	196,000	1,957,000	2,502,000	3,111,000	4,894,000	6,852,000	9,592,000
Annual O&M Cost	255,000	255,000	255,000	294,000	294,000	294,000	534,000	587,000	657,000	248,000	497,000	992,000
Total Annualized Costs	348,000	362,000	379,000	364,000	413,000	490,000	2,491,000	3,089,000	3,768,000	5,142,000	7,349,000	10,584,000

Notes: 1. Costs are present worth in 2000 dollars.
2. Capital costs are based upon sizing to meet peak design flow and a 4-log reduction in fecal coliform.
3. Capital costs are for installation of Spring Creek and are for process equipment only. Costs do not include additional contact tankage (if required) or support facilities.
4. Annual operating costs are based upon an assumed typical 40 CSO events/year at a volume treated of 15 million gallons per event.
5. Annualized costs are based upon a period of 20 years at an interest rate of 8%.

UV Disinfection Dose Requirements and Particle Size Impacts

The dose-response analyses indicated that removal of particles greater than 50-micron in size will improve the efficiency of the UV process because filtration to such levels removes a substantial amount of occluded bacteria. Samples were blended prior to analysis to release occluded bacteria so they could be detected in analysis. Blending the unfiltered samples released fecal coliform and improved recovery of occluded bacteria. Blending samples that had been filtered at retention levels between 1 and 50 microns did not have a significant impact on coliform recovery and did not impact UV dose requirements to accomplish targeted reductions.

The UV dose requirement to accomplish 3-log reduction of fecal coliform in primary-type wastewater (i.e., wastewater of a quality equivalent to a primary-treated wastewater), pretreated to remove particles greater than 50-microns is approximately 20 mJ/cm^2. The results suggest that the maximum reductions that can be expected under practical dose applications up to 40 mJ/cm^2 are 3.5 to 4 logs. With unfiltered effluents and primary-treated wastewaters passed only through the CDS unit, the maximum reductions suggested by the dose-response analyses are approximately 2.5 to 3.0 logs (based on enumeration of blended samples).

CDS Process Performance

The CDS process is capable of accomplishing approximately ten percent TSS removals with a 1200-micron screen. This increases to approximately 30 percent with a 600-micron screen. In both cases, it appears that removals were independent of the flow rate, within the range of flows tested.

The CDS unit, based on visual observations, was effective in capturing and removing debris, including paper and plastics, fibers, and preventing transport to downstream processes. In this respect, the wider aperture screens were as effective as the smaller aperture screens and are more easily maintained. The wider aperture screen tended to be self-cleaning while the smaller aperture screen required manual cleaning and tended to retain the debris on the screen surface. The CDS process can provide protection of downstream filters or other pretreatment devices by removing debris and floatables.

Fuzzy Filter Performance

The filter was effective in removing larger-size suspended solids. The particle size distribution (PSD) and dose-response analyses confirmed that these removals centered on particles greater than 50 microns. The system is more effective in this application at 20-percent compression and at hydraulic loadings between 400 and 800 Lpm/m^2 (10 and 20 gpm/ft^2). At these conditions, TSS removals averaged approximately 40 %. Removals were consistently less at these hydraulic loadings for the 10 and 30 % compressions.

UV Disinfection Performance

The combined results generated with the three UV units indicate that a degree of disinfection with primary wastewaters can be accomplished by UV radiation. Reductions between 2.3 and 2.8 logs can be achieved at hydraulic loadings between 8 and 38 Lpm/kW of lamp input power (2 and 10 gpm/kW) based on the enumeration of blended samples. This is equivalent to approximately 3 to 3.5 logs when enumeration is conducted using standard analyses without blending samples. Doses greater than 40 mJ/cm^2 are required to achieve these reduction levels (U.S. EPA, 2002c).

3.2.6.3 Advanced Demonstration Facility (ADF) in Columbus, GA

Chlorination/dechlorination of CSO, along with several alternative technologies, were tested on over 40 wet-weather events at a full-scale ADF in Columbus, GA. The CSO testing program at ADF was a part of a multi-year watershed study sponsored by the Columbus Water Works Agency with the Wet Weather Engineering & Technology (WWETCO) firm as the principal contractor and with the involvement of the WERF and the U.S. EPA. ADF is comprised of multiple CSO technologies arranged as treatment trains: hydraulic controls, screening, vortex separation, compressed media filtration, and chemical disinfection using Cl_2 as sodium hypochlorite, ClO_2, peracetic acid, and UV disinfection. Multiple technologies were tested side-by-side and in sequential and split stream for determining performance at different loading rates and equipment settings. Performance results and relative costs are summarized below (Columbus Water Works, 2001).

ADF CSO Technology Evaluations

The ADF demonstration facility, with permitted capacity of 48 MGD, consists of coarse screening and flow controls, six 32-ft diameter vortex separators, a compressed media Fuzzy Filter (a 30-inch bed of 1-inch fiber balls contained between two perforated plates), a medium pressure UV system located downstream of the Fuzzy Filter (u-tube arrangement of two banks of 42 bulbs), and other auxiliary equipment. The ADF is fully automated and operates during wet-weather events when runoff exceeds interception. Manned operations include both pre-and post-event activities as well as preventive maintenance. Continuous rainfall monitoring and level instruments automatically initiate operations such as screening, underflow pumps, and disinfection equipment. Post-event activities include residuals removal from screens and grit bins, sodium bisulfite dechlorination, and equipment operation checks.

Testing of three chemical disinfection technologies, Cl_2 as sodium hypochlorite, ClO_2, and peracetic acid, was conducted in designated vortex separators for each technology. The vortex separator is designed to remove grit and concentrated solids but can be and was used for combined solids removal and chemical disinfection. Vortex has no moving parts and acts like a plug-flow reactor providing contact time greater than 70% of theoretical. There is higher usage of chemicals in a vortex than in a separate disinfection tank but the cost of additional chemicals is less than the cost of separate tankage. Sodium bisulfite dechlorination was also conducted in a vortex.

