Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule

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Contents

Appendices

Exhibits

Acronyms

AIDS	Acquired Immunodeficiency Syndrome
AUX 1	Auxiliary 1
AWWARF	American Water Works Association Research Foundation
CC	Cell Culture
COWP	*Cryptosporidium* outer wall protein
CPE	Comprehensive Performance Evaluation
CT	Concentration × Time
CWS	Community Water System
DAPI	Diamidino-2-phenylindole
DBPR	Disinfection Byproducts Rule
DBPs	Disinfection Byproducts
DIC	Differential Interference Contrast
EPA	Environmental Protection Agency
ESWTR	Enhanced Surface Water Treatment Rule
FACA	Federal Advisory Committee Act
$FeCl_3$	Ferric Chloride
FBRR	Filter Backwash Recycle Rule
FS	Flowing Stream
GAC	Granular Activated Carbon
GWR	Ground Water Rule
GWUDI	Ground Water Under the Direct Influence of Surface Water
HAAs	Haloacetic Acids
HAA5	The Sum of 5 Haloacetic Acids
ICR	Information Collection Rule
ICRSS	Information Collection Rule Supplemental Surveys
ID_{50}	Infectious Dose Causing Disease in 50 Percent of the Population
IESWTR	Interim Enhanced Surface Water Treatment
LSP	Lab Spiking Program
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
M/DBP	Microbial/Disinfection Byproduct
MCL	Maximum Contaminant Level
MRDLGs	Maximum Residual Disinfectant Levels Goals
MRDLs	Maximum Residual Disinfectant Levels
NODA	Notice of Data Availability
NPDWR	National Primary Drinking Water Regulation
NTNCWS	Nontransient Noncommunity Water System
NTU	Nephelometric Turbidity Units
OST	Office of Science and Technology
PCR	Polymerase Chain Reaction
PWS	Public Water System
QA/QC	Quality Assurance and Quality Control
RL	Reservoir/Lake
SA-11	Simian Rotavirus
SDWA	Safe Drinking Water Act
SDWIS	Safe Drinking Water Information System
SWTR	Surface Water Treatment Rule
TCR	Total Coliform Rule

TOC	Total Organic Carbon
TRAP-Cl	Thromobospondin-related adhesive protein Cl
TTHM	Total Trihalomethanes
TWG	Technical Work Group

1. Introduction

The United States Environmental Protection Agency (EPA or Agency) Office of Ground Water and Drinking Water (OGWDW) is developing interrelated drinking water regulations to control microbial pathogens and disinfectants/disinfection byproducts in drinking water. These rules are collectively known as the microbial/disinfection byproducts (M-DBP) rules.

The Safe Drinking Water Act (SDWA) Amendments of 1996 require EPA to develop rules to balance the public health risks from pathogens and DBPs. The Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR) and the Interim Enhanced Surface Water Treatment Rule (IESWTR), the first set of M-DBP rules under the SDWA Amendments, were promulgated in December 1998. The Stage 1 DBPR and the IESWTR were the culmination of a 6-year (1992-1998) rule development process that included regulatory negotiations with representatives of the water industry, environmental and public health groups, and local, State, and Federal government agencies. The Amendments also require EPA to publish a Stage 2 DBPR.

To support rule development, EPA expanded its microbial and DBP research program and entered into collaborative efforts with other agencies and the water industry to collect data. This data collection effort included the Information Collection Rule (ICR) and the ICR Supplemental Surveys (ICRSS). In addition, under a joint effort between EPA and the National Rural Water Association (NRWA), NRWA state chapters conducted a survey of byproduct and treatment information at small public water systems (PWSs).

The purpose of the ICR was to provide microbial and DBP occurrence and treatment information from large PWSs to support M-DBP regulations. EPA has worked with stakeholders under the Federal Advisory Committee Act (FACA) to develop the Stage 2 DBPR and Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). These rules are being developed concurrently, using occurrence data from the ICR and other available sources, to ensure that microbial protection is maintained or enhanced while exposure to DBPs is reduced.

This occurrence and exposure assessment provides background information for the LT2ESWTR. This chapter presents an overview of the regulatory background and describes the purpose of this document.

1.1 Regulatory Background

Exhibit 1.1 presents a brief chronology of EPA's rulemaking activities on microbiological contaminants, disinfectants, and DBPs in drinking water, starting with the Total Trihalomethane Rule promulgated in 1979 (USEPA 1979). Following Exhibit 1.1 is a brief description of rulemaking efforts.

Exhibit 1.1 Chronology of EPA's Drinking Water M-DBP Rulemaking Efforts

Year	Regulation	Action
1979	***Total Trihalomethane Rule (TTHM)***	***Promulgated***
1989	***Surface Water Treatment Rule (SWTR)***	***Promulgated***
1989	***Total Coliform Rule (TCR)***	***Promulgated***
1992	Negotiated Rulemaking	Initiated
1994	Stage 1 Disinfectants and Disinfection Byproduct Rule (DBPR)	Proposed
	Interim Enhanced Surface Water Treatment Rule (IESWTR)	Proposed
	Information Collection Rule (ICR)	Proposed
1996	***Information Collection Rule***	***Promulgated***
	Safe Drinking Water Act (SDWA)	***Reauthorized***
1997	Microbial and Disinfectants/Disinfection Byproduct Federal Advisory Committee (M-DBP FACA)	Established
	Stage 1 DBPR and IESWTR Notice of Data Availability (NODA) (November)	Presented new data and M-DBP FACA recommendations
1998	Stage 1 DBPR Notice of Data Availability (NODA) (March)	Presented new health effects data
	Stage 1 Disinfectants and Disinfection Byproduct Rule (DBPR)	***Promulgated***
	Interim Enhanced Surface Water Treatment Rule (IESWTR)	***Promulgated***
1999	Stage 2 M-DBP FACA	Established
2000	Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR)	Proposed
	Filter Backwash Recycle Rule	Proposed
	Ground Water Rule	Proposed
2001	***Filter Backwash Recycle Rule***	***Promulgated***
2002	***Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR)***	***Promulgated***
2003	Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR)	Planned Proposal
	Stage 2 DBPR	Planned Proposal
2005	***LT2ESWTR***	***Planned Promulgation***
	Stage 2 DBPR	***Planned Promulgation***

1.1.1 Total Trihalomethane Rule

In November 1979, EPA promulgated an interim maximum contaminant level (MCL) of 0.10 milligrams per liter (mg/L) for total trihalomethanes (TTHMs): the sum of chloroform, bromodichloromethane, dibromochloromethane, and bromoform. Compliance is calculated as a running annual average of TTHMs (USEPA 1979). The TTHM Rule was based on the need to reduce exposure to DBPs while maintaining disinfection to address microbial risks (USEPA 1979). Certain DBPs have been shown to cause cancer in laboratory animals. The interim TTHM standard applies only to community water systems that use surface and/or ground water, serve at least 10,000 people, and add a disinfectant during any part of the treatment process. At their discretion, States may extend coverage to smaller PWSs (USEPA 1979).

1.1.2 Surface Water Treatment Rule

Between 1979 and 1989, no new regulations related to disinfection or disinfection byproducts were promulgated, however, interim regulations promulgated in 1975 regulating coliform bacteria and turbidity remained in effect. The turbidity limits from the 1975 interim rule still apply to a few water systems that have not installed filtration. In 1989, in response to the requirements of the 1986 SDWA, EPA promulgated the Surface Water Treatment Rule (SWTR) (USEPA 1989a), which established maximum contaminant level goals (MCLGs) of zero for *Giardia lamblia*, viruses, and *Legionella*. The rule applies to water systems that treat surface water or ground water under the direct influence of surface water. The SWTR specifies treatment technique requirements for filtered and unfiltered water treatment systems that are intended to protect against the adverse health effects of exposure to *Giardia lamblia*, viruses, *Legionella*, and many other pathogenic organisms. Briefly, those requirements include the following:

- Maintenance of a disinfectant residual in the distribution system
- Removal and/or inactivation requirements of 3 logs (99.9 percent) for *Giardia* and 4 logs (99.99 percent) for viruses
- Combined filter effluent performance standards of five nephelometric turbidity units (NTU) as a maximum and 0.5 NTU in at least 95 percent of the measurements taken each month, based on samples collected at a 4-hour monitoring interval for treatment plants using conventional treatment or direct filtration (with separate standards for other filtration technologies)
- Watershed protection and other requirements for unfiltered systems.

1.1.3 Total Coliform Rule

In 1989, EPA promulgated the Total Coliform Rule (TCR) (USEPA 1989b) to provide protection from microbiological contamination in the distribution system. Prior to the TCR, the interim regulations required compliance with a MCL based on coliform bacteria density. The TCR established a MCLG of zero for total and fecal coliform bacteria, and a MCL based on the percentage of positive samples collected during a monthly compliance period. Total coliforms are bacteria that are used as an indicator of water treatment effectiveness and distribution system integrity. Fecal coliform bacteria are generally considered indicators of possible fecal contamination. Under the TCR, no more than 5 percent of

distribution system samples collected in any month may contain coliform bacteria. The number of samples to be collected in a month is based on the population served. The location and frequency of sampling is based on a system-specific sampling plan that provides representative coverage throughout the distribution system. Combined, the SWTR and the TCR are intended to address risks associated with pathogens that might be found in source waters or associated with distribution systems.

1.1.4 Regulatory Negotiation Process

Prompted by an interest in balancing health risks from microbial pathogens and DBPs, in 1992 EPA initiated a negotiated rulemaking to address public health concerns associated with disinfectants, DBPs, and microbial pathogens. The negotiators included representatives of State and local health and regulatory agencies, public water systems, elected officials, consumer groups, and environmental organizations. The main concern in developing the rules was to ensure that when utilities changed existing treatment to comply with new requirements for DBPs, they would not compromise microbial protection. Hence, the negotiators agreed that EPA should propose a microbial rule with the DBPR.

Early in the rulemaking process, the Negotiating Committee determined that sufficient plant-specific information on how to optimize the use of disinfectants, while concurrently minimizing pathogen and DBP exposure risk, was not available. Nevertheless, the Negotiating Committee recommended that EPA propose a DBPR to extend coverage to all community water systems (CWSs) and nontransient noncommunity water systems (NTNCWSs) that use disinfectants. CWSs are public systems that serve at least 15 service connections used by year-round residents or that regularly serve at least 25 year-round residents. NTNCWSs are systems that serve at least 25 of the same persons for more than 6 months of the year, where those persons are not full-time residents (e.g., colleges, schools, office buildings).

As a result of the negotiations, the Committee recommended that EPA develop three sets of rules. These rules include a two-staged disinfection byproducts rule, a companion microbial rule, and an information collection rule to gather data on microbial and DBP occurrence.

1.1.5 Information Collection Rule

EPA promulgated the ICR, a monitoring and data-reporting rule, on May 14, 1996 (USEPA 1996a). The ICR data collection results provide EPA and stakeholders with additional information on the national occurrence in drinking water of key influent water quality parameters, disinfectants and disinfection byproducts, and disease-causing microorganisms, including *Cryptosporidium, Giardia,* and viruses. The ICR database also provides engineering data describing how PWSs currently control for such contaminants and information on treatment applications used to reduce DBPs and their precursors.

The ICR data collection focused on large PWSs, which serve populations of 100,000 persons or more. About 300 PWSs operating 501 treatment plants participated in this extensive data collection. Over an 18-month period, these PWSs monitored influent water quality parameters affecting DBP formation and DBP levels in the treatment plant and the distribution system. PWSs also provided operational data and descriptions of their treatment plant design. The surface water systems monitored for bacteria, viruses, and protozoa. Surface and ground water systems conducted monitoring to determine the applicability of treatment study requirements. In addition, ground water systems serving between 50,000 and 100,000 persons were required to perform applicability monitoring. A subset of PWSs performed treatment studies, using either granular activated carbon (GAC) or membrane filtration processes, to evaluate DBP precursor removal and control of DBPs. The systems that were required to

perform treatment studies were selected based on applicability monitoring. All ICR systems began monitoring for treatment study applicability in September 1996. The remaining occurrence monitoring began in July 1997 and was completed in December 1998.

The ICR dataset is the basis for many analyses (for example, *Cryptosporidium* occurrence in source waters) described in this document that support the development of the LT2ESWTR and the Stage 2 DBPR.

1.1.6 Safe Drinking Water Act Reauthorization

In 1996, Congress reauthorized the Safe Drinking Water Act. The 1996 SDWA Amendments include provisions related to the SWTR and the Enhanced Surface Water Treatment Rule. Those provisions established a deadline of November 1998 for the promulgation of both the Stage 1 DBPR and the IESWTR. The Amendments also set deadlines of November 2000 for the LT1ESWTR and May 2002 for the final Stage 2 DBPR. No mandatory deadline was established for LT2ESWTR. However, to ensure a proper balance between microbial and DBP risks, EPA believes it is important to finalize the Stage 2 DBPR in conjunction with the LT2ESWTR.

1.1.7 M-DBP Advisory Committee

In May 1996, EPA initiated a series of public meetings to exchange information on issues related to the development of the IESWTR and the Stage 1 DBPR. EPA established the M-DBP Advisory Committee under the FACA on February 12, 1997, to collect, share, and analyze new information and data, as well as to build consensus on the regulatory implications of this new information. The M-DBP Advisory Committee comprised 20 members representing EPA, State and local public health and regulatory agencies, local elected officials, drinking water suppliers, chemical and equipment manufacturers, and public interest groups. The M-DBP Advisory Committee agreed that the Stage 1 DBPR and IESWTR should:

1) Include proposed MCLs for total trihalomethanes, haloacetic acids, and bromate

2) Require enhanced coagulation and enhanced softening (with an adjustment based on new data)

3) Require microbial profiling to ensure that DBP control does not compromise microbial protection

4) Continue to give credit for complying with disinfection requirements (see section 1.1.2)

5) Establish stricter turbidity limits

6) Establish a *Cryptosporidium* MCLG and requirements for removing *Cryptosporidium*

7) Use a multiple barrier approach

8) Strengthen existing sanitary survey requirements (USEPA 1997)

1.1.8 Stage 1 Disinfectants and Disinfection Byproducts Rule

The Stage 1 DBPR (USEPA 1998a) sets maximum residual disinfectant levels (MRDLs) and MRDL goals (MRDLGs) for chlorine, chloramine, and chlorine dioxide, and MCLs for chlorite, bromate, and two groups of DBPs: TTHMs and five haloacetic acids (HAA5). HAA5 refers to the sum of mono-, di-, and trichloroacetic acids, and mono- and dibromoacetic acids. MCLGs were set for chlorite, bromate, di- and trichloroacetic acids, and each individual trihalomethane. These standards are listed in Exhibit 1.2.

In addition, systems that use surface water or ground water under the direct influence of surface water (GWUDI) and employ conventional treatment or softening must remove a specified percentage of organic materials, measured as total organic carbon (TOC), unless they meet high source water quality standards. This is important because these organic materials, or precursors, react with disinfectants to form DBPs. Precursors can be removed through treatment techniques (enhanced coagulation or enhanced softening), which are described in the Stage 1 DBPR (USEPA 1998a). Exhibit 1.3 lists the percentage reduction of TOC required for various influent TOC and alkalinity levels.

Exhibit 1.2 Stage 1 DBPR Standards

	MRDLG (mg/L)	**MRDL** (mg/L)	**MCLG** (mg/L)	**MCL** (mg/L)
Disinfectants				
Chlorine	4.0 (as Cl_2)	4.0 (as Cl_2)		
Chloramine	4.0 (as Cl_2)	4.0 (as Cl_2)		
Chlorine Dioxide	0.8 (as ClO_2)	0.8 (as ClO_2)		
DBPs				
Bromate			0	0.010
Chlorite			0.8	1.0
TTHMs			--	0.080
Bromoform			0	--
Bromodichloromethane			0	--
Chloroform			N/A	--
Dibromochloromethane			0.06	--
HAA5			**--**	0.060
Monochloroacetic acid			--	--
Dichloroacetic acid			0	--
Trichloroacetic acid			0.3	--
Monobromoacetic acid			--	--
Dibromoacetic acid			--	--

Note: The Stage 1 DBPR included a MCLG of zero for chloroform. The MCLG was challenged and the U.S. Court of Appeals for the District of Columbia Circuit issued an order vacating the zero MCLG (U.S. Court of Appeals, DC Circuit 2000). On May 30, 2000, EPA removed the MCLG for chloroform from its National Primary Drinking Water Regulations (NPDWRs) (USEPA 2000a). EPA is proposing a new MCLG for chloroform in the Stage 2 DBPR (USEPA 2003).

Exhibit 1.3 TOC Percent Removal Requirements for Systems Employing Enhanced Coagulation

	Source Water Alkalinity (mg/L as $CaCO_3$)		
Source Water TOC (mg/L)	**0–60**	**>60–120**	**>120[1]**
>2.0–4.0	35 %	25 %	15 %
>4.0–8.0	45 %	35 %	25 %
>8.0	50 %	40 %	30 %

[1] Requirements apply only to systems using enhanced softening.

1.1.9 Interim Enhanced Surface Water Treatment Rule

The IESWTR serves two purposes: to improve the control of pathogens, specifically *Cryptosporidium*, in drinking water, and to address risk trade-offs with DBPs. Key provisions established in the IESWTR include the following:

- A maximum contaminant level goal of zero for *Cryptosporidium*
- 2 log (99 percent) *Cryptosporidium* removal requirements for systems that use conventional or direct filtration
- Strengthened performance standards for combined filter effluent turbidity and individual filter turbidity
- Disinfection benchmark provisions to ensure continued levels of protection against pathogens while facilities take the necessary steps to comply with the new DBP standards (referred to as risk trade-off)
- Inclusion of *Cryptosporidium* in the definition of GWUDI and in the watershed control requirements for unfiltered PWSs
- Requirements for covers on new finished water reservoirs
- Sanitary surveys of all surface water systems, regardless of size

The IESWTR specifies requirements for turbidity levels in all surface water systems that use conventional treatment or direct filtration, serve 10,000 or more persons, and are required to filter. The turbidity levels in combined filtered water must be no greater than 0.3 NTU in at least 95 percent of samples taken each month; turbidity must not exceed 1.0 NTU at any time. In addition, systems must monitor individual filters and provide an exceptions report to the State monthly. Exceptions to be reported include the following:

- Any individual filter with a turbidity level greater than 1.0 NTU in 2 consecutive measurements taken 15 minutes apart.
- Any individual filter with a turbidity level greater than 0.5 NTU after 4 hours of filter operation, based on 2 consecutive measurements taken 15 minutes apart. If no obvious reason for abnormal filter performance can be identified, a filter profile must be produced within 7 days of the exceedance.
- An assessment by the system of any individual filter that has turbidity levels greater than 1.0 NTU in 2 consecutive measurements taken 15 minutes apart in each of 3 consecutive months.
- A Comprehensive Performance Evaluation by the State, or by a third party approved by the State, of any individual filter that has turbidity levels greater than 2.0 NTU in 2 consecutive measurements taken 15 minutes apart in each of 2 consecutive months.