Chemical disinfectant was delivered by feed pumps according to a developed control algorithm for changeable dosing. At the ADF, the disinfectant demand for CSO was correlated with its ammonia and COD content in conjunction with the continuous flow and time measurements. Chemical disinfection efficiency also correlated with pH, temperature, and TSS. The highest disinfectant dose was given at the beginning of the event and it was diminishing as the event was progressing. A minimum contact time used was three minutes. Disinfectants listed in order of their effectiveness were ClO_2, sodium hypochlorite, and peracetic acid, however all were capable to accomplish a satisfactory disinfection. Chemical dosing under similar conditions requires 15 mg/L sodium hypochlorite, 16 mg/L peracetic acid, and 12 mgL of ClO_2.

Sodium hypochlorite was selected because ClO_2 requires generation onsite with the use of Cl_2 gas and peracetic acid is not licensed in the U.S. for wastewater disinfection. Sodium hypochlorite (Cl_2) dose varied from 4 to 30 mg/L with average concentrations between 8 and 9 mgL. Contact times ranged from 6 to 40 minutes at peak flow rates at events tested. The predominant contact times were between 10 and 20 minutes. Chlorine disinfectant residuals, when operating with variable feed rates, were typically around 1 mg/L. Dechlorination was designed for chlorine residuals exceeding 1 mg/L.

The compressed media filter provided a sufficient pretreatment level for UV disinfection. A double bank of medium pressure high intensity UV lamps (42 lamps per bank) reduced bacteria counts to the hundreds and thousands level (colonies per 100 mL) for flows of 10 to 20 MGD. The contact time for UV disinfection was two minutes. These results were for average conditions of TSS at 50 mg/L, 20% light transmittance and 25 degrees Centigrade water temperature. Transmissivity of treated flow was very important for UV. For example, UV disinfection of *E. coli* bacteria in filtered effluent with about 60% transmissivity was on the order of a magnitude higher (in hundreds of colonies per 100 mL) than in effluent with 40% transmissivity (in thousands of colonies per 100 mL). In contrast, the unfiltered CSO UV transmittance was as low as 20%.

A spreadsheet model was developed to evaluate combinations of intercept, storage, and flow through CSO treatment processes. The evaluation considered removal efficiencies, capital, and operational costs. The ADF findings provided performance criteria for vortex separation, Cl_2, ClO_2, and peracetic acid disinfection, and compressed media filtration followed by UV disinfection.

An optimized model of the ADF facility was developed. The optimized facility includes two 32-ft diameter vortex separators, instead of current six vortex separators, with Cl_2 disinfection followed by dechlorination and 2,000 cubic feet of compressed media filtration, instead of the current 1,000 cubic feet, followed by UV disinfection. The intercept capacity in this example is 10 MGD. The recommended peak flow capacity of the facility is 90 MGD.

Present worth, capital and operation and maintenance (O&M) costs were developed for various treatment trains, including the optimized facility, using 1995 construction costs and annual O&M costs based on several years of operation. Capital costs for a treatment system

designed for 63% removal of TSS were estimated to be approximately $10,000 per acre of combined sewer service area; annual operating costs were estimated to be about $163 per acre. Designing the system for 80% removal of TSS increased the capital cost nearly threefold, with annual operating cost doubling. As discussed above, removal of TSS is representative of disinfection effectiveness, especially for UV (Arnett, 2003. Personal Communication).

3.2.6.4 Washington, DC. Northeast Boundary Swirl Facility (NEBSF) (Disinfection Implementation)

The NEBSF, operated by the District of Columbia Water and Sewer Authority (WASA), provides treatment and disinfection for up to 400 MGD of CSO before discharging to the Anacostia River. The facility provides mechanical screening followed by three 57-ft diameter swirl concentrators. The effluent from swirl concentrators flows to a mixing chamber where sodium hypochlorite is added, usually at a dose of 5 mg/L. Sodium bisulfite is added at the end of the outfall for dechlorination, usually at a dose of 2 mg/L. Flows above 400 MGD are discharged untreated. Samples taken during CSO events at the mixing chamber and at the river outfall are analyzed for *Enterococcus* and fecal coliform. Reported counts range from less than 10 MPN/100 mL to in excess of 250,000 MPN/100 mL. The high numbers are associated with events in excess of 400 MGD and represent blending of treated and untreated CSO.

Annual operating costs for the NEBSF are estimated to about $230,000. This is based on $180,000 for labor and $50,000 for chemicals. The facility discharges on average about 100 times per year, with an average total volume of approximately 1,500 MG (Siddique, 2003. Personal Communication).

3.2.6.5 Birmingham, AL. UV Disinfection at Peak Flow WWTP (Disinfection Implementation under Construction)

The Jefferson County Environmental Services Division for the City of Birmingham and about 20 neighboring communities is in the process of constructing a 350 MGD peak excess flow treatment facility. The new facility, named the Village Creek Peak Flow Wastewater Treatment Plant (PFWWTP), includes a pump station, with 360 MGD capacity, 20 surge basins with surface aeration for mixing (total capacity of 90 MG), granular, monomedia, deep bed filters with 350 MGD capacity, UV disinfection, and a 24 megawatt generating facility (primarily to power the pump station and UV). The UV system will have a total of 2,688 lamps at a peak power requirement of 7,526 kW. The total installation cost of the UV facility is estimated to be $13 million; the cost of UV equipment is about $10.7 million. Operating costs are not available (Chandler, 2003. Personal communication).

3.2.6.6 Oakland County, MI. Chlorine Disinfection at Acacia Park (Disinfection Implementation)

The Office of the Oakland County Drainage Commissioner currently operates three CSO retention basins in southeastern Michigan, all of which provide treatment and disinfection of

flows that exceed their storage capacity. The Acacia Park CSO Retention Treatment Basin (RTB) is a 4 MGD basin that serves a combined area of about 818 acres. Disinfection is by sodium hypochlorite. The feed system provides a dose of 10 mg/L at a CSO flow rate of 426 MGD. There is no dechlorination. The disinfection target is a fecal coliform count of less than 400 cfu/100 mL at a total residual chlorine level of 1.0 mg/L.