1.1.10 Long Term 1 Enhanced Surface Water Treatment Rule / Filter Backwash Recycling Rule

In 2001, EPA promulgated the Filter Backwash Recycling Rule (FBRR) and in January 2002, promulgated the LT1ESWTR to increase protection of finished drinking water supplies from contamination by *Cryptosporidium* and other microbial pathogens (USEPA 2001a; USEPA 2002). These rules apply to PWSs that use surface water or ground water under the direct influence of surface water. The LT1ESWTR extends protection against *Cryptosporidium* and other disease-causing microbes to the 11,500 small surface water systems that serve fewer than 10,000 persons annually. The FBRR, which applies to all systems that recycle filter backwash, thickener supernatant, and liquids from dewatering, regardless of size, establishes filter backwash requirements for certain surface water systems. The filter backwash requirements will reduce the potential risks associated with recycling contaminants removed during filtration. In the 1996 SDWA Amendments, Congress required the Agency to promulgate both rules. A brief description of these rules follows.

FBRR Provisions - These apply to all systems that recycle regardless of population served.

- Recycling systems are required to return certain recycle streams through the processes of a system's existing conventional or direct filtration system or at an alternative location approved by the State.

- All recycling systems must notify the State that they practice recycling and must submit a plant schematic and recycle flow information.

LT1ESWTR Provisions - These apply to systems that are required to filter and that serve fewer than 10,000 persons:

1) Cryptosporidium *Removal*

- All systems must achieve 2 log (99 percent) removal of *Cryptosporidium* by meeting turbidity requirements.

2) *Turbidity*

- Conventional and direct filtration systems must comply with specific combined filter effluent turbidity requirements and individual filter turbidity requirements; the requirements are identical to those in the IESWTR.

3) *Disinfection Benchmarking*

- Public water systems are required to develop a disinfection profile to ensure that if changes are made to the disinfection practices in order to comply with the Stage 1 DBPR, current microbial inactivation treatment is maintained.

4) *Other Requirements*

- Finished water reservoirs for which construction begins after the effective date of the rule must be covered.

- Unfiltered systems must comply with updated watershed control requirements that add *Cryptosporidium* as a pathogen of concern.

1.1.11 Ground Water Rule

In May 2000, EPA proposed a targeted risk-based regulatory strategy for all ground water systems (USEPA 2000b). The requirements provide a meaningful opportunity to reduce the public health risk associated with the consumption of water contaminated with pathogens from fecal contamination for a substantial number of people served by ground water sources.

The strategy addresses risks through a multiple-barrier approach that relies on five major components:

- Periodic sanitary surveys of ground water systems requiring the evaluation of eight elements and the identification of significant deficiencies.
- Hydrogeologic assessments to identify wells sensitive to fecal contamination.
- Source water monitoring for systems drawing from sensitive wells without treatment or with other indications of risk.
- Compliance monitoring to ensure disinfection treatment is reliably operated (where used).
- Correction of significant deficiencies and fecal contamination through one of the following actions:
  - Eliminate the source of contamination
  - Correct the significant deficiency
  - Provide an alternative source of water
  - Provide a treatment which achieves at least 99.99 percent (4 log) inactivation or removal of viruses

1.1.12 Stage 2 M-DBP Advisory Committee

In March 1999, EPA reconvened a M-DBP Advisory Committee to develop recommendations on issues pertaining to the development of the Stage 2 DBPR and LT2ESWTR. The Committee consisted of organizational members representing EPA, State and local public health and regulatory agencies, local elected officials, Indian Tribes, drinking water suppliers, chemical and equipment manufacturers, and public interest groups. The Committee evaluated recent health effects information and the potential benefits of a Stage 2 DBPR and LT2ESWTR. The Committee considered new information from the ICR and other data sources on the occurrence of DBPs and pathogens as well as the treatment performance and costs of various technologies. Technical support for the Committee's discussions was provided by a technical workgroup (TWG) established by the group.

Despite the evaluation of a large amount of data, the Committee recognized that substantial uncertainty remains regarding the nature and magnitude of risk associated with DBPs and pathogens in drinking water. In light of this uncertainty, the Committee recommended steps, based on the extensive analysis discussed in this document and in EPA's economic analysis, to address the areas of greatest concern without placing an undue burden on public water systems.

In September 2000, the Committee signed the Agreement in Principle— a full statement of the consensus recommendations of the group. The agreement was published by EPA in a December 2000

Federal Register notice (USEPA 2000c). The Agreement is divided into Parts A and B, as summarized below.

Part A

Stage 2 DBPR

- MCLs for TTHM and HAA5 will remain at 0.080 and 0.060 mg/L, respectively.
- Compliance with MCLs for TTHM and HAA5 will be based on the locational running annual average (LRAA), in two phases of the rule.
- In Phase 1 of the rule, systems must comply with TTHM and HAA5 MCLs of 0.080 and 0.060 mg/L as a running annual average (RAA) and 0.120 and 0.100 calculated as a LRAA at sample location.
- In Phase 2, compliance with TTHM and HAA5 MCLs of 0.080 and 0.060 mg/L is calculated as a LRAA for each of the new monitoring locations identified in the Individual Distribution System Evaluation (IDSE).
- Systems will carry out an IDSE to select compliance monitoring sites that best capture the highest TTHM and HAA5 levels. The studies will be based either on system-specific monitoring or other system specific data that provides equivalent or better information on site selection.
- MCL for bromate will remain at 0.010 mg/L.

- Additional treatment requirements for *Cryptosporidium* will be based on the results of source water monitoring.

- Systems that are required to provide additional treatment choose technologies from a 'toolbox' of options.

- The monitoring burden for small systems will be reduced through the use of indicators to determine which systems must monitor for *Cryptosporidium*.

- Systems will conduct future monitoring to determine if source water quality has changed following completion of the initial monitoring.

- Unfiltered systems will provide at least 2 logs of *Cryptosporidium* inactivation, and unfiltered systems will meet overall inactivation requirements with a minimum of two disinfectants.

- Systems will cover all uncovered finished water reservoirs unless the reservoir effluent is treated to achieve 4 logs of virus inactivation or the State/Primacy Agency determines that existing risk mitigation is adequate.

- EPA will develop guidance and criteria to facilitate the use of UV light for compliance with drinking water disinfection requirements.

Part B

- Beginning in January 2001, as part of the 6-year review of the Total Coliform Rule, EPA will initiate a stakeholder process to address distribution system requirements related to significant health risks.

- The Committee recommends that EPA develop a national water quality criteria under the Clean Water Act for microbial pathogens for stream segments designated by States/Tribes for drinking water use.

These recommendations reflect the Committee's emphasis on targeted, risk-based rulemaking. They incorporate substantial initial monitoring to identify systems with the highest potential risk. Additional treatment steps are required only where systems exceed limits on locational average DBP concentrations or source water *Cryptosporidium* occurrence levels (USEPA 2000c).

1.1.13 Stage 2 Disinfectants and Disinfection Byproducts Rule

EPA proposed the Stage 2 DBPR in Fall 2003. The rule includes MCLGs for chloroform, monochloroacetic acid (MCAA) and trichloroacetic acid (TCAA). The rule also sets MCLs and monitoring, reporting, and public notification requirements for TTHM—the sum of chloroform, bromodichloromethane, dibromochloromethane, and bromoform—and HAA5—the sum of mono-, di-, and trichloroacetic acids and mono- and dibromoacetic acids. The rule also revises the monitoring requirements for chlorite and chlorine dioxide. The document includes the best available technologies (BATs) upon which the MCLs are based.

- The DBP standards are based on a LRAA compliance calculation, as opposed to a RAA. With the LRAA, each sample point must be in compliance with the standards, rather than all sample points combined.

- Systems must perform an IDSE, which will refocus their sampling plans on points within the distribution system that best represent the highest concentrations of TTHMs and HAA5.

EPA believes the implementation of the Stage 2 DBPR will reduce the levels of DBPs in drinking water supplies and will reduce inequities in exposure across distribution systems, resulting in reduced risk of reproductive and developmental health effects and cancer.

The Stage 2 DBPR applies to public water systems that are CWSs and NTNCWSs that add a primary or residual disinfectant other than ultraviolet light or deliver water that has been treated with a primary or residual disinfectant other than ultraviolet light. In addition, the revised monitoring requirements for chlorite and chlorine dioxide apply to transient noncommunity water systems (TNCWSs).

1.1.14 Long Term 2 Enhanced Surface Water Treatment Rule

The LT2ESWTR provisions follow the recommendations of the M-DBP FACA Advisory Committee Agreement in Principle. Key provisions include the following:

- Source water monitoring for *Cryptosporidium*, with reduced monitoring requirements for small systems.

- Additional *Cryptosporidium* treatment for some filtered systems, based on source water *Cryptosporidium* concentrations.

- At least 2 log inactivation of *Cryptosporidium* by all unfiltered systems, using at least 2 disinfectants.

- Disinfection profiling and benchmarking to assure continued levels of microbial protection while PWSs take the necessary steps to comply with new disinfection byproduct standards.

- Covering, treating, or implementing a risk management plan for uncovered finished water reservoirs.

- Criteria for a number of treatment and management options (i.e., the microbial toolbox) that PWSs may implement to meet additional *Cryptosporidium* treatment requirements. The LT2ESWTR will build upon the treatment technique requirements of the Interim Enhanced Surface Water Treatment Rule and the Long Term 1 Enhanced Surface Water Treatment Rule.

The LT2ESWTR will apply to all PWSs using surface water or GWUDI as a source.

EPA believes that implementation of the LT2ESWTR will significantly reduce levels of *Cryptosporidium* in finished drinking water in systems with high *Cryptosporidium* levels in their source waters. This will substantially lower incidence of endemic cryptosporidiosis associated with drinking

water. In addition, the treatment technique requirements of this document are expected to increase the level of protection from exposure to other microbial pathogens (e.g., *Giardia*).

1.2 Purpose of This Document

The purpose of this document is to summarize available information on *Cryptosporidium* and *Giardia* and use it with statistical models to estimate the occurrence of these pathogens in drinking water systems. This document emphasizes *Cryptosporidium* and *Giardia* because they are important waterborne pathogens that can survive for months in the environment, are resistant to chlorine disinfection (*Giardia* is less resistant than *Cryptosporidium*), and—particularly for *Cryptosporidium*—a small number of oocysts can cause infection.

Many studies support the fact that *Cryptosporidium* oocysts are ubiquitous in the water environment and some types are infectious to humans and many animals. Rivers, lakes, streams, and ground water are potential sources of drinking water contamination. This document characterizes the ICR and ICR Supplemental Survey data on occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in the source waters of large systems and models those data to characterize occurrence in source waters nationwide.

This document uses information from scientific articles related to: (1) the occurrence, health effects, persistence, and transmission of—and the analytical methods for detecting—*Cryptosporidium* and *Giardia*; (2) monitoring studies (from the ICR and ICRSS); and (3) statistical modeling. Its foundation is the *Cryptosporidium* and *Giardia* Occurrence and Exposure Assessment for the Interim Enhanced Surface Water Treatment Rule (EPA 1998c). In addition, EPA conducted literature searches to identify articles and studies to provide supplemental information regarding occurrence and exposure of *Cryptosporidium*, *Giardia*, and other waterborne pathogens.

1.3 Document Organization

This document is organized into eight chapters. A description of each chapter follows.

- Chapter 2—Waterborne Pathogens of Concern: This chapter summarizes the characteristics of common waterborne pathogens, *Cryptosporidium*, *Giardia*, and viruses. Their response to treatment and health effects of specific strains are discussed. Recent waterborne disease outbreaks are described.

- Chapter 3—Characterization of Pathogen Occurrence: This chapter describes the data sources and subsequent analysis methods for the source water microbial occurrence. The challenges encountered in analyzing such occurrences are also discussed.

- Chapter 4—Source Water Occurrence Data: This chapter presents the data collected to document the national occurrence of pathogens in source water. Analyses of co-occurrence and interactions of source water constituents are also presented.

- Chapter 5—Treatment by Physical Removal: This chapter reviews treatment effectiveness of existing and new filtration technologies.

- Chapter 6—Observed Finished Water Occurrence: This chapter presents the data describing pathogen occurrence in finished water.

- Chapter 7—Pre-LT2ESWTR Occurrence Estimates: This chapter provides estimates of pre-LT2ESWTR finished water occurrence of pathogens based on treatments applied.

- Chapter 8—Population Profile: This chapter presents the estimated populations served by public water systems by system size and source of supply. These populations are used to estimate the potential exposure to pathogens in drinking water.

2. Characteristics of Waterborne Pathogens of Concern

This chapter summarizes the characteristics of the waterborne pathogens *Cryptosporidium*, *Giardia*, and viruses. Sections 2.1, 2.2, and 2.3 describe the health effects, variety of strains, person-to-person transmission, and responses to disinfection for these three pathogens. The pathogens discussed are those for which EPA has designated treatment requirements (USEPA 1989a, 1998b) and that were studied as part of the ICR. EPA also designated treatment requirements for *Legionella*, a bacterial pathogen, but *Legionella* was not monitored under the ICR and is not included here. Sections 2.1 and 2.2, on *Cryptosporidium* and *Giardia*, contain descriptions of individual species and their persistence under various environmental conditions. Section 2.3 discusses viruses. Section 2.4 describes recent outbreaks of waterborne disease and their subsequent health implications. Section 2.5 describes the use of coliforms as indicators of contamination.

2.1 *Cryptosporidium spp.* and *Cryptosporidium parvum*

Cryptosporidium is a major concern as a waterborne pathogen because its resistant oocyst form is relatively unaffected by commonly used disinfection methods. For example, 2.5 milligrams per liter (mg/L) of chlorine inactivated *Giardia* after 5 minutes (Jarroll et al. 1981), while it took 80 parts per million of chlorine 90 minutes to inactivate *Cryptosporidium* (Korich et al. 1990) (starting concentrations of organisms differed for each study). *Cryptosporidium* can persist in the environment for extended periods of time. Several biological and epidemiological factors contribute to the potential for waterborne transmission of cryptosporidiosis, including the following (adapted from Casemore 1990):

- Completion of the life cycle in a single host species (i.e, the presence or absence of other species is not necessary for reproduction)
- Excretion of oocysts in large numbers, enhanced by the fact that *Cryptosporidium* can reproduce within the host (autoinfection)
- Immediate infectivity of excreted oocysts (no external “ripening” required)
- Relative resistance of oocysts to most environmental extremes and to common disinfectants
- Low infective dose in humans (DuPont et al. 1995; Chappell et al. 1999; Okhuysen et al. 1999)
- Excretion of oocysts by asymptomatic (displaying no symptoms) carriers
- Long-term excretion of oocysts by animals and chronically ill patients (e.g., immunocompromised individuals)
- Ubiquitous geographic distribution
- Lack of specific therapeutic cure

2.1.1 Description of the Species

Members of the genus *Cryptosporidium* are taxonomically classified in the phylum Apicomplexa, order Eucoccidiorida, suborder Eimeriorina, and family Cryptosporidiidae (Fayer et al. 1997; O'Donoghue 1995). All members of the Apicomplexa are parasitic, and some of them are extremely important as disease agents (Levine 1985). Within the phylum Apicomplexa are several related genera referred to collectively as coccidia. Most coccidia are small protozoa that complete their life cycles intracellularly (within the digestive tract epithelium, liver, kidney, blood cells, or other tissues of the host). *Cryptosporidium* species infect epithelial surfaces, particularly those of the intestines. These species are found in a wide range of vertebrates, including humans (Fayer et al. 1997).

There is uncertainty about the taxonomy (i.e., classification) of species within the genus *Cryptosporidium*. Until 1980, classification was based on the assumption that a particular species infected only one type of animal (i.e., each host species harbored a separate species of *Cryptosporidium*) (Fayer and Ungar 1986). The "single host/single species" assumption is no longer accepted (Tzipori 1985). For example, *C. parvum* has been found to infect both humans and cattle. Hence, other more appropriate taxonomy schemes have been suggested. Molecular characterization techniques provide considerable evidence of genetic variations between isolates of a single species of *Cryptosporidium* from different species of hosts, and increasing evidence suggests that a series of host-adapted genotypes/strains exist (Morgan and Thompson 1998). Khramtsov et al. (1997) discovered a double-stranded RNA in *C. parvum* sporozoite cytoplasm that was not present in any other *Cryptosporidium* species examined. This RNA could be used as an identifier for *C. parvum*. Fayer et al. (1997, 2000) identified 10 species of *Cryptosporidium*, listed in Exhibit 2.1 (isolated from five mammalian, two avian, four reptilian, and one fish species), and refer to several unnamed species isolated from a variety of hosts. The *C. andersoni* species infecting cattle (see Exhibit 2.1) is not the same as the *C. parvum* strain that infects cattle; it more closely resembles *C. muris*.

Depending on the classification scheme in use, virtually all *Cryptosporidium* infections in humans are caused by *C. parvum*. Other species such as *C. felis*, *C. meleagridis*, and other species, however, may infrequently infect humans, especially immunocompromised people (those with weak immune systems) (Pieniazek et al. 1999; Morgan et al. 2000a; Pedraza-Diaz et al. 2001).

Xiao (1999) presented another classification scheme based on the 18S rRNA gene. This scheme separates *C. parvum* and related species into intestinal and gastric genotypes that also exhibit other differences, such as infectiousness versus non-infectiousness to humans. Xiao indicated that *Cryptosporidium* includes at least four distinct species: *C. parvum*, *C. baileyi*, *C. serpentis*, and *C. muris;* he states that *C. wrairi*, *C. felis*, *C. meleagridis*, and *C. saurophilum* are actually strains of *C. parvum*. Champliaud et al. (1998) noted that although *C. meleagridis* is morphologically different from *C. parvum* and does not normally infect humans, the DNA sequences compared by polymerase chain reaction (PCR) amplification were very similar for each species. Therefore, other sequences need to be found to help distinguish between the two.

Exhibit 2.1 *Cryptosporidium* in Host Species

Named species of *Cryptosporidium* proposed as valid	
***Cryptosporidium* species**	**Host species**
C. andersoni	*Bos taurus* (domestic cattle)
C. baileyi	*Gallus gallus* (domestic chicken)
C. felis	*Felis catis* (domestic cat)
C. meleagridis	*Meleagris gallopavo* (turkey)
C. muris	*Mus musculus* (house mouse)
C. nasorum	*Naso literatus* (fish)
C. parvum	*Homo sapiens* (humans) + >100 other mammals
C. saurophilum	*Eumeces schneideri* (skink)
C. serpentis	*Elaphe guttata* (cornsnake) *Elaphe subocularis* (rat snake) *Sanzinia madagascarensus* (Madagascar boa)
C. wrairi	*Cavia porcellus* (guinea pig)

Source: Adapted from Fayer et al. 1997; Fayer et al. 2000.

Tzipori and Griffiths (1998) reviewed the difficulties associated with dividing *Cryptosporidium* into species. These include (1) the lack of clearly defined and fully characterized reference strains for comparative studies to define distinguishing phenotypic (physically observable) and genotypic (genetically determined) parameters; (2) the ability of *Cryptosporidium* to infect a variety of cells, tissues, organs, and vertebrate species; (3) and the often conflicting results of cross-transmission experiments. The authors suggested that *Cryptosporidium* should be considered as a genus that consists of a wide spectrum of isolates whose differences—in host origin, site of infection, and oocyst size—are not as important as virulence and genetic attributes, which have not yet been fully characterized. Tzipori and Griffiths suggest that *Cryptosporidium* species be defined not simply by physical or genetic characteristics, but by linking physical traits (particularly virulence attributes) and genetic markers.

2.1.2 Strains

Considerable genotypic and phenotypic differences exist among and within *C. parvum* isolates (Tzipori and Griffiths 1998). Variations in infectivity to other animals, pathogenicity, antigenicity, protein banding, isoenzyme typing, and genes that code for RNA and certain proteins have been recognized. Xiao et al. (1999) believe, based on such characteristics, that several species that infect different animals are actually strains of *C. parvum*.

Okhuysen et al. (1999) evaluated the virulence of three strains of *C. parvum* in humans. A vast difference in the virulence of the isolates was observed among subjects with presumed infection (diarrhea and/or oocyst excretion): the infectious dose causing disease in 50 percent of the population (ID_{50}) was 87 oocysts for the Iowa (calf) strain; 9 oocysts for the TAMU (horse) isolate; and 1,042 oocysts for the

UCP isolate (calf). For subjects who only excreted oocysts, the ID_{50} was 74.5,125, and 2,788 oocysts for the Iowa, TAMU, and UCP isolates, respectively. Eighty-six percent of the volunteers who received the TAMU isolate developed diarrhea, compared to 52 and 59 percent of those receiving the Iowa and UCP isolates, respectively.

Several studies support the existence of at least two genotypes within the species *C. parvum*: genotype 1, infecting humans only and genotype 2, infecting humans and livestock (Sulaiman et al. 1998; Spano et al. 1998; Pieniazek et al. 1999; Xiao et al. 1998). Widmer et al. (2000), however, did succeed in infecting piglets with genotype 1 and found that virulence increased with each passage. The human genotype was also detected in a dugong (Morgan et al. 2000b). McLauchlin et al. (1999) suggest that the two genotypes of *C. parvum* represent two reproductively isolated populations that are, or behave as, different species within the genus *Cryptosporidium* based on the different chromosome location of the *Cryptosporidium* outer wall protein (COWP) and thrombospondin-related adhesive protein C1 (TRAP-C1). Molecular analysis using TRAP-C2 sequencing for *C. parvum* isolates also differentiated the isolates into two genotypes representing different animal transmission cycles (Peng et al. 1997; Sulaiman et al. 1998). In a review of *Cryptosporidium* taxonomy, Morgan et al. (1999) also suggested separating the two genotypes based on a lack of similarity between the rDNA ITS gene. They also recommended that the pig, dog, and marsupial *Cryptosporidium* genotypes be assigned species names, based on genetic differences even larger than those between *C. parvum* and *C. wrairi,* which infects guinea pigs and has been accepted as a separate species. The authors noted the need to determine how genetic variations in *Cryptosporidium* arise, through studies of reproduction and population, in order to more accurately define what constitutes a separate species.

Contradicting the two-genotype theory, a study by Di Giovanni et al. (1999) identified seven distinct *C. parvum* heat shock protein (*hsp*70) genotypes. Reproducible differences between the *C. parvum* LA-1 (the laboratory control strain) *hsp*70 sequence and the *C. parvum* KSU-1 *hsp*70 reference sequence suggested that *hsp*70 sequences may be useful for differentiating strains of *C. parvum*. The authors also found that the *hsp*70 sequences of raw water or backwash samples of *C. parvum* matched those of LA-1 in some cases and KSU-1 in others. In other samples, the *hsp*70 sequences differed from both the laboratory and reference strains.

2.1.3 Fate and Transport

The fate and transport of pathogens in the environment are major issues with respect to the exposure of humans to waterborne pathogens. The ability of microorganisms to survive in the environment permits their transport either by water, food, or personal contact with a human host (Hurst 1997). The human exposure pathway of concern to EPA is drinking water from surface water and from ground water supplies that are under the influence of surface water. Surface water sources include lakes, rivers, reservoirs, and cisterns. Ground water supplies are extracted for drinking water by vertical and horizontal wells, infiltration galleries, and springs.

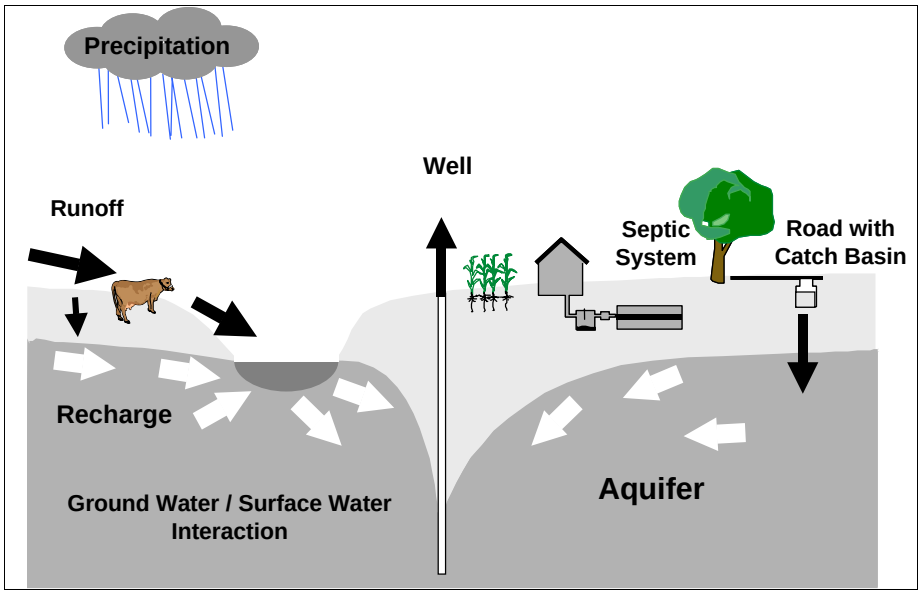
2.1.3.1 Surface Water

When more rain falls than can be absorbed immediately by the soil or soil cover, water will pond on the surface. With increasing rainfall, the water will flow to a lower level on the surface, such as a river, lake, or reservoir, as shown in Exhibit 2.2. As water travels, it may pick up contaminants on the

soil surface (e.g., *Cryptosporidium* oocysts from deposited fecal matter). These particles are then suspended in the surface water and can be transported as runoff. The microorganisms (including parasitic protozoa) associated with the soil can be transported as individual organisms, aggregates of organisms, or within an aggregate of soil particles and organisms. Partly for this reason, incidences of waterborne cryptosporidiosis tend to vary temporally, with a higher prevalence during the warmest, wettest months (Current 1986). In most areas of North America, *Cryptosporidium* occurrence generally becomes a concern in surface waters during the spring, when rains increase runoff and many newborn animals, which are more susceptible to *C. parvum*, are present in the environment. However, cryptosporidiosis cases in the United States are most prevalent in late summer (Wolfson et al. 1985). The seasonal pattern of cryptosporidiosis cases is suggestive of recreational water transmission in late summer and fall.

The character (topography, plant cover) and uses (urban, farming) of a watershed also influence the occurrence or concentration of *Cryptosporidium* in surface water (Hansen and Ongerth 1991). For example, one survey found that a mountainous, forested watershed with little or no human activity had the lowest surface water oocyst concentrations and oocyst production, while downstream sample sites influenced by dairy farming and urban runoff had oocyst concentrations and production rates almost 10 times higher than the upstream sites (Hansen and Ongerth 1991). In contrast, modeling by Walker and Stedinger (1999) suggests that dairy oocyst loads are minor compared with treated wastewater oocyst loads from humans.

Exhibit 2.2 Ground Water/Surface Water Interaction



Cryptosporidium may also directly enter surface water via waterfowl. Canada geese fed large doses of *C. parvum* have been shown to pass intact oocysts that then caused severe illness in newborn mice (Graczyk et al. 1997), although the geese did not develop cryptosporidiosis. Oocysts were also found in goose feces collected in the environment (Graczyk et al. 1998). Canada geese, some of which no longer migrate, could cause contamination of surface water sources and finished water reservoirs.

Cryptosporidium also can be transported through soil and ground water (Mawdsley et al. 1996; Hurst 1997). Movement of *C. parvum* through soil and ground water is affected by sedimentation and

filtration of the surrounding soil and aquifer matrix (Brush et al. 1999; Harter et al. 2000). Harter et al. measured oocyst pore velocities of 16.8 meters per day (m/day) in a coarse sand laboratory column.

Adsorption of oocysts to soil and aquifer matrix particles affects filtration. Adsorption depends on the magnitude and distribution of the electrical charge on the organism and the surrounding soil and aquifer matrix. Walker et al. (1998) summarized research on the effect of oocyst charge on surface transport of *Cryptosporidium*. They reported that, because oocysts are approximately the size of clay/silt particles, the amount of kinetic energy needed to entrain and suspend oocysts in overland flow may be quite small. Studies of oocyst sorption to simplified analogs of soil mineral fractions and hydrophobic materials suggested that the effective diameter of the oocyst may be the same as the oocyst itself in the absence of positively charged particles. However, Brush et al. (1998) reported that the charge distribution can be altered by the purification method and that the hydrophobicity can change as oocysts age.

The buoyancy of oocysts also affects their fate and transport in environmental media. Oocysts that are not bound to particles may have a tendency to float. Researchers have found that 65 to 84 percent of oocysts floated up into the supernatant of a homogenized fecal matter/distilled water mixture after 18 and 23 hours (Swabby-Cahill et al. 1996). Also, after six 10-minute centrifugations at 1,500 rotations per minute (rpm), 16 percent of the oocysts were detected in the top third of the supernatant, suggesting some resistance to settling (Swabby-Cahill et al. 1996). *Cryptosporidium* oocysts have a very low density (about 1.05 grams per cubic centimeter (g/cm^3)) and a very low settling rate (2 millimeters (mm) per hour or less), which suggests that sedimentation without coagulation may not be an effective means of oocyst removal (Gregory 1994). Rose et al. (1997) and Sreter and Szell (1998) also noted the low sedimentation rate for oocysts. Medema et al. (1998) found that oocysts attached to wastewater effluent particles settled more quickly than those that were freely suspended and that sedimentation velocity increased with particle size. In source waters, many oocysts are likely to be adsorbed to organic or other suspended material and would probably settle more quickly than free-floating oocysts (Medema et al. 1998).

2.1.3.2 Ground Water Under the Direct Influence of Surface Water

Some ground water—extracted for drinking by wells, infiltration galleries, or springs—is regulated as surface water. Infiltration galleries are collection devices characterized by buried perforated pipe in which water collects and is directed towards pumps. They are often used with shallow ground water sources. Ground water that is considered to be under the direct influence of surface water (GWUDI) is usually immediately adjacent to surface water or to the discharge point of a spring. These ground water supplies are considered especially vulnerable to contamination by parasitic protozoa. GWUDI may be contaminated by direct infiltration of oocysts from the surface as a result of rain. More commonly, however, ground water is contaminated by the action of pumping wells (see Exhibit 2.2). Given sufficiently high pumping rates, wells can reverse the direction of ground water flow. In this case, surface water is induced to flow from a river, lake, or reservoir into the adjacent ground water, where it may be extracted by one or more pumping wells. If the surface water is contaminated with oocysts, the adjacent ground water may also become contaminated. Because of the potential for contamination, GWUDI is regulated as if it were surface water.

Surface water sediments and the aquifer matrix material are believed to play significant roles in minimizing oocyst transport to water supply wells. Not enough is known, however, about the hydrogeology of aquifer matrices and sediments to determine their significance in preventing contamination. Also, as discussed in section 3.3.1, problems with detection methods complicate the

accuracy of data collection. Therefore, whether the oocysts are present and not recovered or simply not present in hydrogeologic settings such as alluvial aquifers is not known. Furthermore, little information is available to elucidate which hydrogeologic settings are sensitive to oocyst contamination, because ground water flow and oocyst transport through fractures or dissolution conduits can effectively bypass the protective action of most of the aquifer matrix. In one study examining riverbank filtration, oocysts were recovered at a well 200 feet from the Ohio River (Arora et al. 2000).

Harvey et al. (1995) modeled the transport of protozoa in ground water systems, using free-living flagellates (protozoa with flagella, tail-like organelles used for locomotion) (2 to 3 micrometers (µm) in size) and microspheres (0.7 to 6.2 µm in size). The authors observed the movement of flagellates and microspheres through a column of sediment containing layers of varying grain size. They noted that physical straining was particularly important in porous media, such as coarse sands, with grain diameter greater than 100 µm. Adsorption effects appeared to be related to the size of the microspheres. The largest microspheres (2.8 to 6.2 µm) were not significantly transported, but 83 percent of the concentration of 1.7-µm-size microspheres added to the column were present in the effluent.

To examine the mechanisms by which oocysts may be transported through soil to ground water, Mawdsley et al. (1996) studied transport of *Cryptosporidium parvum* oocysts through three soil types using 35-centimeter-long cores of each soil type. *C. parvum* transport was greater in a silty loam and a clay loam soil than in a loamy sand soil. Because these results contradict other evidence that suggests clay soils exhibit greater adsorption and smaller micropores than sandy soils, factors other than adsorption and micropore size appear to have influenced the oocyst movement. The authors cited their use of intact soil cores to maintain the natural soil structure and macropores, and concluded that the rapid flow of water through macropores, which are representative of natural field conditions, largely bypasses the filtering and adsorptive effects of the soil and greatly increases the risk of this pathogen's transport to ground water (Mawdsley et al. 1996).

Hancock et al. (1998a) investigated the correlation of *Cryptosporidium* and *Giardia* occurrence in GWUDI. They found no correlation for either pathogen between the distance of the ground water source from adjacent surface water and occurrence. However, Hancock et al. (1999) reported that microbiota in eight major groups were indicative of *Cryptosporidium* and *Giardia* contamination of ground water. *Cryptosporidium* were found in seven of 149 vertical wells and five of 14 horizontal wells.

2.1.3.3 Transmission of Cryptosporidiosis

Cryptosporidiosis is primarily transmitted by fecal-oral transmission through direct contact (e.g., hand-to-hand) with an infected person or through contact with a fecally contaminated item (e.g., door knobs or sink faucets). It is also commonly spread by swimming or playing in contaminated water and sometimes through contaminated food. Cryptosporidiosis resulting from contaminated drinking water is relatively rare, but large outbreaks have occurred as a result of drinking water contamination. People infected with *Cryptosporidium* in an outbreak associated with a particular source, such as a day care center or a contaminated swimming pool, can infect others, such as family members, who were not in contact with the original source. This is called secondary transmission.

Secondary spread of *Cryptosporidium* infection among individuals may occur in homes, daycare centers, hospitals, and urban environments, anywhere people are highly concentrated (Baxby et al. 1983; Brown et al. 1989; Ribeiro and Palmer 1986; Heijbel et al. 1987; Melo Cristino et al. 1988; Fayer et al.

1990; Casemore et al. 1997). Juranek (1995) explains that children in diapers are at especially high risk for direct transmission of cryptosporidiosis because of intimate play or careless diaper changing practices. Infection rates greater than 60 percent in urban day care centers have been reported (Fayer and Ungar 1986). Asymptomatic family members living with infected individuals are sometimes found to excrete small numbers of oocysts. Outbreaks are often associated with confirmed secondary cases among family members and other people who had recent contact with infected individuals. For example, in 1989, an outbreak of cryptosporidiosis occurred at a daycare center in Atlanta. Forty-nine percent of the children and 13 percent of the staff members who submitted stool samples were found to be infected with *Cryptosporidium* (Tangermann et al. 1991). Most of the cases likely were transmitted through the fecal-oral route.

Documented hospital cross-infection with *C. parvum*, such as from patient to staff, is further evidence of human-to-human transmission (Tzipori et al. 1983; Crawford and Vermund 1988; Casemore et al. 1997). *Cryptosporidium* has been found in sputum and in vomit (Tzipori et al. 1983). Nosocomial cryptosporidiosis infections (those contracted in hospitals) have been reported in both hospital staff and patients (Juranek 1995). Fecal-oral exposure during sexual contact has been implicated as a transmission (exposure) route for direct-contact transmission in homosexual males with acquired immunodeficiency syndrome (AIDS). Respiratory cryptosporidiosis has also been reported in AIDS patients, sometimes in the absence of diarrhea (Mifsud et al. 1994; Clavel et al. 1996; Dupont et al. 1996). Symptoms may include fever, pneumonia, bronchitis, sore throat, and difficulty breathing.

2.1.4 Health Effects

Members of the genus *Cryptosporidium* are parasites of the intestinal tracts of fishes, reptiles, birds, and mammals. *C. parvum* is the species commonly associated with self-limiting infections in healthy humans characterized by mild to severe diarrhea, dehydration, stomach cramps, and/or a slight fever, all generally lasting less than 2 weeks. Infection in unhealthy humans (especially those who are immunocompromised) typically is more severe and may persist for months and result in death. A *Cryptosporidium* outbreak in Milwaukee caused at least 46 deaths of individuals with AIDS and 8 deaths among people with underlying medical conditions (Hoxie 1997). A few other *Cryptosporidium* species (e.g., *C. felis)* have been found to infect immunocompromised humans, (Piezanek et al. 1999).

Cryptosporidium infection may result from ingestion of oocysts in food or water contaminated by feces, or by person-to-person contact. In the gastrointestinal tract of suitable hosts, four sporozoites excyst from each oocyst and parasitize epithelial cells. Thick-walled oocysts that are shed from the host in fecal material are resistant to environmental stressors and may persist until they enter a new host by the oral route. Approximately 20 percent of the oocysts produced in the gut have been shown to fail to form a thick oocyst wall and instead release "thin-walled oocysts." These thin-walled oocysts allow the excystation (release) of sporozoites within the gut and result in accelerated infection of new cells without infection from external sources (Kansas State University 1997). This process of reproduction within the same host is called autoinfection. Infection may or may not result in clinical symptoms of cryptosporidiosis, for which no therapeutic cure is available.

The first symptoms of cryptosporidiosis appear 2 to 10 days after a person becomes infected. The symptoms may include profuse, nonbloody, watery diarrhea that generally resolves spontaneously within 2 weeks; however, variability in clinical symptoms exists. Diarrheal symptoms generally are not distinguishable from those caused by other common enteric pathogens. Other symptoms reported by

individuals afflicted with cryptosporidiosis include abdominal cramps, vomiting, lethargy, and general malaise (USEPA 1998d).

Human volunteer studies in which subjects ingest known doses of *C. parvum* (also called challenge studies) have been conducted to assess the infectivity and dose-response of *C. parvum* in humans (DuPont et al. 1995). In this study, infectivity was defined as causing excretion of oocysts (regardless of whether symptoms were present). Sixty-two percent (18 of 29) of healthy subjects who ingested became infected. The oocyst dose ranged from 30 to 1 million. Eleven of the 18 infected individuals had enteric symptoms; seven of these individuals had diarrhea and, by clinical definition, cryptosporidiosis. Only 1 of 5 subjects receiving a dose of 30 oocysts became infected, while 14 of 16 who received doses of 300 or more oocysts did, and all of those receiving doses of 1,000 or more oocysts did. The ID_{50} for the Iowa strain of *C. parvum* was calculated at 132 oocysts in humans, compared with an ID_{50} of 60 oocysts in neonatal mice; however, the test strain of *C. parvum* in this case was adapted to a mouse model before challenge studies began, which may account for the disparity in ID_{50} values. The mean and median incubation periods for cryptosporidiosis in the study were 9.0 and 6.5 days, respectively. Infected humans developed clinical enteric symptoms that were associated with excretion of oocysts, although 1 of the 11 subjects who did not pass oocysts passed a single soft stool on day 10 and exhibited enteric symptoms on days 23 through 31. Symptoms of clinical illness included abdominal pains, cramps, and diarrhea (in six subjects); nausea (in six subjects); vomiting (in one subject); and moderate dehydration (in one subject).