Annual operating costs for the Acacia Park facility are estimated to be $120,000. This includes $58,000 for labor, $24,000 for energy and utilities, $26,000 for chemicals, and $10,500 for laboratory and other services. Over the three-year demonstration period, the facility captured 60% of the flow it received; that is treated overflows represent 40% of flow into the facility. The total volume of flow into the facility was estimated at 146 MG, with 88 MG retained and returned to the sewer system and 58 MG treated and discharged. Overflows occurred on average four to five times per year, and ranged in volume from 0.13 to 17 MG (Mitchell, 2003. Personal Communication).

3.2.6.7 Bremerton, WA. UV Disinfection at CSO Treatment Facility (Disinfection Implementation)

The City of Bremerton has recently constructed a CSO treatment facility that uses high-rate clarification, followed by UV disinfection, to treat flows up to 45 MGD. The facility uses a medium-pressure, high-intensity UV system that employs a total of 90 lamps. A 500-kW generator supplies power to the UV system as well as pumps, mixers, and other equipment. The clarification system uses a polyaluminum chloride coagulant. The primary reason for choosing UV over chlorination was to avoid degradation of hypochlorite between discharge events, which occur about 20 times a year. Bremerton installed a UV system at a a cost of about $600,000 to disinfect CSO discharges. The annual operation cost for the entire facility is estimated to be about $50,000 (Poppe, 2003. Personal Communication).

3.2.6.8 Disinfection of Collected Stormwater and Dry Weather Urban Runoff

New Orleans, LA - Stormwater Disinfection

The city of New Orleans, LA evaluated a prototype disinfection facility for stormwater using sodium hypochlorite in the late 1960s and early 1970s; (Pavia and Powell, 1968) however, they did not adopt the practice permanently.

Santa Monica Urban Runoff Recycling Facility (SMURRF)

Santa Monica's urban runoff recycling facility (SMURRF) project, completed in December 2000, in Santa Monica, CA, treats dry weather runoff water from excessive irrigation, spills, construction sites, pool draining, car washing, the washing down of paved areas, and some wet weather runoff. SMURRF treats an average of 0.5 MGD of the above urban runoff with solids, and oil and grease removing technologies prior to UV disinfection for removal of pathogens. The treated runoff is reused for landscape irrigation and for in dual-plumbed

buildings for flushing of toilets. For more information, see the Internet site at: http://Epwm.Santa-Monica.Org/Epwm/Smurrf.html.

Moonlight Beach Urban Runoff Treatment Facility

Moonlight Beach Urban Runoff Treatment Facility in the City of Encinitas, CA has been treating dry season urban runoff since September 2002. The facility accepts flows up to 150 gpm. The technologies used are filtration followed by UV disinfection. Coliform bacteria were reduced by over 99%. The facility does not operate during the wet season (Rasmus and Weldon, 2003).

3.3 Best Management Practices (BMPs) for Control of Pathogens in Urban Stormwater

3.3.1 Introduction

Practices to control and manage the quality and quantity or urban runoff have become widespread. This set of practices has been labeled best management practices or BMPs. Structural BMPs are designed to function without human intervention at the time a storm event occurs (Urbonas, 1999). Wet ponds, dry ponds, constructed wetlands, filters, rooftop storage, and swales are examples of structural BMPs that can be applied to urban stormwater. Eliminating illicit cross connections between the sanitary sewage system and separate stormwater drainage system is another structural BMP. Similarly, reduction of stormwater volume that enters combined or sanitary sewer systems aids in reducing CSO and SSO volumes. These measures are distinct from the others because they involve repairing the stormwater or sewerage system, rather than erecting a structure to manage or control stormwater quality. Other practices that reduce stormwater volume known as inflow reduction techniques include disconnection of roof leaders and redirection of area and foundation drains and basement sump pumps to soils where the flow will infiltrate to the ground or groundwater. Nonstructural BMPs are generally good housekeeping practices or measures designed to institute good housekeeping for reducing or preventing pollutant deposition in a watershed, e.g., public education or regulation (Urbonas, 1999).

This section provides a detailed discussion of the application of structural and nonstructural BMPs to stormwater microbial contamination. Available data on performance of BMPs for removing microorganisms from stormwater are presented. However, quantitative results are inconclusive or unavailable for many of the BMPs.

3.3.2 Structural BMPs

Wet ponds, dry ponds, constructed wetlands, filters, rooftop storage, and swales exhibit varied effectiveness for volume reduction and removal of suspended solids, metals, and nutrients. Structural BMPs have been applied to control pathogens to a lesser extent than to the other pollutants, and have produced mixed results. Often, controlling pathogens or

microorganisms is a secondary goal for these BMPs, which are more routinely implemented for reducing flow volume, sediment, or nutrients. Some environmental professionals are of the opinion that these practices do not affect pathogens to a meaningful degree and, therefore, should not be implemented to obtain the goal of reducing microbial concentrations. Microorganism or pathogen removal has been reported most frequently by sand filters, wetlands, and wet detention ponds. EPA Storm Water Technology Fact Sheets for these BMP types are available on EPA's web site at http://cfpub.epa.gov/npdes/ (U.S. EPA, 2003b). The fact sheets include the following information:

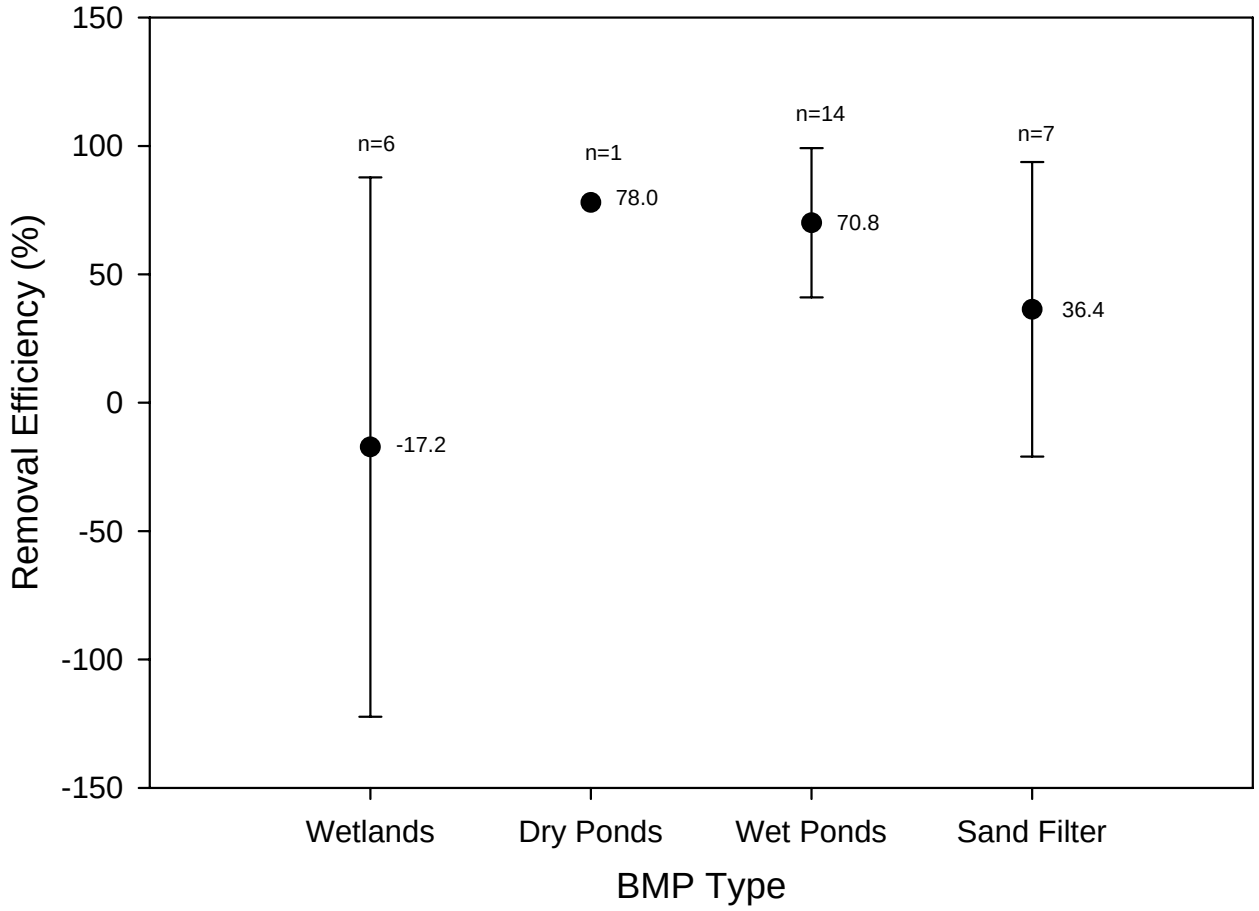
- description
- applicability
- advantages and disadvantages
- design criteria
- performance
- operation and maintenance
- costs

Limited research has been conducted on the effectiveness of structural BMPs for controlling stormwater pathogen loads to receiving waters. Much of the existing information has been compiled by Winer (2000) and by ASCE (2002) in U.S. EPA-sponsored projects. The data is compiled in database format, therefore, it is general in nature. It is included here to provide the reader with the range of BMP effectiveness and the database reference information. For more detailed information on a particular site, the reader should go to the original reference cited in the database. Reported fecal coliform removal efficiencies range from 99% at a wet pond in Ontario, Canada to –134% in a Fremont, CA wetland. These data show that while there are cases where microorganism reduction can be achieved to some extent by employing BMPs, BMPs also serve as environments where microorganisms are generated, presumably from increased wildlife populations and resuspension of bottom deposits. Table 3-3 presents performance data on the effectiveness of four types of BMPs for treating stormwater: wetlands, dry ponds, wet ponds, and sand filters (ASCE, 2002; Kurz, 1998; Winer, 2000). Figure 3-1 illustrates the variability of fecal coliform percent removal efficiencies reported. For each case study, the removal efficiencies are calculated using the average inlet and outlet fecal coliform concentrations.

Table 3-3. Stormwater BMP Effectiveness Data.							
BMP Type	**Total Coliform (CFU/100 mL)**			**Fecal Coliform (CFU/100 mL)**			**Location and Reference**
	Influent	Effluent	% Removal	Influent	Effluent	% Removal	
Wetlands						78	Lake Beardall, FL. Submerged gravel wetland. Egan *et al*., 1995, in Winer, 2000 (Study 91).
				2516	5882	-134	Fremont, CA. ASCE, 2002.
				2516	4581	-82	Fremont, CA. ASCE, 2002.
	3	2120	-706	2	236	-117	Sea Pines Plantation, SC. Surface flow, full scale, natural marsh, abundant wildlife, runoff and manure from horse trail. MacClellan, 1989, referenced in Table 17-3 of Kadlec and Knight, 1996.
				690	20	97	Kingston, MA. Shallow marsh (natural or constructed not specified). Horsley, 1995, in Winer, 2000 (Study 79).
				1350	768	55	Glenwood, WA. Shallow marsh (natural or constructed not specified). Koon, 1995, in Winer, 2000 (Study 80).
Dry Pond						78	Maple Run III, TX. ASCE, 2002.
Sand Filter						37	Joleyville, TX. City of Austin, Texas, 1990, in Winer, 2000 (Study 105).
						83	Brodie Oaks, TX. City of Austin, Texas, 1990, in Winer, 2000 (Study 106).
						36	Barton Creek, TX. City of Austin, Texas, 1990, in Winer, 2000 (Study 107).
						37	Highwood, TX. City of Austin, Texas, 1990, in Winer, 2000 (Study 108).
				5695	18528	-85	Barton Ridge Plaza, TX. City of Austin, Texas, 1990, in Winer, 2000 (Study 109).
						81	Barton Creek Square, TX. City of Austin, Texas, 1991, in Winer, 2000 (Study 110).
			59.4			66	Madeira Beach, FL. Kurz, 1998.