Follow-up studies indicate that the number of excreted oocysts and the pattern and duration of oocyst shedding vary widely among immunocompetent individuals (Chappell et al. 1996). In the volunteer challenge study, high variability in shedding patterns was observed. Oocysts were observed intermittently in consecutive stool samples, implying that production of oocysts is not uniform and may be influenced by unknown factors. Thus, when single stool samples are submitted for diagnostic analysis, the test for a patient with cryptosporidiosis may be negative.

Chappell et al. (1999) conducted additional volunteer studies to determine whether *Cryptosporidium* infectivity varied with prior exposure to *Cryptosporidium*, as determined by positive tests for anti-*C. parvum* serum immunoglobulin G. Study participants ingested doses of 500 to 500,000 oocysts. Infection and diarrhea correlated with higher oocyst doses. The authors determined that the *Cryptosporidium* ID_{50} for previously exposed individuals was 1,880 oocysts, 20 times higher than the ID_{50} for non-exposed people. In addition, oocysts were detected in the feces of only 54 percent of test subjects with symptoms. These results suggest that previous *Cryptosporidium* exposure provides some immunity to further infection at low oocyst doses.

2.1.5 Persistence

Several factors influence oocyst survival. This section presents the findings from several studies describing oocyst inactivation due to temperature and dessication.

The survival pattern of oocysts suggests that, once an initial contamination has occurred, water can remain a source of viable oocysts for days (Heisz 1997; Lisle and Rose 1995). Lisle and Rose reported a duration of 176 days to produce die-off rates of 96 percent in tap water and 94 percent in river water. After 2 days, a realistic contact time in most water distribution systems, only 37 percent of the oocysts were nonviable.

Olson et al. (1999) compared oocyst survival in different media at temperatures likely to occur in the natural environment. They examined survival in -4°, 4°, and 25° C in distilled water, soil, autoclaved (sterilized) soil, and feces. Unlike *Giardia*, which died off quickly at low temperatures, *Cryptosporidium* oocyst survival was best at -4°C, with close to 50 percent of oocysts remaining viable for 12 weeks in all media except feces. Survival was lowest at 25°C, but oocysts were still viable at six weeks in all media. Survival rates were best in water and worst in feces. Viability was determined by dye exclusion tests.

Cordell and Addiss (1994) noted that oocyst survival decreases in extreme temperatures and arid conditions. Laboratory studies show that *Cryptosporidium* oocysts stored in airtight containers can remain viable for 8 to 9 months, and excystation seems to occur soon after exposure to air (Tzipori 1983). Robertson et al. (1992) reported that air drying an oocyst suspension at room temperature for 4 hours eliminated viability. Oocysts in fecal material are protected from desiccation, however, so their viability in the environment is prolonged (Rose et al. 1997).

Inactivation of oocysts is generally determined by assessing the viability or infectivity of the oocyst. Viability means the organism is alive, but with many methods, one cannot be absolutely sure this is the case. Viability can be estimated (with varying results, as described below) by testing an organism's capability to exhibit metabolic activity or respond to biochemical stimuli through *in vitro* excystation, infection of cell lines, changes in parasite morphology observed by light microscope, and uptake or exclusion of fluorescent dyes. Infectivity is the ability of an organism to complete its life cycle within a host and is the only way to be sure an organism is alive.

The correlation between viability and infectivity is important for assessing potential exposure. Campbell et al. (1992) and Neumann et al. (2000) demonstrated that the fluorescence intensity from nucleic acid binding dyes correlates with viability and infectivity for untreated oocysts, although some controversy surrounds the use of fluorescent dyes to assess viability (Robertson et al. 1998). The controversy arises because some oocysts that are not shown by such assays to be viable may appear viable after exposure to a trigger, such as acid. Oocysts that are shown to be viable through other methods, such as excystation or *in vitro* cultured cell infectivity, may not necessarily be able to infect live animals (Neumann et al. 2000). In addition, correlation may be affected by chemical treatment. Bukhari et al. (2000) found slight inactivation of *C. parvum* after treatment with ozone, as determined by dye permeability assays and excystation. But they found much higher inactivation when they assessed the same ozonated *C. parvum* via mouse infectivity.

Many studies describe inactivation in terms of "log inactivation" or "log reduction." Each unit of log removal is a factor of 10. Therefore, "a disinfectant achieved 1 log inactivation" means 10 percent of the original number of viable oocysts were present after treatment. In other words, 90 percent of the oocysts have been inactivated. Two log inactivation means that the viable oocyst concentration is 1 percent of the concentration present before treatment, or that 99 percent of oocysts have been inactivated. Determining the amount of inactivation is difficult due to the problems with assessing viability.

Exhibit 2.3 shows the results of several studies of physical *Cryptosporidium* inactivation by dessication and exposure to extreme temperatures. The studies in Exhibit 2.3 are not strictly comparable, because the researchers did not assess inactivation in the same manner—they began with different concentrations of oocysts and may have processed the samples differently. The results are thus presented as a brief summary of the range of conditions found to inactivate *Cryptosporidium.*

Exhibit 2.3 Physical Inactivation of *Cryptosporidium* Oocysts

Agent	Conditions	Results	Test	Reference
Note: Ex = Excystation, I = Infectious, NI = Noninfectious, DEP = Dielectrophoresis. *In vivo* testing performed in mice.				
Heat	121°C, 10 minutes	Protein changes	DEP	Archer et al. 1993
Heat	50–55°C, 5 minutes	NI	*In vivo*	Blewett 1989
Heat	45°C, 20 minutes 60°C, 6 minutes	NI NI	*In vivo* *In vivo*	Anderson 1985
Heat	59.7°C, 5 minutes 64.2°C, 5 minutes 67.5°C, 1 minute 72.4°C, 1 minute	I NI I NI	*In vivo* *In vivo* *In vivo* *In vivo*	Fayer 1994
Heat	71.7°C, 5-15 seconds	NI	*In vivo*	Harp et al. 1996
Freezing	-196°C, 10 minutes -20°C, 3 days	NI NI	*In vivo* *In vivo*	Sherwood et al. 1982
Freezing	-70°C, 1 hour -20°C, 8 hours; 1 day -15°C, 24 hours; 1 week -10°C, 1 week	NI I; NI I; NI I	*In vivo* *In vivo* *In vivo* *In vivo*	Fayer and Nerad 1996
Freezing	Liquid nitrogen -22°C, ≤32 days	100% reduced 98% reduced	Ex/dyes Ex/dyes	Robertson et al. 1992
Drying	Air dried, 2 hours Air dried, 4 hours	97% reduced 100% reduced	Ex/dyes Ex/dyes	Robertson et al. 1992
Drying	Air dried in feces, 1–4 days	NI	*In vivo*	Anderson 1986

Source: Adapted from Fayer et al. 1997.

Temperature is a key factor affecting survival (Rose 1997). Although most of the temperatures shown in Exhibit 2.3 are above what *Cryptosporidium* would be exposed to in nature, they indicate the extent to which oocysts can survive adverse conditions. For instance, Fayer and Nerad (1996), testing oocysts frozen at -10°, -15°, -20°, and -70°C for 1 to 168 hours, demonstrated that oocysts of *C. parvum* in water can be both viable and infective after freezing, although survival rates decrease with decreasing temperature. Blewett (1989) observed a 92-percent reduction in oocyst viability (assessed by excystation) following exposure to a temperature of 55°C for 5 minutes. Harp et al. (1996) tested the effect of pasteurization on infectivity of oocysts in water and milk and confirmed that exposure to temperatures of 71.7°C for 5 to 15 seconds is sufficient to destroy the infectivity of *C. parvum* oocysts in water and milk.

2.1.6 Response To Disinfection

Most *Cryptosporidium* oocysts are removed through the filtration of surface water, along with coagulation, flocculation, and sedimentation. For the remaining oocysts, disinfection becomes important for inactivation. Disinfectants commonly used to treat drinking water, however, are not very effective for inactivating oocysts. Exhibit 2.4 summarizes the results of several studies regarding the effectiveness of disinfectants, including ultraviolet (UV) radiation, on *Cryptosporidium* oocyst inactivation. In general, chlorine/hypochlorite required the largest concentrations and longest contact times. UV radiation is now feasible as a disinfectant; smaller doses are needed for inactivation than initially thought. The studies in Exhibit 2.4 may not be directly comparable, because they did not assess inactivation in the same way. Also, each began with different concentrations of oocysts and may have processed their samples differently. The results are provided to summarize the effectiveness of different levels of disinfectant under various water conditions. More detail on selected studies follows Exhibit 2.4.

Exhibit 2.4 Disinfectants Tested Against *Cryptosporidium* Oocysts

Disinfectants	Conditions	Results	Test	Reference
Note: Ex = Excystation, I = Infectious, NI = Noninfectious. NR=No marked reduction. *In vivo* testing performed in mice.				
Chlorine	2.5 mg/L, 30 min. 5 mg/L, 30 min.	NR	Ex	Quinn and Betts 1993
Chlorine	867 – 5118 mg/L, 24 hours	72.5% to 88.1% reduction	Ex	Ransome et al. 1993
Chlorine	16,000 mg/L, 12 hours 28,000 mg/L, 24 hours	90% reduction 100% reduction	Ex	Smith et al. 1990
Chlorine	80 ppm, 90 minutes	90% reduction	Ex	Korich et al. 1990
Chlorine followed by chloramine	2.5 – 7.5 mg/L Cl_2, 45 minutes; 4:1 NH_3 to Cl_2, 2 – 3 hours	7 – 75% reduction	*In vivo*	Oppenheimer et al. 1997
Hypochlorite	2.8%, 30 minutes, 25°C	89% reduction	Ex	Sundermann et al. 1987
Hypochlorite	1%, 30 minutes, 22°C 1%, 30 minutes, 37°C	55% reduction 69% reduction	Ex	Blewett 1989
Hypochlorite	3%, 18 hours	I	*In vivo*	Campbell et al. 1992
Hypochlorite	5.25%, 2 hours, 20°C	I	*In vivo*	Fayer 1995
Chlorine dioxide	0.007 mg/L, 16 minutes 0.22 mg/L, 30 minutes	97% reduction 94.3% reduction	Ex *In vivo*	Peeters et al. 1989
Chlorine dioxide	1.3 mg/L, 1 hour	92.7% reduction	Ex	Korich et al. 1990

Exhibit 2.4 Disinfectants Tested Against *Cryptosporidium* Oocysts

Disinfectants	Conditions	Results	Test	Reference
Chlorine dioxide	4.03 mg/L, 15 minutes	96% reduction	Ex	Ransome et al. 1993
Chlorine dioxide	1.52 mg/L, 10-60 min, pH 6 1.52 mg/L, 10-60 min, pH 8	50–80% reduced 80–95% reduced	Ex, cell culture	LeChevallier et al. 1996
Chlorine dioxide	1.4 mg/L, 120 min., 1°C 1.2 mg/L, 120 min., 22°C 4.7 mg/L, 30 min., 1°C 4.5 mg/L, 30 min., 22°C	68.4% reduction 99.2% reduction 74.8% reduction 98.4% reduction	*In vivo*	Li et al. 1998
Chloramine	3%, 24 hours	I	*In vivo*	Pavlasek 1984
Monochloramine	80 ppm, 90 minutes	90% reduction	Ex	Korich et al. 1990
Monochloramine	0.066 mg/L, 48 hours 3.76 mg/L, 24 hours	76.8% reduction 80.5% reduction	Ex	Ransome et al. 1993
Ozone	0.3 – 2.3 mg/L, 5-15 minutes, 3-22°C	21-99.998% reduction	Ex *In vivo*	Finch et al. 1993a
Ozone	0.4–2.4 mg/L, 22°C, 3-30 minutes, pH 6-8	68.4–99.997% reduction	*In vivo*	Gyürék et al. 1999
Ozone	0.36 – 2.2 mg/L, up to 42 minutes, 20°C	2–100% reduction	Ex	Rennecker et al. 1999
Ozone	0.3–0.4 mg/L, 2 minutes	NR–90+%	Dye, ex, *in vivo*	Bukhari et al. 2000
Ozone followed by chloramine	0.8 – 4 mg/L O_3 12-30 minutes; 0.5 – 2.5 mg/L chloramine, 30 – 120 minutes	49.9% to 96% inactivation	*In vivo*	Oppenheimer et al. 1997
Mixed-oxidant solution	5 mg/L, 4 or 24 hours	99.9% inactivation	*In vivo*	Venczel et al. 1997
UV	80 mJ/cm^2 120 mJ/cm^2	90% reduced 99% reduced	Ex Ex	Ransome et al. 1993
UV	8748 mJ/cm^2	100% reduced	Ex/dyes	Campbell et al. 1995
UV	41–246 mJ/cm^2 19–159 mJ/cm^2	NI, 10.7–98.9% reduced NI, I, 24.7–95.1% reduced	Ex/dyes /*In vivo*	Bukhari et al. 1999

Exhibit 2.4 Disinfectants Tested Against *Cryptosporidium* Oocysts

Disinfectants	Conditions	Results	Test	Reference
UV	3–9 mJ/cm^2, medium pressure UV 11–20 mJ/cm^2 medium pressure 3–9 mJ/cm^2, low pressure 16–33 mJ/cm^2, low pressure	99.96– >99.99% inactivation >99.998% inactivation 99.9–99.97% inactivation 99.995–>99.999% inactivation	*In vivo*	Clancy et al. 2000
UV	3 mJ/cm^2	99.8% inactivation	*In vivo*	Shin et al. 2000a
Pulsed UV UV	0.25–9.5 mJ/cm^2 0.5–6 mJ/cm^2 medium pressure	0–99.95% reduced 0-99.95% reduced	Cell culture--PCR Same	Mofidi et al. 1999
Pulsed light	1 mJ/cm^2	100% reduced	*In vivo*	Dunn et al. 1995

Source: Adapted from Fayer et al. 1997

Finch et al. (1997) evaluated the effects of several disinfection methods on the inactivation of *Cryptosporidium*, noting that chlorine and monochloramine alone at practical plant levels are not effective. Current (1986) also concluded in his review of *Cryptosporidium* biology that chlorine and sodium hypochlorite (chlorine bleach) in typically used concentrations are poor disinfectants for *Cryptosporidium*, although full-strength bleach (5.25 percent sodium hypochlorite) destroyed oocyst infectivity to mice after 10 minutes.

Korich et al. (1990) exposed *Cryptosporidium parvum* to ozone, chlorine dioxide, chlorine, and monochloramine and tested viability using a comparison of excystation and mouse infectivity. Ozone and chlorine dioxide inactivated oocysts more effectively than did chlorine and monochloramine. Ozone at 1 mg/L for 5 minutes produced greater than 90 percent inactivation. Chlorine dioxide (1.3 mg/L) inactivated 90 percent after 1 hour, and chlorine (80 mg/L) and monochloramine (80 mg/L) required 90 minutes for 90-percent inactivation.

Oppenheimer et al. (1997) studied the relationship between *Cryptosporidium* oocyst inactivation and CT, the product of disinfectant residual (C) and disinfectant contact time (T), for disinfectants applied to a wide range of source waters. The disinfection practices investigated included ozonation, addition of chlorine followed by chlorine, addition of chlorine followed by chloramines, and addition of ozone followed by chloramines. The data suggest no appreciable biocidal effect, either for chlorine alone or for chlorine followed by chlorine. No more than 0.5 log inactivation was achieved using chlorine followed by chloramines, even at impracticably high CT levels. However, chloramination immediately following ozonation provided some enhancement of inactivation.

Recent research on chlorine dioxide inactivation of *Cryptosporidium* has demonstrated moderate effectiveness at feasible concentrations (considering disinfection byproducts) and reasonable contact times. LeChevallier et al. (1996) showed that chlorine dioxide was moderately successful at disinfecting *Cryptosporidium*. They achieved up to about 1.3 log of disinfection at pH 6 when the treatment was carried out at 20°C, and less than 1 log if disinfection occurred at pH 8 or at 10°C. Li et al. (1998) investigated temperature effects on *Cryptosporidium* inactivation at pH 6 and found that log inactivation increased three- to four-fold as temperature was raised from 1 to 22°C.

Of all the disinfectants used in water treatment plants, ozone is the most effective in inactivating *Cryptosporidium* in terms of the short contact time and lower residual concentration needed to achieve a significant level of inactivation. Disinfection effectiveness, however, decreases with increasing pH. Finch et al. (1997) reported a 1.5 log inactivation at 22°C and pH 6 and 0.5 log at pH 8. Gyürék et al. (1999) developed models for predicting inactivation of oocysts up to 3 logs, concluding that ozone was an effective disinfectant for *Cryptosporidium*, but that results would vary with raw water source. One problem with ozone disinfection is that at low levels the observed inactivation varies depending on the method used to determine viability. Dye assays showed little difference between viability of ozonated oocysts and controls at low doses and contact times, while mice infectivity tests showed a significant increase in inactivation (Bukhari et al. 2000).

Venczel et al. (1997) evaluated an electrolytically produced mixed-oxidant solution (containing free chlorine, chlorine dioxide, ozone, hydrogen peroxide, and other short-lived oxidants) for inactivating *Cryptosporidium parvum* oocysts. The disinfection efficacy of the mixed-oxidant solution was compared with that of free chlorine. The mixed-oxidant solution was considerably more effective, with a 5 mg/L-dose of mixed oxidants producing a greater than 3 log inactivation of *C. parvum* oocysts (Iowa strain) in 4 hours. The same dose of free chlorine produced no measurable inactivation in 4 or 24 hours.

UV radiation works by damaging the DNA, RNA, or both of an organism, preventing it from reproducing. Four types of UV treatment have been developed to inactivate microorganisms. In the first, low-pressure radiation, constant UV radiation of a particular wavelength (254 nanometers (nm)) is applied to water passing through a tube. In advanced UV treatment, similar UV lamps are used, but the process is combined with a filter that temporarily traps organisms, lengthening the time they are exposed to UV radiation. Medium-pressure radiation uses lamps that emit radiation of wavelengths between 200 and 300 nm. In pulsed UV treatment, water flows through a chamber filled with lamps that provide flashes of high-intensity radiation of multiple wavelengths, including visible wavelengths.

Turbidity, color, and dissolved salts all prevent UV energy from penetrating the water and therefore affect the amount of UV radiation required to disinfect a volume of water. UV light is generally not used for disinfecting turbid water because of interference; it is used to disinfect ground water, however, which is not prone to turbid conditions. Unlike chlorine, UV light has no residual disinfecting capability and cannot prevent recontamination (USEPA 1999b), but it causes minimal disinfection byproduct (DBP) formation.