Table 3-3 continued. Stormwater BMP Effectiveness Data.							
BMP Type	**Total Coliform (CFU/100 mL)**			**Fecal Coliform (CFU/100 mL)**			**Location and Reference**
	Influent	Effluent	% Removal	Influent	Effluent	% Removal	
Wet Ponds						70	Monroe Street, Wisconsin. Bannerman and Dodds, 1992, in Winer, 2000 (Study 91).
				83633	1324	98	St. Elmo, TX. City of Austin, Texas, 1996, in Winer, 2000 (Study 26).
						86	Unqua, NY. Driscoll, 1983, in Winer, 2000 (Study 34).
					1779	90	Heritage Park, Ontario, Canada. Liang, 1996, in Winer, 2000 (Study 43).
	470	395.6	16				Jacksonville, FL. ASCE, 2002.
				6937	2516	64	Fremont, CA. ASCE, 2002.
				17619	4764	73	Davis, NC. FC Mass Removal Efficiency reported 48.1%. Borden *et al*., 1996, in Winer, 2000 (Study 11).
						-6	Piedmont, NC. Borden *et al*., 1996, in Winer, 2000 (Study 12).
						46	Woodhollow, TX. City of Austin, Texas, 1991, in Winer, 2000 (Study 13) and ASCE, 2002.
					783	64	Harding Park, Ontario, Canada. Fellows *et al*., 1999, in Winer, 2000 (Study 16).
						56	East Barrhaven, Ontario, Canada. Ontario Ministry of the Environment, 1991, in Winer, 2000 (Study 19).
						99	Kennedy-Burnett, Ontario, Canada. Ontario Ministry of the Environment, 1991, in Winer, 2000 (Study 20).
						97	Uplands, Ontario, Canada. Ontario, Canada. Ontario Ministry of the Environment, 1991, in Winer, 2000 (Study 21).
			64			98	Tampa, FL. Kurz, 1998.
Influent and effluent data provided in table when available.							

Figure 3-1. Fecal Coliform % Removal Efficiency by BMP Type.



Legend: ● is mean; error bar is standard deviation. n = number of BMPs reported
ASCE, 2002; Kurz, 1998; Winer, 2000.

There are many factors affecting variability including stormwater characteristics, BMP design, and environmental factors contributing to microorganism die-off.

3.3.2.1 Ponds and Wetlands

In contrast with the fact that better performance was observed in wet ponds over wetlands in the studies reviewed above, a number of research studies show that wetlands may provide advantages over ponds for indicator microorganism removals. One study found greater removal of thermotolerant coliforms, enterococci, and heterotrophic bacteria from stormwater in a wetland system (80-87%) than in a pond (–2-22%) (Davies and Bavor, 2000). The researchers attribute greater bacteria removal in the wetland to increased sedimentation aided by vegetation and increased removal of fine suspended particles (< 2 microns) with attached bacteria. Pond and wetland performance on microorganisms in sewage is an indicator of their performance on stormwater. A wastewater treatment wetland removed 97-99.9% of fecal coliform and *Enterococcus* and 70% of coliphage (Stenstroem and Carlander, 2000). The investigators

attribute the bacteria concentration reductions to the wetland's ability to remove suspended particles. Viruses have been shown to accumulate in wetland biofilms resulting in their removal from the effluent (Flood and Ashbolt, 2000).

The University of Arizona sponsors a research program on constructed wetlands treatment of secondary sewage effluent at the Pima County Constructed Ecosystem Research Facility in Tuscon. Although the research examines the effect of constructed wetlands on reducing microbial pathogen and indicator concentrations in secondary sewage effluent, the results provide useful information that can be applied to stormwater. A duckweed-covered pond, a multi-species subsurface flow wetland, and a multi-species surface flow wetland reduced concentrations of *Giardia* cysts, *Cryptosporidium* oocysts, total coliform, fecal coliform, coliphage, and enteric viruses in secondary sewage effluent (Gerba *et al*., 1999; Karpiscak *et al*., 1996; Thurston *et al*., 2001). Removal of the larger microorganisms, i.e., *Giardia* and *Cryptosporidium*, was the greatest in the duckweed pond, with sedimentation thought to be the primary removal mechanism. In contrast, the greatest removal of coliforms and coliphage occurred in the subsurface flow wetland, which may be related to the large surface area available for adsorption and filtration (Gerba *et al*., 1999). When supplying potable water to a wetland at the facility, Thurston *et al*. (2001) showed that total and fecal coliform concentrations increased. The researchers attribute the greater densities found in the summer months to the flora and fauna in and around the wetland. Warm waters promote the growth of bacteria contained in the animal feces deposited in the wetland. Increased plant growth may increase root exudates, oxygen to the rhizosphere, and accumulation of organic matter, believed to increase microorganism growth (Thurston *et al*., 2001). The results of these studies are summarized in Table 3-4.

Performance of constructed wetlands treating dairy farm wastewater for use in irrigation provides another source of information related to the effectiveness of constructed wetlands on removing pathogens from stormwater runoff. Kern *et al*. (2000) conducted a seasonal effects study at a side-by-side wetland constructed at the Institute of Agricultural Engineering in Potsdam, Germany. The subsurface flow wetland with a horizontal water flow reduced fecal coliform densities by 99.3 and 95.8% in the summer and winter, respectively. The principal mechanism in eliminating fecal coliform seemed to be adsorption to soil particles followed by die-off and predation (Kern *et al*., 2000). During the summer months, vertical distribution of fecal coliform densities in the control wetland bed, which did not receive wastewater, was equivalent to the levels in the treatment bed. In the winter, fecal coliform counts were three orders of magnitude higher in the treatment bed. High counts in the control bed in the summer were attributed to the presence of warm-blooded animals.

Table 3-4. Results of Wetlands Effectiveness Studies on Secondary Sewage Effluent at Pima County, AZ Constructed Ecosystem Research Facility.							
Reference	**Wetland Type**	**Percent Reduction**					
		TC	**FC**	***Giardia***	***Cryptosporidium***	**Enteric Viruses**	**Coliphage**
Karpiscak *et al*. (1996)	Multi-Species Surface Flow	98	93	73	58	98	N/A
Gerba *et al*. (1999)	Duckweed Covered Pond	62	61	98	89	38*	40
Gerba *et al*. (1999); Thurston *et al*. (2001)	Multi-Species Subsurface Flow	99	98	88	64	95	N/A
* from Karpiscak *et al*. (1996) reporting July - December 1994; other duckweed results reported by Gerba *et al*. (1999) for period July 1994 - December 1995							

Karpiscak *et al*. (1999) studied the effectiveness of an integrated wastewater treatment facility, consisting of solids separators, anaerobic lagoons, aerobic ponds and constructed wetlands, on dairy waste in Glendale, Arizona. In the aerobic pond, fecal coliform and *Listeria* concentrations decreased by 98.5 and 96.6%, respectively. Total coliform, however, increased by approximately 40%. Concentrations of all three organisms were decreased in the wetlands, total coliform by 79%, fecal coliform by 82.8%, and *Listeria* by 99.1%. Reductions are attributed to UV radiation, degradation of organic matter, solids settling, competition from other microorganisms, phytoremediation, and residence time.

3.3.2.2 Sand Filters

Sand filters operate by trapping suspended particles or adsorbing pollutants. Sand filters can be constructed in underground trenches or in above-ground, pre-cast concrete boxes. Advantages include the lower areal requirements than ponds and the ability to install them out of public view (Kurz, 1998), both of which facilitate their use in ultra-urban environments where ponds are more difficult to site.

3.3.2.3 Illicit Discharge Detection and Elimination

Improper connections to storm drainage systems convey contamination to receiving-water bodies. Sources of microbial contamination transported through this route include sanitary wastewater and septic tank effluent (Pitt *et al*., 1993). Since the 1980s, many municipalities initiated programs to identify and correct illicit connections in response to information highlighted by EPA's Nationwide Urban Runoff Program (U.S. EPA, 1983) and the 1987 Clean Water Act. The Clean Water Act requires municipal separate storm sewer system discharge permits to effectively prohibit non-stormwater discharges into storm drains. EPA has an Internet

site that presents information about illicit discharges, how specific municipalities are working to address them, and methods for identifying them: http://cfpub2.epa.gov/npdes/stormwater/menuofbmps/illi_2.cfm (U.S. EPA, 2003b). Pitt *et al*. (1993) published an EPA User's Guide on investigating inappropriate pollutant entries into storm drainage systems available at http://www.epa.gov/ednnrmrl/repository/cross/cross.pdf. An update of this manual has been funded by EPA and will be published in the near future. It is a collaborative effort between Pitt and the Center for Watershed Protection. The new manual will include information on optical brightener monitoring, a quick and effective way for screening large watersheds for illicit wastewater connections.

Procedures for identifying potential illicit discharges to storm drain systems include reviewing existing drainage area maps, surveying building storm drain connections, and inspecting sewer lines (U.S. EPA, 2003b). Visible flow during dry periods is a sign of a possible cross connection that should be further investigated. Visual inspection of the insides of a sewer system can be done with television equipment. Differences between known connections shown on maps and those revealed by the television should be further investigated. Tracers are often used to investigate suspected illicit connections (Pitt *et al*., 1993). A tracer is a parameter not characteristic of the base flow; the particular tracer present is dependent on the content of the illicit discharge. Tracers include water temperature, specific conductivity, fluoride and/or hardness, ammonia and/or potassium, surfactants and/or fluorescence (including optical brighteners from laundry detergents), chlorine, color, odor, turbidity, and flotables. Tracers for microbial contamination would include sanitary wastewater parameters such as BOD or suspended solids. Tracers can also be artificial, such as a dye. Smoke testing is another investigative method for illicit connections. Zinc chloride smoke injected into the sewer lines emerges from all breaks in the sewer line, vents in connected buildings, and outfalls (U.S. EPA, 2003b).

3.3.3 Nonstructural BMPs

Nonstructural BMPs include institutional and educational practices with the goal of changing behaviors so that the amount of pollutants entering the stormwater drains and receiving waters are reduced (Urbonas, 1999). These common sense measures for addressing microbial contamination include limiting public and animal access to sensitive watershed or riparian areas, public education on the role of storm drains, erosion control, vegetative buffers, street sweeping, animal waste management, and pet waste or pooper-scooper ordinances. While quantitative data on nonstructural BMP effectiveness are limited, a number of these practices have been shown to reduce receiving-water bacteria levels in rural and agricultural settings, primarily by controlling sources. They are provided here because some of the practices may apply to urban watersheds, particularly developing rural areas. Several demonstrations are described in the report prepared for EPA entitled *Section 319 Nonpoint Source National Monitoring Program – Successes and Recommendations* (Lombardo *et al.*, 2000). The types of practices reported to be successful are riparian/livestock exclusion fencing, riparian zone vegetation establishment and/or restoration, improved grazing management including stream crossings, improved handling of barnyard runoff and manure, campground educational programs on waste disposal, and upgrading septic systems. Project updates included in the 2002 update report (Lombardo *et al*., 2002) available at

http://h2osparc.wq.ncsu.edu/02rept319/indexframe.html show mixed results associated with using BMPs for reducing nonpoint source microbiological contamination. Some of the relevant results are presented below.

- The following BMPs were implemented in Arizona's Oak Creek Canyon Watershed: erecting permanent barricades along a highway to significantly reduce visitor access to the watershed's state park and campground, improving restroom facilities at the park and campground, and educational outreach. While limited improvement to the water quality in Oak Creek is attributed to these BMPs, the watershed task force is investigating additional sources of fecal coliform that, if addressed, can result in further improvement.

- Reductions in fecal coliform in California's Morro Bay Watershed are attributed to measures used to restrict or eliminate cattle access to riparian pastures.