Several independent data sets now exist for inactivation studies with *Cryptosporidium parvum* using either animal infectivity or cell culture infectivity endpoints (mouse—Clancy et al. 2000; Bukhari et al. 1999; and cell culture — Shin et al. 1999; Shin et al. 2000a; Mofidi et al. 1999). All the data conservatively suggest that a pulsed UV dose of 20 millijoules per square centimeter (mJ/cm^2) will result in at least a 3 log inactivation of *Cryptosporidium parvum*. In fact, in many studies, a dose of 10 mJ/cm^2 was so effective the assay detection limit was reached.

Bukhari et al. (1999) reported that medium-pressure UV was effective at inactivating *C. parvum* up to 3.9 logs at a dose of 19 mJ/cm^2 in a 200-gallon per minute (gpm) pilot system, using a mouse infectivity assay. This medium-pressure UV work demonstrated that a continuous wave UV source could perform as well as a pulsing source.

UV irradiation from a monochromatic low-pressure UV source for inactivation of *C. parvum* was subsequently investigated by Shin et al. (1999, 2000b), who demonstrated that a dose as low as 4 mJ/cm^2 resulted in a greater than 3 log inactivation. Thus, monochromatic UV appeared to be as efficient at inactivation of *C. parvum* as medium-pressure and pulsed UV sources.

Collectively, these studies—using low-pressure (monochromatic), medium-pressure (polychromatic) and pulsed (polychromatic) UV systems on the bench and pilot scale in a variety of water matrices—clearly indicate that UV light is effective in inactivating *C. parvum*. *Cryptosporidium* is perhaps the pathogen most studied for UV effectiveness, and studies continue.

2.2 *Giardia* and *Giardia lamblia*

Giardia is found worldwide, and giardiasis is one of the most prevalent intestinal diseases of humans (Meyer 1990; Craun 1986). *Giardia* infections are also common among domestic animals, such as cats, dogs, birds, horses, rabbits, sheep, cattle, and goats, as well as other mammals and birds (Meloni et al. 1995). Meloni et al. documented evidence of zoonotic transmission (transmission of disease from animals to humans) in their study of genetic variation of *Giardia*, based on the fact that the same genotypes were detected in humans and animals.

Members of the genus *Giardia* are flagellated, single-celled, binucleate protozoa that exist as parasites in the intestinal tract of virtually every class of vertebrates. These protozoa have a two-stage life cycle, the trophozoite (vegetative form) and the cyst (dormant, resistant form). Trophozoites of *Giardia lamblia* (synonyms: *G. duodenalis* and *G. intestinalis*) inhabit the upper small intestine of the vertebrate host (Filice 1952). The trophozoites are 9 to 21 µm long and 5 to 15 µm wide, and cysts are 10 to 15 µm long and 7 to 10 µm wide (Daly 1983; USEPA 1998e).

2.2.1 Description of the Species

Host specificity and morphological characteristics have been used to distinguish species of *Giardia* (Meloni et al. 1995). Both approaches, however, have limitations—the same *Giardia* species may be found in different host species, and *Giardia* that appear identical may be different genetically. Additional research to provide a taxonomic interpretation of genetic variation is currently underway. Thompson and Lymbery (1996) examined genetic variability in *Giardia* and *Cryptosporidium*. They noted the lack of understanding of within-host interactions among genetically different parasites, both within the same species of parasite and among different species of the same genus.

Sil et al. (1998) cloned and characterized the ribosomal RNA genes from an Indian isolate of *G. lamblia* to develop a method to differentiate *Giardia* from other enteric pathogens. Of the gene regions studied, all were found to be genus-specific and not strain-specific.

2.2.2 Strains

Studies of *Giardia* have identified genetic variations both among and within species. Hopkins et al. (1997) compared small subunit ribosomal RNA sequences from 13 human and 9 dog isolates of *Giardia duodenalis*, which revealed 4 distinct genetic groups. Groups 1 and 2 contained all of the human isolates, whereas groups 3 and 4 consisted entirely of *Giardia* samples recovered from dogs. A genetic basis for the differences observed between the groups was supported by sequence analysis of nine *in vitro* cultured isolates that were placed into the same genetic groups established by enzyme electrophoresis.

G. lamblia strains have been separated into two groups: group A, also called Polish, and group B, also called Belgian (Paintlia et al. 1999). Ey et al. (1998) studied variant surface protein genes of genetic groups I and II within group A. They identified a third type of gene found exclusively in group II isolates.

2.2.3 Fate and Transport

The factors that influence the transport of *Giardia* in the environment are the same as those affecting *Cryptosporidium* (see section 2.1.4): adsorption, filtration, and sedimentation. The other main feature affecting transport of *Giardia*, especially in soil and aquifer materials, is its size. *Giardia* cysts are 10 to 15 µm in length and 7 to 10 µm in width, larger than the 4-to 6-µm diameter *Cryptosporidium* oocyst. The cyst's larger size potentially restricts movement through some soils and aquifer materials, except in the presence of natural pathways such as macropores, fractures, and conduits. As with *Cryptosporidium*, *Giardia* cysts in feces deposited on soil surfaces are readily transported by surface runoff during rainfall into surface water and, in some hydrogeologic settings, to ground water.

2.2.4 Transmission of Giardiasis

The most common method of *Giardia* transmission in the United States is through the fecal-oral route, particularly in day care centers. Overturf (1994) reviewed studies of giardiasis in daycare centers and reported that the occurrence of *Giardia* infections among children in daycare ranges from 17 to 90 percent. A study in Wisconsin reported the rates of *Giardia* infection among children were 17 to 47 percent, with rates among tested staff and household contacts of 9 to 35 percent and 5 to 18 percent, respectively (Overturf 1994). Because *Cryptosporidium* and *Giardia* are distributed worldwide, data from daycare centers in Salamanca, Spain, are applicable to the question of human transmission. Rodriguez-Hernandez et al. (1996) studied 170 children younger than 4-years-old who regularly attended daycare centers. *Giardia* was the most frequently identified parasite (found in 25.3 percent, or 43 children); 10 percent of children (17) had *Cryptosporidium* parasites. Children infected at daycare centers may transmit giardiasis to other family members.

Giardia can also be transmitted within hospitals or other institutions. Transmission has also been documented through sexual contact (USEPA 1998e). *Giardia* can be transmitted through drinking water or recreational water. Between 1965 and 1996, 108 outbreaks occurred in public water systems; 15 outbreaks occurred in association with recreational water use (USEPA 1998e).

2.2.5 Health Effects

Giardia cysts enter a host through ingestion. They excyst in the stomach, subdivide, and attach to the intestinal wall. The incubation period can last from 2 to 12 days, after which *Giardia* can lead to weight loss and dehydration and may cause any of the following symptoms: diarrhea, abdominal cramps, headaches, nausea, vomiting, and low-grade fever. For the average healthy adult, the symptoms last approximately 2 weeks and, if untreated, can become chronic or cause intermittent diarrhea. For immunocompromised individuals, the disease can last for months.

In 1997, 25,389 cases of giardiasis were reported by states to the Centers for Disease Control (CDC) nationwide (Furness et al. 2000). This number is thought to underestimate the actual number of cases, since most cases of diarrhea are not reported. Furness et al. estimate that anywhere from 500,000 to 2.5 million giardiasis cases occur annually. However, it is unlikely that these cases are associated with drinking water contamination. Although states do not report the source of infection to CDC, the authors hypothesize that most cases are due to recreational water use, since most cases occur during the summer.

2.2.6 Persistence

Environmental conditions contributing to the persistence of *Giardia* cysts are similar to those described for *Cryptosporidium* in section 2.1.5. Surface water sources are more likely to be contaminated with *Giardia* than are ground water sources (Craun 1990). Marginally treated or untreated surface water supplies pose a high risk of transmitting *Giardia* because cysts can survive for several months in cold water, and relatively few *Giardia* cysts are required for an infective dose (Craun 1990). Exhibit 2.5 summarizes some representative examples of the effects of environmental conditions on the persistence and viability of *Giardia* cysts. Some of the conclusions of these studies are described below.

The occurrence of *Giardia* cysts in water is well documented (Madore et al. 1987; Craun 1990; Hancock et al. 1998b; LeChevallier and Norton 1995), as is persistence. *Giardia* cysts survive relatively long periods in water, particularly at temperatures below 20°C; above 20°C, cyst inactivation is rather rapid. Evidence suggests that *Giardia* cysts in fresh water survive best at 4 to 8°C (Wickramanayake 1985; Jakubowski 1990). Kayed and Rose (1987) reported survival of protozoan cysts in water in the laboratory for more than 140 days. Jarroll et al. (1984) reported that cysts did not survive when *Giardia* were exposed for 24 hours to artificial sea water at 4°C or to air-drying at 4°C or 21°C. Johnson et al. (1997) studied the survival of *Giardia*, *Cryptosporidium*, and other enteric pathogens in marine waters. Using excystation as the viability assay, they demonstrated a 3 log reduction of cysts in marine waters after 3 hours in direct sunlight; but cysts in the dark required 77 hours to show a 3 log reduction (Johnson et al. 1997).

Exhibit 2.5 Effects of Environmental Conditions on the Viability of *Giardia* Cysts

Environmental Conditions	Conditions	Effect	Viability Assay	Reference
Note: Ex = Excystation, I = Infectious, NI = Noninfectious, DEP = Dielectrophoresis. *In vivo* testing performed in mice.				
Liquefied feces	Stored at 4°C	Infective for 1 yr	*In vivo*, counterimmuno-electrophoresis of feces for antigen	Craft 1982
Distilled water Tap water	Stored at 37°C Stored at 8°C 100°C -13°C	Viable ≤4 days Viable 77 days 100% reduction >99% reduction after 14 days	Ex, dye Same Same Same	Bingham et al. 1979
Distilled water Environmental waters (lake and river) Minneapolis tap water	*G. muris* cysts in fecal pellets stored in water at 5–7°C Same stored in water at 5–7°C Same stored in tap water at 20–28 °C	100% viability at 7 days; 17–100% at 28 days; 0% at 56 days Viable 2–3 months Loss of viability within 3 days; 0–17% at 7 days; 0% at 14 days	Dye, *in vivo*, and cyst morphology by microscopy Same Same	deRegnier et al.,1989
Artificial sea water	24 hr at 4°C	No cysts survived	Ex	Jarroll et al. 1984
Distilled water	1–20+ days at -6°C 5°C 20°C 37°C	 99.2– >99.8% reduced 2–9% reduced 6– >99.8% reduced 92– >99.8% reduced	Ex	Wickramana-yake et al. 1985
Distilled water Soil Autoclaved Soil Feces	-4°C 4°C 25°C Same Same Same	Viable <1 wk. 80% reduced after 11 wks. (Ex), noninfective after 11 wks. Viable 4 wks. (Ex), noninfective after 2 wks. Viable/infective 1-9 wks Viable/infective 1-9 wks Viable/infective 1-9 wks	Dye, *in vivo*	Olson et al. 1999

Environmental Conditions	Conditions	Effect	Viability Assay	Reference
Marine waters	3 hr in sunlight 77 hr in the dark	99.9% reduction 99.9% reduction	Ex Ex	Johnson et al. 1997
Air	Cysts exposed to air drying at 4°C or 21°C for 24 hr	No cysts survived	Ex	Jarroll et al. 1984

2.2.7 Response to Disinfection

Giardia cysts are not as resistant to disinfection as *Cryptosporidium* oocysts; thus, treatment designed to inactivate oocysts will effectively inactivate *Giardia* cysts. Korich et al. (1990) reported that *C. parvum* oocysts are 30 times more resistant to ozone and 14 times more resistant to chlorine dioxide than *Giardia* cysts exposed to the same disinfectant under the same conditions. Data collected by Owens et al. (1994) indicated that *Cryptosporidium parvum* was 10 times more resistant to ozone than *G. muris* protozoa. Oppenheimer et al. (1997) noted that the temperature-adjusted simulated CT values for ozone inactivation of oocysts were 5 to 20 times the CT values for *Giardia* inactivation listed in the *Surface Water Treatment Rule Guidance Manual*.

Jarroll (1988) and Jakubowski (1990) reviewed *Giardia* cyst disinfection. Some of the studies they reviewed and data from additional studies are summarized in Exhibit 2.6. These studies may not be comparable because they did not all use the same methods for assessing inactivation. Each also began with different concentrations of cysts and may have processed samples differently. The results are presented to provide a brief summary of the disinfectant levels found to inactivate *Giardia*.

Many of these studies are further described in the text following Exhibit 2.6. Some of the studies described in the text are *not* listed in Exhibit 2.6 because insufficient data on the experimental conditions or results were available.

Exhibit 2.6 Disinfectants Tested Against *Giardia* Cysts

Disinfectant	Conditions	Results	Viability Assay	Reference
Note: Ex = Excystation, I = Infectious, NI = Noninfectious, DEP = Dielectrophoresis. *In vivo* testing performed in mice.				
Chlorine	1 mg/L at 5°C for 10 minutes 1.5 mg/L at 25°C for 10 minutes 2 mg/L at 5°C for 60 minutes 2.5 mg/L at 15°C for 10 minutes	35% (at pH 6) to 56% (at pH 8) cyst (*G. lamblia*) survival No cyst survival No cyst survival No cyst survival at pH 6, but 1.8% survival at pH 7	Ex	Jarroll et al. 1981
Chlorine	0.3 – 2.5 mg/L at 0.5°C to 5.0°C at pH 6 to pH 8	Mean CT to produce 99.9% to 99.99% inactivation: 185 to 280 To produce >99.99% inactivation: 220 to 290	*In vivo*	Hibler et al. 1987
Chlorine	0.3 mg/L, 6.5 hours, pH 9 3.6 mg/L, 3 hours, pH 9 16.3 mg/L, 1 hour, pH 9	99% inactivation 99% inactivation 99% inactivation	Ex	Rubin et al. 1989
Ozone	0.15 mg ozone/L for 0.97 minute at 25°C, or 0.48 mg ozone/L for 0.95 minutes at 5°C 0.18 mg ozone/L for 1.3 minutes at 25°C, or 0.70 mg ozone/L for 2.5 minutes at 5°C	99% inactivation of *G. lamblia* cysts 99% inactivation of *G. muris* cysts	Ex Ex	Wickramanayake et al. 1985
Ozone	0.02 – 1.3 mg/L, 0.25-5 minutes, 22°C	1.4%-99.996% inactivation of *G. muris* cysts	Dye, ex, *in vivo*	Labatiuk et al. 1991

Disinfectant	Conditions	Results	Viability Assay	Reference
Ozone	0.26–0.82 mg/L for 5 min., pH 6.85 1.10–2.52 mg/L for 2–13 min., pH 6.7	99.92– >99.9992% inactivation of *G. muris* cysts 99.5–99.996% inactivation of *G. lamblia*	*In vivo* *In vivo*	Finch et al. 1993b
UV	63 mJ/cm^2	Less than 90% reduction	Modified ex	Rice and Hoff 1981
UV	Up to 85 mJ/cm^2 Up to 95 mJ/cm^2	94.99– 99.87% reduction of *G. muris* 0–68.3% reduction of *G. muris*	*In vivo* Ex	Finch and Belosevic 1999
UV	10–40 mJ/cm^2	99–99.9% reduction	*In vivo*	Campbell 2000
UV	2 mJ/cm^2	>99.99% reduction	*In vivo*	Shin et al. 2000a

Chemical disinfection with chlorine is dependent on temperature and pH. Hibler et al. (1987) determined the chlorine CT required to inactivate *Giardia* cysts at 0.5°C to 5.0°C. Reaction rates decrease with temperature, which contributes to higher CT values necessary to inactivate cysts at lower temperatures. Loss of biocidal activity occurs at pH above 7.5. CT values were determined for final chlorine concentrations of 0.3 to 2.5 mg/L. The data were inconsistent for concentrations above 2.5 mg/L. The authors did not recommend using concentrations above 2.5 mg/L to compensate for a shorter contact time, because the cyst wall may be able to resist extreme adverse environmental conditions for a short period of time.

Ozone proved to be much more efficient at inactivating *Giardia* cysts than chlorine, based on a CT comparison with results from Jarroll et al. (1981) by Wickramanayake et al. (1985). Increasing temperature from 5°C to 25°C decreased the CT requirements of ozone necessary to achieve a 99-percent inactivation; this temperature effect was similar to findings for chlorine by Hibler et al. (1987). Labatiuk et al. (1991) and Owens et al. (1994) reported that ozone effectively inactivates *Giardia muris* cysts. *G. muris* and *G. lamblia* did not exhibit significantly different responses to ozone in a comparative study by Finch et al. (1993b).

Najm et al. (1998) evaluated the effect of the turbidity level on ozone inactivation of *Giardia muris* cysts in natural water samples. They observed that cyst inactivation was higher in a low-turbidity sample (1.3 nephelometric turbidity units (NTU)) than in samples having turbidity levels of 11 NTU and 19 NTU (Najm et al. 1998).

Limited studies have been completed on the effectiveness of UV light in inactivating *Giardia* cysts. As with *Cryptosporidium*, initial studies of the UV inactivation of *Giardia* were done using viability assays (excystation); these studies probably overestimated the dose required to inactivate

Giardia. Rice and Hoff (1981) reported UV doses of 63 mJ/cm^2 were necessary for less than 1 log inactivation, based on excystation viability assays. Although these doses are not extremely high, they were considered impractical at the time because UV equipment available at the time was designed for smaller doses. In addition, *Giardia* could be controlled effectively with moderate doses of chlorine. *In vivo* and cell culture infectivity assays are being used in current studies to more accurately determine UV doses necessary for inactivation (Linden 2000); these doses are several times smaller than those in earlier studies. Using medium pressure UV doses of less than 25 mJ/cm^2, Finch and Belosevic (1999) achieved 2 to 3 log inactivation of *G. muris* measured via mouse infectivity; inactivation did not increase with increased dose, even at 75 mJ/cm^2. This inactivation was similar to that achieved for *Cryptosporidium*. Shin et al. (2000a) achieved much better results with *G. lamblia* and low pressure UV. They attained >4 logs inactivation of *Giardia* with a dose of only 2 mJ/cm^2. Campbell (2000) reached an inactivation of 3 logs. He stated that this was a conservative estimate because shielding and variability in resistance could decrease inactivation.

2.3 Viruses

Waterborne pathogenic enteric viruses are among the organisms that continue to be regulated under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). Enteric viruses, which are shed via the intestinal tract and may be ingested through drinking contaminated water, have been shown to cause a variety of diseases, including poliomyelitis, heart disease, encephalitis, aseptic meningitis, hepatitis, and gastroenteritis. Little information is available about viruses' association with waterborne disease because viruses are not detected frequently in drinking water during waterborne disease outbreaks (a list of outbreaks is shown in Appendix A). The viral pathogens of concern include adenoviruses, astroviruses, caliciviruses, hepatoviruses, enteroviruses, and rotaviruses; each is discussed in section 2.3.2 below. Because viruses are so diverse, each type is affected differently by disinfection and effectiveness of disinfection will not be described in as much detail as in the previous sections.

EPA's Office of Science and Technology (OST) recently prepared final drafts of two drinking water criteria documents for viruses: *Drinking Water Criteria Document for Viruses: An Addendum* (USEPA 1999b) and *Drinking Water Criteria Document for Enteroviruses and Hepatitis A: An Addendum* (USEPA 1999c). They update EPA's draft drinking water criteria document for enteric viruses, which was compiled in 1985 (USEPA 1985). These two documents present available information regarding virus properties, occurrence, health effects, detection, and water treatment technologies.

2.3.1 Health Effects

Humans and a variety of animals throughout the world may be infected by adenoviruses. Adenoviruses are pathogenic only within the species in which they originate (USEPA 1999b). Only a few adenoviruses are enteric. Adenoviruses, astroviruses, and caliciviruses all can cause gastroenteritis.

Enteroviruses are associated with a variety of diseases, including poliomyelitis, aseptic meningitis, heart disease, respiratory disease, gastroenteritis, and diabetes mellitus (USEPA 1999c). Enteroviruses also account for approximately 10 to 20 percent of encephalitis cases with proven viral etiology. Infants and young children experience the highest rates of serious enteroviral disease, and males are infected at a 50 percent higher rate than females (Modlin 1997; USEPA 1999c).

Hepatoviruses cause hepatitis; of which types A and E can be transmitted through drinking water.

Rotaviruses are the most significant cause of severe gastroenteritis in young children and infants. Although these viruses cause gastroenteritis in young children, non-Group A rotaviruses also can cause outbreaks in older children and adults. Rotaviruses consistently outrank other known etiologic agents of severe diarrhea in scores of illnesses and hospitalizations reported annually (USEPA 1999b). Fecal-oral transmission is the primary mode of transmission for rotaviruses.

2.3.2 Viral Pathogens

2.3.2.1 Adenoviruses

Some enteric adenoviruses are fastidious, or difficult to grow in cell cultures; they are thus more difficult to detect, and their role as an agent of gastroenteritis may be underestimated. Adenoviruses most commonly cause respiratory illness through routes other than drinking water. These viruses can also cause conjunctivitis, cystitis, and rashes. However, Ad40 and Ad41, the two serotypes of "enteric" adenoviruses, occur in large numbers in stool samples of individuals suffering from gastroenteritis (USEPA 1999b, CDC 2001a). Common symptoms of gastroenteritis include watery diarrhea, vomiting, headaches, fever, and abdominal cramps (CDC 1998b). Enteric adenoviruses are thought to be a significant cause of childhood diarrhea (USEPA 1999b). Currently, however, no direct evidence associating "enteric" adenoviruses with transmission of disease via drinking water exists, because no studies of adenoviruses in drinking water have been conducted (USEPA 1999b).

2.3.2.2 Astroviruses

Seven serotypes of human astrovirus have been identified. Extensive seroepidemiological studies in the United Kingdom between 1975 and 1987 revealed that serotype 1 astrovirus accounted for 65 to 72 percent of the cases of astrovirus-induced gastroenteritis, while each of serotypes 2 through 5 accounted for 6 to 8 percent of the cases, respectively (Kurtz and Lee 1987; USEPA 1999b). This single-stranded RNA virus can cause gastroenteritis, predominantly in children under the age of 5. Transmission occurs mainly through fecal-oral contact. Water has been suggested as another possible route of transmission, but there has been no strong evidence to support this assertion (AWWARF 1997).

2.3.2.3 Caliciviruses

Norwalk viruses are classified in the family Caliciviridae and the genus Calicivirus (ICTV 1995). The Norwalk virus is the prototype strain of a group of fastidious 26- to 35-nm non-enveloped, single-stranded RNA viruses associated with outbreaks of gastroenteritis. The Norwalk-like viruses were named after the location of the 1968 Norwalk, Ohio, outbreak from which they were first isolated (Kapikian et al. 1996; USEPA 1999b).

Since the original report of Norwalk virus as the cause of an acute gastrointestinal illness, several morphologically similar agents have been detected and shown to be associated with gastroenteritis. These viruses, named after the locations where they were first found, include the Hawaii, Snow Mountain, and Taunton agents, and have been designated as small round structured viruses (SRSV).

Human caliciviruses may fall into a range of antigenic types, which have been associated with outbreaks in all age groups in North America, Australia, Asia, Africa, and Europe (Matson et al. 1989). The more prevalent antigenic types appear to be those that infect primarily infants and young children, such as HCV (Sapporo), although strains that produce symptomatic infections in adults have been identified (Matson et al. 1989; USEPA 1999b). Studies on the environmental occurrence and susceptibility to treatment of human caliciviruses have been hindered by the inability to grow them in cell culture.

2.3.2.4 Hepatoviruses

Hepatitis A virus causes liver disease (CDC 2000b). Symptoms include jaundice, fatigue, abdominal pain, loss of appetite, intermittent nausea, and diarrhea. These symptoms can last up to 6 months, but regularly last less than 2 months. The virus is transmitted orally, through food or water outbreaks, and through feces. Those at risk are international travelers, persons living in regions of endemic hepatitis A, such as American Indian reservations or Alaska Native villages, and people in frequent contact with an infected person, either sexually or through another form of contact. During outbreaks, the most common groups at risk are people who frequent day care centers, homosexually active men, and intravenous drug users (CDC 2000a). Cases of hepatitis E, whose symptoms include abdominal pain, dark urine, fever, enlargement of the liver, jaundice, and vomiting (CDC 2001b), are usually transmitted through fecally contaminated food and water (CDC 2001c). Hepatitis E cases have been identified in the United States among people with no known risk factors, and serology data suggest that hepatitis E is endemic to the United States (Karetnyi et al. 1999).

2.3.2.5 Enteroviruses

Enteroviruses include the polioviruses, coxsackieviruses, and echoviruses; they are the second most common viral pathogen known to infect humans. Although adults are less likely to become infected, everyone is at risk. Infection can occur through contact with a contaminated surface, secretions from an infected person, or through contact with feces. Most of the time, infected individuals are asymptomatic, but those who become ill express symptoms of a common cold or flu, such as mild upper respiratory illness. In rare cases, an infected individual can develop aseptic or viral meningitis, or even more serious illnesses that affect the heart, brain, or organs. Infections in the United States are most likely to occur during the summer and fall (CDC 1998a).

2.3.2.6 Rotaviruses

Rotavirus is a double-stranded RNA virus, common among children and infants, that can cause severe diarrhea and can result in hospitalization or possibly death due to dehydration. Symptoms include watery diarrhea and vomiting for 3 to 8 days with frequent abdominal pains and fever. The incubation period for the disease is about 2 days (CDC 2001d). Transmission occurs by contact with contaminated surfaces, ingestion of contaminated food or water, or fecal-oral contact, that is, oral contact with something that has been contaminated by stool. In temperate climates, the disease is most common in the winter and early spring (CDC 2001d).

2.3.3 Persistence

Human pathogenic viruses of concern that are common in wastewater include hepatitis A virus (HAV), hepatitis E virus (HEV), rotavirus, astrovirus, caliciviruses, enteric adenoviruses, and enteroviruses such as poliovirus, echovirus, and coxsackievirus. These viruses can contaminate source waters, along with recreational waters and waters used for growing shellfish. Limited research has been performed recently on virus persistence in the environment. Much of the research on persistence of viruses in the environment is based on studies of marine waters because of the potential for beach and shellfish contamination. However, some data on viruses in fresh water and ground water are available, and some research has been conducted on the ability of viruses to survive wastewater treatment. Data on persistence of viruses in the environment are presented below.

Sewage contains culturable virus concentrations between 5,000 and 28,000 plaque-forming units per liter (PFU/L), and plant effluents contain about 50 PFU/L (Metcalf et al. 1995). Sediments typically contain concentrations 2 to 4 logs higher than those in water. Because cell culture methods are not yet available for many enteric viruses, the actual number of viral pathogens present in a sample is probably much higher than these numbers reflect. The implications for source waters that receive wastewater treatment plant effluent are significant.

Alvarez et al. (2000) found that, in ground water environments, MS2 coliphages (a virus that infects coliform bacteria) kept at 27°C were inactivated in 8 days. When the temperature was reduced to 4°C, the viruses were reactivated. The same experiment performed with poliovirus resulted in no reactivation. Dahling and Safferman (1979) studied survival of enteric viruses in an Alaskan river and found that they survived for 7 days (34 percent) in an ice-covered river. In their review of hydrogeological characteristics that protect against ground water contamination, Robertson and Edberg (1997) report that viruses can travel up to 250 to 408 meters in glacial silt and sand aquifers and up to 1600 meters in karst aquifers. A study of animal viruses in nonaerated animal wastes found that virus inactivation could take anywhere from 1 week to 6 months depending on the virus type, temperature, pH, and other conditions (Pesaro et al. 1995).

Marzouk et al. (1979) examined ground water samples for the presence of enteroviruses and indicators following land application of sewage. Indicators included heterotrophic plate count bacteria, fecal coliforms, and fecal streptococci. Enteroviruses were found in 20 percent of ground water samples, including 12 samples that contained no detectable fecal organisms.

Survival of viruses and bacteria in ground water was examined by Keswick et al. (1982). Coxsackievirus was the most stable, followed by poliovirus, fecal streptococci, echovirus, *E. coli*,

rotavirus, and F2 coliphage. Enteroviruses survive longer than 24 days in ground water. Fecal streptococci proved to be a better indicator of viruses in ground water than *E. coli*.

Alhajjar et al. (1988) used poliovirus, total coliforms, fecal coliforms, and fecal streptococci to model the infiltration of effluent from a septic system. Poliovirus (vaccine strain) was tracked from a single inoculum introduced through a toilet. No tracer bacteria reached the ground water; however, poliovirus was recovered at a concentration of 62 PFU/100 mL from an inoculum of 108 PFU of virus.

Vaughn et al. (1979) discussed virus response to wastewater treatment and lack of correlation between bacterial fecal indicators and viruses in the environment. Viruses survived in chlorinated wastewater effluents treated with combined chlorine doses sufficient to effect a 5 log reduction in bacterial populations (Berg et al. 1978).

Aulicino et al. (1996) reported reduction of indicator bacteria and viruses between influent and effluent of a sewage treatment plant. Enteric viruses in raw sewage were reduced 2 to 3 logs by treatment. Fecal coliforms, fecal streptococci and total coliforms were reduced less than 2 logs.

Land application of sewage sludge has been shown to introduce enteroviruses into the environment (Sagik et al. 1978). Enteroviruses survive for 28 days (Wellings et al. 1975) and poliovirus survive 11 days in summer and 96 days in winter in effluent-irrigated soil (Tierney et al. 1977). Viruses attach to soil particles and are transported through the environment by water (Hurst et al. 1980a). Virus concentrations in upper layers of soil are higher than in subsurface layers by at least 1 log. Viral adsorption to particles is variable between viruses (Goyal and Gerba 1979). Echovirus moved through the soil at a slower rate than poliovirus, and did not survive as long. Drying soil greatly affected virus survival. The maximum depth to which viruses migrated in soil was 60 cm. Temperature, moisture content, presence of aerobic microorganisms, adsorption affinity for a particular virus, pH, and mineral content were the chief influences affecting virus survival in soil (Hurst et al. 1980b).

2.3.4 Response To Disinfection

While some viruses are known to be resistant to disinfection, the general assumption is that viruses are less resistant than *Cryptosporidium*. For this reason, treatment processes designed to control for *Cryptosporidium* are thought to be adequate for the inactivation of viruses.

Enteroviruses are thermolabile and rapidly destroyed when exposed to a temperature greater than 50°C (Melnick 1996; USEPA 1999c). Some viruses have been shown to be resistant to chlorine disinfection. EPA (USEPA 1999b) has available a table summarizing inactivation of viruses in water by free chlorine, based on information adapted from Sobsey (1989). The table indicates levels of disinfectant concentration and contact time in minutes required for a given level of inactivation. According to Sobsey, a chlorine residual of 0.5 mg/L or less was sufficient for 2 to 4 log inactivation of viruses in buffered demand-free water.

In general, enteric viruses are more resistant to free chlorine than are enteric bacteria (SDWC 1980). Recent data for rotaviruses, hepatitis A virus, and virus indicators such as MS2 coliphages support previous results for other enteric viruses, showing that they can be substantially inactivated by free chlorine. Norwalk virus is relatively resistant to chlorination, however, compared with other enteric viruses, such as poliovirus type 1, human rotavirus (Wa), and simian rotavirus (SA-11) (Keswick et al.

1985; USEPA 1999b). Some human volunteers became ill from Norwalk virus after drinking water with a chlorine dose of 3.75 mg/L, a dosage similar to that in most municipal water treatment systems. The same dose of chlorine was effective against other viruses. The authors concluded that chlorine treatment alone cannot be relied on to inactivate Norwalk viruses and that doses up to 10 mg/L might be required to inactivate the virus.

Peterson et al. (1983) evaluated the effect of chlorine treatment on the infectivity of hepatitis A virus in marmoset monkeys and concluded that the virus was more resistant to chlorine than were other enteroviruses (USEPA 1999c). Sobsey et al. (1991) studied the chlorine and monochloramine inactivation kinetics of cell-associated hepatitis A virus (viruses inside cells) and found that cell-associated hepatitis A was always inactivated more slowly than dispersed hepatitis A virus (viruses unattached to solids or cells). The authors concluded that disinfection criteria for inactivation of hepatitis A and other enteric viruses should be based on viruses associated with particulates, because they are better models for viruses found in water than are free-floating viruses (Sobsey et al. 1991). Payment and Armon (1989) reported on a virus sampling program in seven drinking water treatment plants; all plants delivered finished water in which the average cumulative reduction of viruses was 95.15 percent with disinfection using chlorine or ozone (USEPA 1999c). Melnick (1996) reported that a free residual chlorine treatment of 0.3 to 0.5 mg/L chlorine can cause rapid inactivation of enteroviruses, but that the viruses can be protected from such inactivation by organic substances (USEPA 1999c).

Ma et al. (1994) compared chlorine inactivation of poliovirus using cell culture and PCR methods. They found that with cell culture, 1 minute of exposure to 0.5 mg/L of chlorine was necessary for inactivation. With PCR, 6 minutes were needed for the same concentration of chlorine. The authors suggested that PCR was detecting inactivated viruses with undamaged nucleic acids. More recently, Blackmer et al. (2000) determined that researchers had previously underestimated the contact time necessary for chlorine to inactivate viruses. Many previous experimenters had observed only one cycle of cell culture. Blackmer et al. noted that if seemingly inactive viral material from one culture was applied to a second cell culture, virus activity appeared (infected cultured cells changed morphology). However, it took several weeks for the results of the cultures to become apparent. The authors also performed PCR early during the first cell culture, detecting viral nucleic acids within a few days and eliminating the need to wait for changed cell morphology. With both methods, they found that with a concentration of 0.5 mg/L of chlorine, 10 minutes of exposure was necessary for complete inactivation.

Through a monitoring program, Dee and Fogleman (1992) examined the ability of monochloramine to inactivate coliphage viruses at a full-scale plant. They determined that monochloramine alone was insufficient for even 2 log removal of coliphages. Berman et al. (1992) compared MS2 coliphage inactivation by monochloramine prepared three different ways. They found that inactivation was most effective when ammonia was applied after chlorine. Inactivation using preformed monochloramine was ineffective, resulting in only a 1 log reduction after 4 hours.

Little information is available on the mode of action by which chlorine dioxide inactivates viruses. Viruses react rapidly to chlorine dioxide; when chlorine and chlorine dioxide are combined, the inactivation appears to be synergistic. Inactivation of poliovirus type 3 by chlorine dioxide has been documented at rates that increase as pH increases from 5.6 and 8.5 (SDWC 1980). Chen and Vaughn (1990) found that chlorine dioxide doses of 0.05 to 0.5 mg/L effectively inactivated human and simian rotaviruses within 20 to 120 seconds, with more efficient inactivation occurring with increasing pH.

White (1986) concluded that ozone was a superior virucide. Payment and Armon (1989) maintain that viruses not eliminated by prechlorination–coagulation–sedimentation–filtration were relatively insensitive to the ozonation process. Finch and Fairbairn (1991) found that MS2 coliphage experienced a 4 log reduction when exposed to a residual of 40 µg/L for 20 seconds. However, poliovirus type 3 was inactivated to a lesser extent (2.4 logs), suggesting that MS2 may not be an effective enterovirus indicator. As with other oxidizing agents, the efficiency of ozonation is reduced when dissolved organic matter and reduced inorganic constituents are present (USEPA 1999b).

Viruses in nonturbid water and on exposed surfaces can be inactivated with UV light (Cliver 1997). Viruses as a group appear to be the most UV-resistant organisms and will likely be the limiting organism in determining a reactor design dose for systems requiring virus inactivation. Currently, California Title 22 standards for design of UV systems for water reclamation require a dose of 140 mJ/cm^2 based on a 4 log inactivation of poliovirus (including a safety factor).

Maier et al. (1995) and Meng and Gerba (1996) reported that a dose range of 20 to 30 mJ/cm^2 was required for a 4 log inactivation of poliovirus type 1. Battigelli et al. (1993) reported that coxsackievirus required a dose of 29 mJ/cm^2 for a 4 log reduction, while hepatitis A virus required a dose of 16 mJ/cm^2 for a 4 log reduction. Wiedenmann et al. (1993) reported similar results, indicating that the hepatitis A virus required a UV dose of 22 mJ/cm^2 for a 4 log reduction. Rotaviruses are more resistant than hepatitis. Battigelli et al. reported that rotavirus strain SA 11 required a dose of 42 mJ/cm^2 for a 4 log inactivation (Battigelli et al. 1993). The most resistant virus type appears to be adenoviruses. Meng and Gerba (1996) reported that adenovirus required doses of up to 120 mJ/cm^2 for a 4 log inactivation, even more than the 62 mJ/cm^2 required to inactivated MS2 coliphage. MS2's resistance to UV radiation (Malley et al. found that MS2 required doses of 64 to 93 $mJ/cm^{2,}$ depending on water quality) has led some researchers to consider it a good indicator of the extent of inactivation (Meng and Gerba 1996; Malley et al. 1999).

Zhang et al. (1991) examined the levels of indicator bacteria and viruses in source water and treated drinking water. Indicator bacteria (HPC, total coliforms and fecal coliforms), coliphage, and enteroviruses were reduced but not eliminated by complete treatment (prechlorination, coagulation-sedimentation, sand filtration and final chlorination). Reductions were 97.95-99.99 percent for standard bacterial indicators, 90.63 percent for coliphage, and 53.18 percent for enteroviruses. Complete water treatment was more effective at reducing turbidity, coliforms, and coliphage than enteroviruses.

Differences in virus susceptibility may be related to the morphology of the virus, such as the type of nucleic acid present (single- vs. double-stranded RNA or DNA) or the lipid/protein envelope. Studies are currently underway to systematically investigate the effects of virus morphology on disinfection effectiveness. Because viruses exhibit the most resistance to UV of the pathogens considered, numerous studies are underway to determine the fundamental basis for the relative resistance of viruses compared to other types of microorganisms.