- BMPs implemented in Washington's Totten and Eld Inlets are repair of failing on-site wastewater treatment systems and implementation of farm plans on farms that potentially threaten receiving-water quality. "Freshwater fecal coliform count and loading results suggest that for Burns, Pierre, and McLane creeks, the degree of BMP installation and maintenance is inadequate, and/or that unfactored demographic change may be eroding what might otherwise be improved conditions. For Schneider and Perry creeks, where water quality improved, the ability to link the improvement to pollution-control programs is hampered by lack of a control in one case, by non-BMP land-use change in the other case, and by inadequate BMP data in both cases. If effectiveness is measured by significant lasting decreases in pollution, then the results allow the possibility of effectiveness in these two cases. In those cases where pollution decreased, it appears to be on the rise again, which suggests that nonpoint pollution-control programs need to be at least cyclical if not continuous." (Batts and Seiders, 2003).

- A system of BMPs designed to exclude livestock from critical areas of streams and riparian zones has contributed to a reduction in indicator bacteria counts from 29 to 40% in Vermont's Lake Champlain Basin Watershed. Indicator bacteria counts exhibited pronounced seasonal cycles – low in winter and high in the growing season beginning in May. Additional experiments confirmed that indicator bacteria survive in stream sediments during the warmer months and can be resuspended when the sediments are disturbed. Decreases in *E. coli* and fecal coliform occurred during all seasons in the two watersheds studied, while fecal streptococcus decreases were significant in one of the watersheds. (Meals *et al*., 2001).

- Erosion control and animal waste management practices were implemented in Alabama's Lightwood Knot Creek Watershed. Although water quality improved for a number of characteristics, fecal bacterial concentrations were not improved. Fecal coliform concentrations decreased to some extent, but not to a significant degree. Fecal streptococcus concentrations increased in the watershed. The relatively small change was attributed to a design flaw in the constructed cattle crossing that encourages cows to congregate on the crossing during dry periods. (Cook and O'Neil, 2003).

- BMPs were shown to decrease indicator bacteria concentrations in North Carolina's Long Creek Watershed. The 70% decrease in median fecal coliform levels in one part of the creek is attributed to livestock exclusion. The installation of exclusion fencing in the pasture of the area's largest dairy farm is believed to be responsible for 90% and 80% decreases in fecal coliform and fecal streptococci levels.

Aside from farm animals, indigenous wildlife, rodents, and pets can increase indicator microorganism concentrations to levels that exceed water quality standards. In Northern Virginia's Four Mile Run Watershed, microbial source tracking identified a number of species (waterfowl, raccoon, human, dog, deer, and Norway rat) as the *E. coli* sources (Simmons, Jr. *et al.*, 2000; NVRC, 2002). The TMDL developed for fecal coliform requires that loadings from waterfowl, raccoon, dog and other wildlife, as well as humans, be reduced by significant percentages (NVRC, 2002). Although nonstructural BMPs will likely be used, the TMDL document does not address how achieving the TMDL goal will be accomplished. The approach will be presented in the TMDL implementation plan to be developed.

Instituting pet waste management or pooper-scooper laws is the traditional way communities have dealt with pet waste, which can contaminate water bodies or pose a potential threat to residents through direct contact. Waye (2003) cites the success of dog parks as BMPs. These parks should be located away from water bodies, and provide fencing, public education on managing waste, and disposal bags and receptacles. Having a local community pet group take responsibility for a park and establishing the norm of picking up after one's own pets help to ensure success of these parks.

Other nonstructural BMPs include modifying storm drain inlets to impede rodent access, public education, labeling storm drain inlets, and street sweeping.

3.3.3.1 Managing Waste from Resident Canada Geese

In recent years, geese populations have grown in many areas in the U.S. The problems encountered by local communities are the health and cosmetic problems associated with the fecal material generated, as well as the number of geese, and related traffic and safety concerns as these large birds cross traffic. Municipalities are instituting measures to protect public health from the impacts associated with this waste. The coastal town of Spring Lake, in New Jersey's Monmouth County, is experiencing high bacteria levels in a pond occupied by many Canada geese. During rain events, the pond overflows into the ocean, resulting in beach closures. The municipality automatically bans swimming at the nearby ocean beaches for 24 hours after it rains at least one-tenth of an inch (Bates, 2003). Restricting contact with recreational waters during wet-weather events is practiced by many municipalities as a precautionary measure because of the potential for waterborne illnesses to result in swimmers in contact with pathogens in the wet weather discharges.

Colts Neck, another Monmouth County community, recently contracted with the U.S. Department of the Interior's Fish and Wildlife Service to asphyxiate Canada geese at local

ponds. The local health officer defended the action based on the nuisance and potential health hazards posed by the geese droppings in and around the ponds (Jordan, 2003). Some citizens and animal rights advocates opposed the action and proposed alternatives. Waye (2003) names the possible alternatives identified by GeesePeace (www.geesepeace.org), including egg addling, vegetative barriers around water bodies, border collie patrols, goose repellants, and "no feed" zones.

Most Canada geese populations are migratory, wintering in the U.S. and migrating north to summer breeding grounds in the Canadian Arctic. The availability of park-like open spaces with short grass adjacent to water bodies have resulted in growing numbers of locally-breeding geese in the U.S. known as resident Canadian geese. There are an estimated 3.5 million resident Canada geese in the U.S. (U.S. Fish and Wildlife Service, 2002). Resident geese are protected under the Migratory Bird Treaty Act of 1918 and the Migratory Bird Conservation Act of 1929, and cannot be legally taken during a hunting season, unless a special federal permit is obtained from the Service. The proposed draft Environmental Impact Statement (EIS) released March 4, 2002, by the U.S. Fish and Wildlife Service grants the States the authority to implement approved population control strategies, such as nest and egg destruction, and trapping and culling programs, without having to go through the permit process. Until the draft EIS is finalized, scheduled for the fall 2003, states must obtain a special permit from the Service for resident Canada geese population control strategies.