2.4 Waterborne Disease Outbreaks (1997 - Present)

In 1997 and 1998, 10 drinking water-related waterborne disease outbreaks occurred in public water systems in the United States. One was a *Cryptosporidium* outbreak, three were *Giardia* outbreaks, two were caused by bacteria, and four were characterized by acute gastrointestinal illness of unknown

cause (Barwick et al. 2000). Of the 10 outbreaks, two occurred in systems with surface water sources (including a giardiasis outbreak at an unfiltered system). Three outbreaks occurred in systems served by springs, and the remaining five occurred in ground water systems.

Several other outbreaks of different types occurred throughout the United States at private water systems, and 18 outbreaks associated with recreational water occurred (nine of which were caused by *Cryptosporidium*) (Barwick et al. 2000).

The largest outbreak associated with drinking water during this period occurred in 1998 in Williamson County, Texas, where 160,000 gallons of sewage spilled into Brushy Creek. The sewage infiltrated a karst aquifer and contaminated four wells, one-quarter mile from the creek, with *Cryptosporidium*. An estimated 1,400 people were infected. Under normal conditions, the wells, which were 100 feet deep and encased in cement, would not be influenced by surface water. Extreme drought and high water demand, however, had lowered the water table, allowing the sewage to be drawn down into the aquifer (Bergmire-Sweat et al. 1998).

In 1999 and 2000, 20 drinking water-related outbreaks occurred in PWSs, where the outbreaks were caused by microbes or were manifested by acute gastrointestinal illness of unknown cause (AGI) (Lee et al. 2002). AGI could be caused by chemical as well as biological causes, although symptoms might differ for chemical agents. One AGI outbreak known to be caused by a chemical but where the chemical was not identified is excluded from the group of 20 outbreaks. During 1999–2000, *Cryptosporidium* caused only one outbreak, and *Giardia* caused two. Norwalk-like viruses, *E. coli* O157:H7, and other bacteria were identified as causes of some of the other outbreaks. AGI was listed as the source for 8 of the 20 outbreaks.

The largest of the outbreaks during this period was an *E. coli* outbreak. In 1999, an *E. coli* O157:H7 outbreak occurred at the Washington County Fair in New York. More than 900 people were affected, including 2 who died from kidney failure caused by toxins produced by the O157:H7 strain. The contaminated water came from a shallow unchlorinated well (CDC 1999).

A list of drinking water-associated outbreaks in PWSs from 1991–2000, based on CDC data, is shown in Appendix A. This list excludes outbreaks associated with chemical ingestion. It is possible that additional outbreaks associated with microbiological causes occurred in PWSs during this time, but they were not reported to CDC.

2.5 Indicators of Fecal Contamination

Monitoring for *Cryptosporidium* and *Giardia* is difficult in part due to their low concentrations in source water. Inaccuracies of current detection methods, discussed in section 3.3.1, also contribute to this difficulty. Therefore, other organisms are commonly used to indicate the possible presence of pathogens in drinking water and source water. This section discusses the use of total coliforms, fecal coliforms, and *Escherichia coli* (*E. coli*) as indicators of *Cryptosporidium* and *Giardia*.

2.5.1 Total Coliforms

Most coliform bacteria are harmless. Total coliform bacteria are common inhabitants of soil and vegetative material, and their presence in drinking water suggests that treatment was incomplete or a breach exists in the well or distribution system. For this reason, they are used as indicators. Traditionally, total coliform bacteria were identified by characteristics common to many species of bacteria from different genera. For example, coliform bacteria are rod-shaped, do not form spores, are gram-negative, and break down lactose under certain conditions. More recently, tests for the presence of certain enzymes have been added as criteria for determining whether bacteria are coliforms (Toranzos and McFeters 1997). All total coliforms belong to the family Enterobacteriaceae, and include members of the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia*. Although total coliform bacteria can indicate fecal contamination, their presence may be due to other causes. In distribution systems, for instance, coliform bacteria grow in the organic matter that sometimes accumulates on the interior walls of distribution pipes (biofilm). Biofilm growth can and does occur when no fecal contamination exists.

2.5.2 Fecal Coliforms

Fecal coliforms compose a subgroup of total coliform bacteria commonly found in the feces of warm-blooded animals. They grow at a higher temperature than most total coliforms, which explains their ability to colonize and survive in mammalian intestines (Toranzos and McFeters 1997). Fecal coliforms typically do not cause disease, but their presence in the environment correlates with that of several waterborne pathogens. In some cases, however, fecal coliforms, such as some *Klebsiella* bacteria, have been found in industrial effluent or other waters with high carbohydrate or plant content and no apparent fecal contamination. Some fecal coliforms, including *E. coli*, also have been found in distribution systems growing in biofilm, and in water from pristine sources (Toranzos and McFeters 1997).

2.5.3 *Escherichia coli*

E. coli is the primary species of fecal coliform bacteria that normally inhabits the gastrointestinal tract. Most strains do not cause disease, but a few strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, and kidney failure. For example, *E. coli* O157:H7 has been responsible for several waterborne disease outbreaks in recent years. Considered a better indicator of fecal contamination than other coliforms (Edberg et al. 2000), *E. coli* can be differentiated from other fecal coliform by biochemical tests.

2.5.4 Persistence

The ability of indicator bacteria to survive in the environment can affect their usefulness as indicators of fecal contamination and associated pathogens. If indicator bacteria die off before pathogens do, and if no pathogens are monitored, contamination can go undetected. If indicators outlast the pathogens, the indicators' presence is a false alarm. The survival of bacteria in aqueous environments is dependent upon a variety of factors, including aggregation, adsorption to particles, sedimentation, coagulation, flocculation, solar radiation, availability and competition for nutrients, predation by other microorganisms, lysis by bacteriophage, presence of algal and bacterial toxins, chemical toxicity, and physicochemical effects such as pH, temperature, and salinity (Evison and Tosti 1980).

Coliform bacteria demonstrate a significant growth potential when held in darkness (Grigsby and Calkins 1980). The effect of visible light on *E. coli* in natural waters was examined by Barcina et al. (1989), who showed that biosynthetic processes in ^{14}C labeled glucose uptake experiments were inhibited by visible light.

Sunlight also affects the survival of fecal coliforms and fecal streptococci in seawater (Fujioka et al. 1981). Both fecal coliforms and fecal streptococci suspended in seawater were reduced by 1 to 2 logs within 1 to 4 hr. at 24° C. Fecal streptococci were slightly more stable in seawater than fecal coliforms. Sunlight can penetrate up to 3.3 m beneath the surface of seawater with bactericidal effects. In a similar study, when sewage samples were exposed to sunlight, 90 percent of fecal coliforms were inactivated within 28 to 38 minutes, whereas 90 percent of fecal streptococci were not inactivated after a 2-hour exposure to sunlight. Ninety to 99 percent of fecal coliforms and fecal streptococci retained upon membranes were inactivated when the membranes were exposed to sunlight for 10 to 15 minutes (Fujioka and Narikawa 1982).

Survival of total coliforms, fecal coliforms and fecal streptococci were measured at 0° C for 7 days (Davenport et al. 1976). The percentage of survivors was 8.4 percent for total coliforms, 15.7 percent for fecal coliforms, and 32.8 percent for fecal streptococci. The fecal coliform/fecal streptococci ratio was > 5 at all locations, suggesting that the ratio may not reliably indicate source of contamination at low water temperature. The minimum growth temperature of *E. coli* is 7.5 to 7.8° C (Shaw et al. 1971).

Fecal coliforms, fecal streptococci, and *C. perfringens* were tested in freshwater and seawater for survival using diffusion chambers (Davis et al. 1995). A 1 log reduction of fecal coliforms and fecal streptococci occurred within 85 days in freshwater and marine sediments. *E. coli* in sediments remained culturable throughout a period of 68 days, suggesting that sediments provide both protection and nutrition. Use of diffusion chambers simulates natural conditions, and results in markedly longer survival times than use of free cell suspensions of *E. coli*, where reported survival times are considerably shorter.

Bacteria in natural environments are subjected to lethal and sublethal stresses, which impact the ability to culture them. Injured cells may make up as much as 90 percent of bacterial populations (Domek et al. 1984). Injured bacteria may be sensitive to agents in culture media such as bile salts, surface tension reducing agents, dyes, etc., all of which may reduce their recovery. Bacteria exposed to disinfectants frequently require resuscitation or enrichment before they can be recovered on culture media. When mEndo and mT7 agar were compared, mT7 consistently recovered more coliforms than mEndo (Du Preez et al. 1995). Selection of media and methods affects recovery of bacteria from the environment and the interpretation of results.

Enrichments have been used to resuscitate injured bacteria prior to enumeration from environmental samples (Davies et al. 1995). There is ample evidence from acridine orange or immunofluorescence stains that the number of intact bacterial cells far exceeds the numbers cultured by various media. Culturable but non-viable bacteria continue to respire at low levels and their presence may be detected by use of electron transport indicators or the activity of inducible enzymes such as beta-D-galactosidase.

Payment (1999) studied the inactivation of *E. coli,* other bacteria, and several types of viruses by chlorine. He concluded that chlorine in water rapidly inactivates *E. coli* and thermotolerant coliforms, but the most resistant pathogens can be unaffected for hours.

2.6 Summary

Although some *Cryptosporidium* and *Giardia* species can survive in different hosts, and some nonhuman hosts (e.g., cattle) contribute to the widespread occurrence of these protozoa, the primary species affecting humans are *C. parvum* and *G. lamblia*. On occasion, *C. felis* and *C. meleagridis* have infected immunocompromised humans. Transmission is typically from person-to-person, although outbreaks caused by contaminated drinking water do occur. Several types of viruses can cause illness in humans in the United States, including adenoviruses, enteroviruses, and hepatoviruses. All of these viruses can cause gastrointestinal illnesses and may be transmitted from person to person as well as through drinking water. It is difficult to estimate viruses' contribution to waterborne disease, as they are often not detected in drinking water during outbreaks.

Extensive research has been performed on the ability of protozoa to withstand environmental conditions and disinfection; the data obtained are difficult to compare, due to questionable reliability of methods used to assess viability and infectivity. In general, however, *Cryptosporidium* oocysts are resistant to extreme temperatures, particularly freezing, and halogen-based disinfectants have been shown to be ineffective against *Cryptosporidium* in practical doses, due to the impermeability of oocyst walls. Ozone and UV disinfection have been shown to be effective against *Cryptosporidium*. *Giardia* are more susceptible than *Cryptosporidium* to environmental stress and to most chemical disinfectants. While only one *Cryptosporidium* and one *Giardia* species typically affect humans, numerous types of viruses causing different illnesses are found in drinking water. Viruses are assumed to be inactivated by the disinfection required under the Surface Water Treatment Rule; however, specific disinfection needs for each type of virus vary due to the varied resistance of different viruses to chemical disinfection. Data on UV disinfection of viruses are limited; however, viruses appear to be more resistant to UV radiation than *Cryptosporidium* and *Giardia*.

3. Methods for Characterizing the Occurrence of Pathogens

In an effort to determine the occurrence of pathogens in source water, *Cryptosporidium* and *Giardia* in particular, EPA conducted two surveys, the Information Collection Rule (ICR) and ICR Supplemental Surveys (ICRSSs). This chapter describes the data sources, laboratory analytical methods used in the surveys, and data analyses. The data analyses include statistical analyses of observed data and a Bayesian model that incorporates both observed data and known relationships between data parameters. A model was desired to account for limitations in laboratory analytical methods, among other difficulties in accurately estimating the occurrence of pathogens, *Cryptosporidium* in particular, in source water.

3.1 Data Sources

To assess microbial occurrence and human exposure via drinking water, data were obtained from various sources. Before the ICR, data were obtained from various small scale studies; these data were used in the development of the IESWTR and the LT1ESWTR and are characterized in the regulatory support documents for those rules.

Microbial occurrence data were collected through the ICR and ICRSS monitoring programs for use in the development of the LT2ESWTR. The ICR was promulgated in 1996, 2 years before the IESWTR.

3.1.1 Pre-ICR Occurrence Data

Before the ICR data collection effort, no federal monitoring programs required routine monitoring of PWS source waters to determine the occurrence of protozoa. Previously, evidence of occurrence of *Cryptosporidium* and *Giardia* in drinking water supplies was gathered only through epidemiological surveillance reports and a limited number of surveys and individual monitoring studies. *Cryptosporidium* and *Giardia* have been shown to exist in drinking water supplies, as indicated by various studies of waterborne disease outbreaks, but these studies are site-specific and may not be representative of nationwide occurrence. In addition, comparing results from the various studies is difficult because the studies have different recovery rates, different sample volumes ("detection limits"), and they use different detection methods. Some studies also have small sample sizes, which can increase error. For summaries of outbreak incidences, the reader is referred to the *Occurrence Assessment for the Interim Enhanced Surface Water Treatment Rule* (USEPA 1998c) and the *Occurrence Assessment for the Long Term 1 Enhanced Surface Water Treatment and Filter Backwash Rule* (USEPA 2000d).

3.1.2 ICR Monitoring Program

The ICR obtained plant-level data sets that link water quality and treatment from source to tap. Additionally, the ICR described the relationships between treatment conditions and actual population served (USEPA 2000e). Systems included in the ICR monitoring program are surface and ground water PWSs serving populations of at least 100,000 people. However, only surface water and GWUDI systems serving populations of at least 100,000 were required to conduct microbial monitoring. These large plants sampled source water for *Cryptosporidium*, *Giardia*, viruses, and coliforms on a monthly basis for 18 months. These systems also were required, with some exceptions permitted, to monitor their finished water if 10 or more oocysts or cysts per liter were detected in their raw water during any of the first 12

months of monitoring. More detailed information, such as sampling locations and frequencies, can be found in the *ICR Data Analysis Plan* (USEPA 2000e).

The data were reported and tracked through the ICR Data Management System (DMS), which contains information on treatment processes used, water source type, and sample data from the PWSs participating in the ICR monitoring program. The ICR DMS consists of three data systems: the ICR Water Utility Database System used by PWSs to report data; the ICR Laboratory Quality Control Database System used by independent laboratories to analyze and report water utility sample quality control information; and the ICR Federal Database System, which can upload and maintain data from utilities and laboratories to a central database. For use in the data analysis, several auxiliary databases were created using the data from the ICR Federal Database System after quality assurance and quality control (QA/QC) measures were performed. The Auxiliary 1 (AUX1) database was the primary source of data for the analyses that are discussed in this document. The information in the other auxiliary databases was generally not needed for this document.

3.1.3 ICR Supplemental Surveys

The ICRSSs were conducted to complement the data collected through the ICR. Additional data were needed to characterize the distribution of protozoa in source waters because (1) the ICR collected microbial data only from systems serving at least 100,000 people and (2) the ICR Method has low and variable recovery. The systems included in the ICRSSs are large (47 systems with more than 100,000 people served), medium (40 systems with 10,000–100,000 people served), and small (40 systems with fewer than 10,000 people served). Protozoa data were not collected from small systems.

A stratified random sample of 40 plants for medium and large system size categories was monitored in the ICRSS, for a total stratified random sample of 80 plants. The selection of the stratified random sample of 40 plants for each system size was conducted in two steps. The plants were first stratified into two source water categories: plants served primarily by flowing stream–type surface waters and plants served primarily by reservoir/lake–type surface waters. A random sample of plants was then selected from each of these strata and recruited to participate in the surveys; the number of plants selected from each stratum reflected the proportion of plants served by each source water type across the United States for each plant size.

The surveys also collected protozoa data from a "certainty sample" of seven of the largest surface water plants along with 40 large randomly sampled plants. The water quality of these 7 plants could be very different or very similar to the 40 large randomly sampled plants. Because these 7 plants were not randomly selected, the results from these plants are not included in the national occurrence modeling discussed in this document.

The ICRSSs were designed to meet four objectives, two primary and two secondary. The primary objectives are shown below:

1. Characterize the national distributions of protozoa concentration (*Cryptosporidium* and *Giardia*) for plants at large and medium water systems. Also, accurately characterize protozoa concentrations at individual plants (e.g., characterize the mean, median, and 90th percentile). These characterizations will support development of national estimates of the impacts (costs and benefits) of various regulatory options.

2. Compare the national distributions of protozoa concentrations for plants in large systems to the national distribution for plants in medium systems. This comparison will help determine whether national impacts of required treatment estimated for large systems might be expected for medium systems and possibly small systems.

The secondary objectives are below:

1. Characterize the distribution of protozoa by source water body type (e.g., reservoirs/lakes vs. flowing streams) and watershed attributes (e.g., coliform density). This will support a potential classification of systems into categories of relative risk.

2. Collect data on water quality parameters and disinfection byproduct (DBP) precursors in source water that will support regulatory impact analyses for medium and small systems.

For medium and large plants, sampling occurred twice per month at each plant for 12 consecutive months, beginning in March 1999. The samples were analyzed for concentrations of *Cryptosporidium* using Method 1622 for the first 4 months; then Method 1623 was implemented for the completion of the ICRSS so that *Giardia* concentrations could also be determined. For the large plants, pH, temperature, turbidity, and coliform measurements were taken with every sample; total organic carbon (TOC) measurements were taken with every other sample (once a month); and additional water quality measurements were taken with every matrix spike sample. In addition to these parameters, medium plants collected additional water quality parameters monthly, including alkalinity, ammonia, bromide, and UV-254 absorbance. Also, split samples were collected at each plant on five of the sampling dates for spiking and analysis to estimate the recovery rate of Method 1622/1623.

Small plants were included in the ICRSSs but did not test for protozoa. Twice a month, samples were analyzed for coliform, pH, temperature, and turbidity. Monthly samples were tested for TOC, alkalinity, bromide, ammonia, and UV-254.

A comparison of the monitoring programs for the ICR and ICRSS is shown below in Exhibit 3.1. The data set for medium plants participating in the ICRSS is called the ICRSSM set, the set for large systems in the ICRSS is called the ICRSSL set.

Exhibit 3.1 ICR and ICRSS Comparison

	ICR	ICRSSM	ICRSSL
System size of plants participating (population served)	≥100,000	10,000-99,999	≥100,000
Number of plants participating	350	40	40
Method for selecting participating plants	All plants in size category	Stratified random selection	Stratified random selection
Sample frequency	Monthly	Semi-monthly	Semi-monthly
Sampling period	July 1997-December 1998	March 1999-February 2000	March 1999-February 2000
Total number of samples per plant	18	24	24
Laboratory method	ICR Method	Method 1622/23	Method 1622/23
Required sample volume for *Cryptosporidium*	100 L	10 L	10 L
Median sample volume analyzed for *Cryptosporidium*	3.2 L	10 L	10 L
Sample concentration process	Percoll-sucrose density gradient centrifugation	Immunomagnetic separation	Immunomagnetic separation
Microscopic examination process	Immunofluorescent assay, differential interference contrast (DIC)	Immunofluorescent assay, DAPI staining, and DIC	Immunofluorescent assay, DAPI staining, and DIC
Average recovery rates for lab method for *Cryptosporidium*	12%	43%	

Notes: DAPI stands for 4',6-diamidino-2-phenylindole.
The recovery rates for the ICR Method and Method 1622/23 were calculated in spiking studies described in Sections 3.3.2.1 and 3.3.2.3. The recovery rates for plants using Method 1622/23 were not calculated separately for each plant size category.