3.3.4 Effects of BMPs on Receiving-Water Quality

From the available information on structural and nonstructural BMPs, it is evident that more research is needed on their effectiveness in reducing microbiological loads in stormwater runoff. Further, there should be a distinction between the effectiveness of structural and nonstructural BMPs. The highly variable effectiveness data exhibited by structural BMPs indicate that a variety of conditions affect the behavior of microorganisms and thus performance. These include BMP volume, temperature, light intensity, wetland plant type, filter design, and maintenance scope and frequency. As research in these areas progresses, BMP designs and O&M requirements can be aimed at achieving improved results. With even less quantitative information available for nonstructural BMPs, studies of their effectiveness in watersheds will provide information for health and environmental managers in other watersheds.

A concern with using BMPs to treat stormwater is that the microbial densities in the effluents may exceed water quality standards, even in BMPs considered to be performing well. For example, Davies and Bavor (2000) report a geometric mean for *Enterococcus* concentrations of 9.0×10^2/100 mL for the wetland's outflow, which is much higher than the U.S. recreational fresh water standard of 33/100 mL. In a case like this, the receiving water will need to have a high enough flow rate or volume to achieve the water quality target through dilution. Therefore, using a single BMP may not provide the level of treatment needed, in which case other options will need to be considered. These include incorporating a preliminary treatment step upstream of a structural BMP to create a treatment train or disinfecting the stormwater. Reducing runoff volume and source control are the most reliable ways to decrease microorganism loads to receiving waters from stormwater.

3.4 Conclusions

Managing microbial contamination in urban watersheds presents unique challenges. A primary reason for this is that some of the microorganism content in runoff and waterways occurs naturally because microorganisms are components of waste products deposited by animals residing in these watersheds. The populations of these organisms vary with animal population and are affected by environmental factors such as temperature, sunlight, and nutrient availability. Also, quantitative effectiveness results of the BMPs used to manage this diffuse source pollution are often unavailable or inconclusive. Therefore, managers relying on BMPs for allocating nonpoint source loads to achieve a TMDL goal need to be prepared to revise management plans and even allocations if monitoring data reveals that the desired results are not achieved.

Although some quantitative information on the effectiveness of structural BMPs for managing microbial contamination in stormwater is available, the amount of information is less plentiful than it is for other contaminants. Microorganism or pathogen removal has been reported most frequently for sand filters, wetlands, and wet detention ponds. However, the results are highly variable. The available wet pond fecal coliform data shows removals between 46 and 99 percent, except for one site where the removal was –5.8%. The wetlands efficiency data reviewed has an even greater range of removal efficiencies, from –134% to 97%. These results contradict some research studies with findings that show wetlands have better removal efficiencies than ponds. Research to understand the key biological, chemical, and physical processes controlling microorganism behavior in commonly used stormwater BMPs is necessary (Sullivan and Borst, 2001). This research would better define the relationships between design parameters and effectiveness and will contribute to the development of models that will predict effluent quality over a BMP's lifetime, temporal variations of effluent quality, and differences in performance due to differences in events (Sullivan and Borst, 2001). Also useful would be increased understanding of the relationships between common water quality parameters, e.g., TSS, and microbial indicators and pathogens.

Less quantitative information is available on the effectiveness of nonstructural BMPs than on the structural BMPs discussed above. EPA's Nonpoint Source National Monitoring Program generates some data that shows decreases, increases, and no change after BMP implementation. The watersheds described in the available program summaries are primarily rural in nature. Public education and pet waste management regulations and programs are other nonstructural BMPs that show promise for urban watershed management but for which quantitative performance data are needed.

Disinfection of CSO and other WWF types achieves a much greater degree of microorganism removal than BMPs. It's also been the subject of a much greater amount of research and investigation. Disinfection has been demonstrated to reduce microorganism concentrations in WWFs with high concentrations (10^5 to 10^7 organisms/100 mL) by several orders of magnitude and produce effluents meeting permit discharge requirements (10^2 to 10^3

organisms/100 mL). WWF disinfection generally occurs within shorter contact times than conventional wastewater disinfection, i.e., less than 30 minutes, with intense mixing to ensure disinfectant contact with the maximum number of microorganisms, and increased disinfectant dosage. Effective use of this high-rate disinfection process requires use of a treatment train, with an initial treatment of either filtration or inertial separation (e.g., sedimentation and vortex) to remove suspended solids. This is to address the phenomena of microbial aggregation and particle association/occlusion that cause decreased disinfection efficiency.

Chlorination is the only chemical disinfection technology currently used for disinfection of WWF. Although effective, this technology generates formation of chlorinated hydrocarbons, i.e., chloroform and THMs, which are suspected carcinogens. To address this concern and remove excess free Cl_2, the chlorination process can be augmented by dechlorination with either gaseous sulfur dioxide or sodium bisulfite solution. Other disinfection technologies investigated for CSO include UV light irradiation, ClO_2, and O_3. Of these three technologies, only UV disinfection has recently entered commercial use for WWF disinfection. Chlorine dioxide and O_3 have not been put to commercial use in the U.S. Removal efficiencies for the disinfection technologies discussed (Cl_2 , UV, ClO_2 , and O_3) achieve bacterial reductions of 99.9% to 99.99%. This is a significantly greater level of contaminant reduction than is achieved by BMPs. Although just beginning to be used for treating stormwater, disinfection of stormwater may be necessary to achieve water quality objectives in some watersheds.

A final point that should be considered is the uncertainty associated with the use of indicator microorganisms to determine pathogen reductions resulting from the use of a control technology or a BMP. Chapter 1 explores the relationships between indicators, pathogens, and waterborne illness. Although the desired reduction of an indicator microorganism density, TMDL, or water quality target is achieved by a certain technology or a management approach, there is still a possibility of public health impact due to the presence of disease-causing microorganisms, i.e., pathogens. Alternatively, the indicators may have provided a false or exaggerated indication of the presence of disease-causing pathogens and, thus, no benefit to human health was achieved through the control or management practice implemented. Watershed managers need to be aware of the limitations associated with indicators and remember the primary goal of protecting public health.

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