3.1.4 Representativeness of ICR and ICRSS Plants

Exhibit 3.2 and 3.3 show locations within each watershed of the 80 ICRSS randomly selected plants and the 350 ICR plants broken down by filtered or unfiltered status (there are no unfiltered medium ICRSS plants). All systems serving 100,000 or more people were included in the ICR. The seven large ICRSS plants that were not randomly selected (four filtered and three unfiltered) are not included in the exhibit because these plants were not used in the data analysis presented in this document. Note, the plants not included in the survey are assumed not to be fundamentally different from those included in the survey. In both maps, there appear to be fewer than the actual number of plants because those plants that share zip codes are superimposed on one another.

Based on the maps, both the medium and large ICRSS plants appear to be geographically representative of the whole set of ICR plants. This is to be expected, since the ICRSS plants were randomly selected.

Exhibit 3.2 Distributions of ICRSS Plants

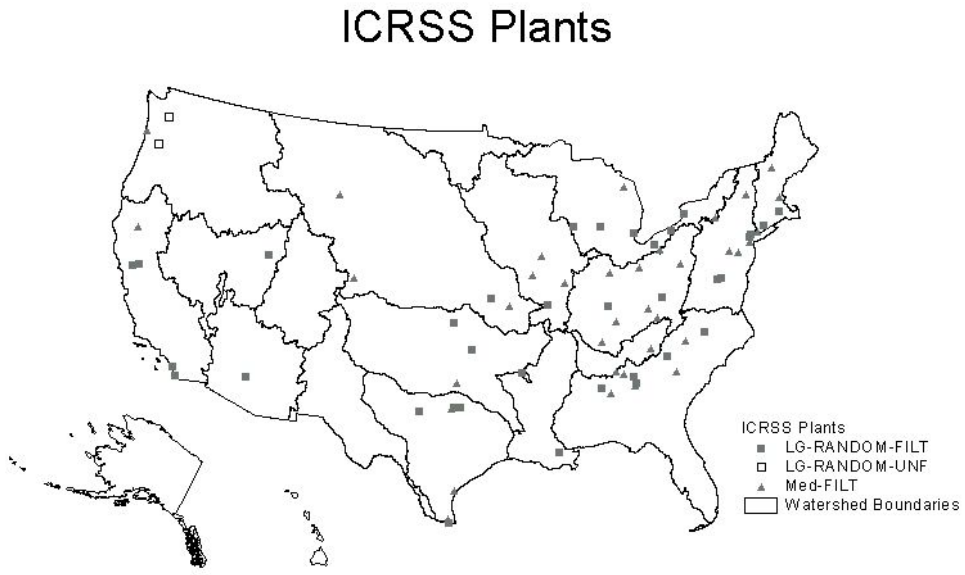
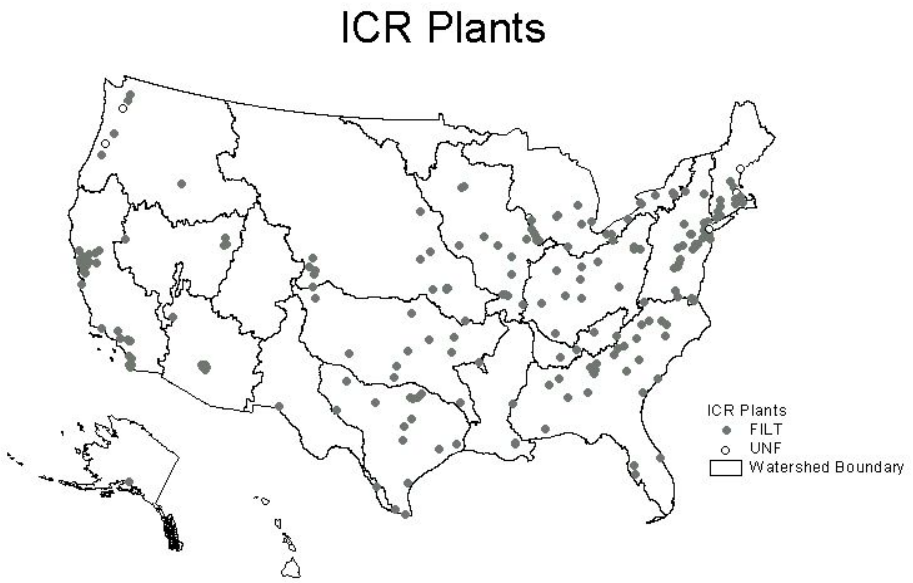


Exhibit 3.3 Distributions of ICR Plants



3.2 Analytical Methods

Sections 3.2.1 and 3.2.2 review three analytical methods used to determine *Cryptosporidium* and *Giardia* concentrations. Section 3.2.3 describes the method used to detect viruses.

3.2.1 ICR Method

The ICR specified a method for sampling and analysis of protozoa referred to as the ICR Method (USEPA 1996b), which provided for the quantitative measurement of *Cryptosporidium* oocysts and *Giardia* cysts in surface water. Four basic steps constituted the method: sample collection, concentration, purification, and assay. Each step offers ample opportunity to introduce error and reduce the efficiency of recovery.

The protocol calls for samples to be collected by passing water through a polypropylene yarn-wound filter or Filterite cartridge with a nominal pore size of 1 micrometer (µm) at a rate of 1 gallon per minute and pressure of 30 pounds per square inch. The method specifies volumes of 100 liters (L) (26.4 gallons (gal)) of raw water or 1,000 L (264 gal) of finished water to be passed through the filters. These filters retain oocysts, cysts, and particulate matter. After the appropriate volume of raw or finished water has been passed through the filter, the filter is delivered to a qualified ICR laboratory for analysis. At the laboratory, sample preparation begins with removal of the material retained by the filter fibers by washing the filter with an eluting solution. The material removed (the eluate) is then concentrated by centrifuging. Next, the *Cryptosporidium* oocysts and *Giardia* cysts are separated from other debris by Percoll-sucrose density-gradient centrifugation. The oocysts and cysts are placed on a cellulose acetate membrane filter and, using a technique called immunofluorescence assay (IFA), are stained with fluorescent antibodies specific to *Cryptosporidium* oocysts and *Giardia* cysts. The stained oocysts and cysts are then viewed under a microscope and counted.

To ensure that accurate and valid data were collected for the ICR, the United States Environmental Protection Agency (EPA) evaluated participating chemical and microbiological laboratories to ensure they were qualified to perform analyses required for the ICR. To be accepted for the ICR, a laboratory had to submit an application for EPA's approval, satisfactorily analyze unknown performance evaluation (PE) samples (described in section 3.3.2.2), and pass an on-site evaluation. In addition, laboratory personnel assigned to perform the protozoa analyses had to be approved individually.

Any of the several steps of the ICR Method—from collection to analyses—can contribute to variability in analytical results. Oocysts and cysts can be lost during filtration, elution, concentration of the eluate, purification, or staining of the concentrate. Optimal recovery of oocysts and cysts through these steps requires the technician to be well trained and follow the method carefully. Exceeding the specified flow rate may reduce recovery by forcing cysts and oocysts through the filter. Exceeding the specified sample volume can result in an excessive amount of debris and reduced recovery during the elution, concentration, and other steps of the method. Interferences can occur during staining and counting of the mounted specimens. Identification of the immunofluorescent oocysts and cysts requires a skilled microscopist and expensive equipment. Even for skilled microscopists, field samples are more difficult to read than spikes because of the different kinds and amounts of artifacts in the samples. Many samples contained large amounts of autofluorescent debris, such as algae, diatoms, and other protozoa, which could obscure *Giardia* and *Cryptosporidium*. The assay is complex; each assay requires several hours of sample preparation. In addition, method specifications requires sample elution to be completed

within 96 hours of sample collection to minimize potential changes of the physical and biological characteristics of the oocysts and cysts in the sample.

The ICR Method specifies the volume of sample to be *collected*; however, the amount of the sample *analyzed* is not specified. As a result, laboratories often analyzed varying volumes when the ICR Method was applied for analysis of ICR samples. The volume analyzed depended on the sample and pellet volume and was based on the volume needed to meet the desired detection limit. It also depended on the workload at the laboratory, the traditional volumes analyzed by a laboratory for a particular customer, the discretion of the laboratory analyst, and the amount of interfering turbidity or debris in the sample.

3.2.2 Method 1622 and Method 1623

EPA developed and validated improved analytical protozoan methods, Methods 1622 and 1623, to detect and enumerate *Cryptosporidium* oocysts and *Giardia* cysts in water (USEPA 1999d). The primary difference between the predecessor method, Method 1622 (USEPA 1999e), and Method 1623 is that the Method 1622 immunomagnetic separation (IMS) kit includes only reagents for *Cryptosporidium* purification, whereas Method 1623 uses the *Giardia*/*Cryptosporidium*-combination kit, which includes reagents for both *Cryptosporidium* and *Giardia* purification. The methods were developed to collect source water protozoan occurrence data during the ICRSSs. The strength of Methods 1622 and 1623 is that they are performance-based and can be adapted to changes in technology. The principal components of Methods 1622 and 1623 are the same as the ICR Method; however, there are significant changes in sample size, filtration medium, filter elution buffer, concentration technique, and the staining technique.

The procedure for analyzing water samples using Method 1623 consists of several components: sample collection, concentration, purification, and assay. The method involves filtration of a 10-L water sample through an absolute porosity capsule filter. *Cryptosporidium* oocysts and *Giardia* cysts are eluted from the filter with aqueous buffered salt and detergent solution, and immunomagnetic separation is used to separate the target organisms from extraneous materials in the water sample. In immunomagnetic separation, magnetic beads coated with pathogen-specific antibodies react with the pathogens and the sample is exposed to a magnetic field to separate the beads and attached pathogens from the sample debris. The magnetic beads used in the immunomagnetic separation procedure are then treated to release the oocysts and cysts for assay. The assay procedure for the method is performed by staining the oocysts and cysts with immunofluorescent antibodies and a mixture of 4, 6-diamidino-2-phenylindole (DAPI). Oocysts and cysts are identified by fluorescence and differential interference contrast (DIC) microscopically and are classified on the basis of fluorescence of the oocysts and cysts (apple-green fluorescence) and uptake of DAPI (blue nuclei or blue internal staining). Qualitative and quantitative analyses are performed by viewing the microscope slide to determine if the microorganisms meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts and by counting the total number of microorganisms on the slide confirmed to be oocysts or cysts, respectively.

Method 1623 helps analysts identify and enumerate *Cryptosporidium* oocysts and *Giardia* cysts and minimizes false positives. The method also has a higher recovery (an average of 43 percent of *Cryptosporidium* and 53 percent of *Giardia* in matrix spike water) than the ICR Method (12 percent of *Cryptosporidium* and 26 percent of *Giardia* in matrix spike water). It is postulated that Method 1623 has higher recovery than the ICR Method due to enhanced features such as optimized filtration procedures and immunomagnetic separation. The ability to detect *Cryptosporidium* oocysts and *Giardia* cysts at low concentrations depends on the concentration of interfering particles. Interferences from extraneous

particles should be less than in the ICR Method because immunomagnetic separation removes most non-protozoan particles from the sample prior to microscopic observation.

Although Method 1623 has several advantages over the ICR Method, neither method distinguishes among species of the *Cryptosporidium* oocysts and *Giardia* cysts, nor do they determine viability and infectivity. The method still requires a skilled microscopist and expensive microscopic equipment for the identification of immunofluorescent oocysts and cysts. Furthermore, each assay requires several hours of sample preparation.

3.2.3 ICR Method for Viruses

The sample volume that must be collected for viruses is 200 L, and the volume that must be assayed is 100 L. Samples are collected by passing water through a positively charged filter designed to trap viruses. Beef extract solution is then poured through the filter under pressure to wash off the filtered material. The solution containing the filtered virus material, the eluate, is mixed with hydrochloric acid, which causes a precipitate to form to entrap the virus particles. The precipitate is centrifuged, and the supernatant is discarded. The pellet is redissolved and centrifuged again. At this stage, the supernatant is retained; this concentrated sample is divided and used to inoculate cultured cells derived from monkey kidney (also called BGM) cells. The cells are observed under a microscope for cytopathic effects, such as cell disintegration and changes in cell morphology (but not cell death). If actual cell death occurs, a portion of the sample is diluted, and the inoculation is repeated in a new culture until only cytopathic effects occur. Each flask of cultured cells exhibiting cytopathic effects is counted, and the total is entered into EPA software that determines the most probable number (MPN) of culturable viruses per milliliter. This number is later converted to MPN per liter.

3.3 Data Analysis Procedures

This section describes the procedures used to analyze the ICR and ICRSS data. The AUX1 database contains the ICR data for 18 monthly samples from approximately 300 large systems (surface water and GWUDI systems serving a population of over 100,000 people), representing approximately 500 plants. The ICRSS database contains semi-monthly data collected over 12 months from 47 large systems, 40 medium systems, and 40 small systems (coliform data only). The data analysis and statistical calculations used data from the AUX1 and ICRSS databases on *Cryptosporidium*, *Giardia*, viruses (ICR data only), and coliforms.

EPA developed a hierarchical model using Bayesian parameter estimation techniques to describe the uncertainty of individual assays, the variability of occurrence over space and time, and the contributions of source water type, turbidity, and month. This model used observed data to better characterize the national distribution of protozoa occurrence in source water. "Occurrence" is the term used to describe the nationwide distribution of concentrations of organisms. The model is described in section 3.3.3.2 and detailed in Appendix B.

3.3.1 Challenges in Analyzing Microbial Occurrence Data

Analysis of the ICR microbial data is complex due to multiple factors. This section discusses the challenges in analyzing microbial occurrence data and indicates reasons for data discrepancies.

3.3.1.1 Low Occurrence and Concentration of Microbes

One way to increase the likelihood that a sampling program's findings will be statistically representative of a system with rare, low-concentration events is to obtain a large number of samples and large sample volumes. Although the ICR analyses used data from about 5,600 samples, the ability to predict future sample events of low concentrations at individual plants depends on having sufficient data available for each plant. The ICR goal was to collect 18 samples of 100 L at every plant and analyze at least 10 L from each sample. However, the median volume analyzed was 3 L and only 44 percent of plants had 18 usable observations of *Cryptosporidium* (after QA/QC controls).

Small numbers of samples and small sample volumes analyzed make accurate predictions of low concentration sampling events difficult. Consider the following example that uses a Poisson distribution to estimate the likelihood that samples with zero counts (non-detects) contain *Cryptosporidium*: in 18 samples of 3.33 L (similar to the median volume analyzed in the ICR analyses), 18 non-detects would still represent more than a 50-percent chance of having at least 0.01 oocysts/L in the source water and a 30-percent chance of having 0.02 oocysts/L. Thus, although no *Cryptosporidium* oocysts were reported through laboratory analysis in more than 90 percent of the samples in the ICR, it cannot be concluded that *Cryptosporidium* oocysts were not present in the source waters. These factors are discussed in more detail in section 3.3.1.3. To better estimate a more likely true distribution, data were modeled using a hierarchical model discussed in section 3.3.3.2.

3.3.1.2 Sample Variability in Laboratory Technique

The detection of the true number of *Cryptosporidium* oocysts and *Giardia* cysts in a laboratory sample is complex and can lead to variable results. Average recovery in spiking studies in which a known amount of oocysts and cysts were added to a sample ranged from 12 to 43 percent for *Cryptosporidium* and 26 to 53 percent for *Giardia*, depending on the condition of the cysts and oocysts, the spiking method, and the detection method. A detailed discussion can be found in section 3.3.2. Recovery is calculated as the number of oocysts or cysts detected in the matrix sample less the detects from the unspiked field sample, divided by the amount in the original spike. *Cryptosporidium* and *Giardia* spiking studies used in the ICRSSs indicate that recovery varies from laboratory to laboratory and from analyst to analyst.

Some spiking studies resulted in recoveries of more than 100 percent. Sample error may be the strongest contributing factor to these results; that is, the analyzed portion of the sample may have actually contained a concentration that exceeded the total sample concentration.

Algae or other constituents in the water could have been counted as protozoa during laboratory analysis, leading to "false positives" or higher reported concentrations in some of the ICR and ICRSS observed data. Analyses of blanks in the spiking studies sometimes produced non-zero results, which supported the existence of false positives in the observed data. Inaccuracy also occurred in counting spike stocks—oocysts or cysts may clump, and aggregates of 10 or more oocysts may appear as a single oocyst.

3.3.2 ICR and ICRSS Recovery Studies

To determine the percentages of the *Cryptosporidium* oocysts and *Giardia* cysts captured in laboratory analyses, spiking studies were performed. The results of these studies are shown in Exhibit 3.4.

3.3.2.1 ICR Lab Spiking Program

The purpose of the ICR Lab Spiking Program (LSP) was to assess the recovery of oocysts and cysts from field samples analyzed with the ICR Method (described in section 3.1). At the time of the ICR sample collection, a duplicate 100 L sample was collected on two sampling dates from 70 plants during the last 8 months of ICR monitoring. The duplicate samples were spiked with a known quantity of *Giardia* cysts and *Cryptosporidium* oocysts (based on the use of hemacytometer counting methods) and filtered. Spiking doses targeted 5,000 oocysts/cysts per 100 L (low-spiking period) and 10,000 oocysts/cysts per 100 L (high-spiking period). Mean recovery using the chamber-counting procedure was calculated to be 12 percent for *Cryptosporidium* and 26 percent for *Giardia* (see Exhibit 3.4 below).

3.3.2.2 ICR Performance Evaluation Study

EPA implemented the ICR PE Study to ensure the ICR laboratories' ability to perform the ICR Method throughout the duration of the ICR. To become an approved protozoa principal analyst for the ICR, an analyst was initially required to correctly analyze a set of eight PE samples (seven spiked, one blank). Ongoing approval required the analyst to correctly analyze two samples per month throughout the ICR monitoring period. Filters were spiked with mixtures of *Cryptosporidium* oocysts and *Giardia* cysts enumerated using a hemacytometer chamber-counting procedure and analyzed along with unspiked filters. Mean recovery using this method was 41 percent for *Cryptosporidium* and 48 percent for *Giardia* (see Exhibit 3.4 below). Results were higher for the PE study than the LSP because the filter, not the water sample, was spiked, and organism losses through the ICR filter are reported to be a significant source of the overall losses of organisms through the entire method.

3.3.2.3 ICRSS Matrix Spiking Program

As with the LSP, the purpose of the ICRSS Matrix Spike Program was to assess the recovery of oocysts and cysts from field samples using Method 1622/1623 (described in section 3.1.2). Method 1622 was used for *Cryptosporidium* analysis only. During five sampling events during the year-long program, each plant collected an additional 10 L sample at the same time as the routine sample and shipped both samples to its assigned protozoa laboratory. The laboratory spiked the additional sample with a known quantity of *Cryptosporidium* oocysts and *Giardia* cysts (the quantity was unknown to the laboratory performing the analysis) and filtered and analyzed both samples using Method 1622/1623. The spikes were enumerated using a flow cytometer procedure. Recovery using this method averaged 43 percent for *Cryptosporidium* and 53 percent for *Giardia* (see Exhibit 3.4).

Exhibit 3.4 Spiking Study Results

	Study / Program		
	ICR Lab Spiking Survey	**ICR PE Study**	**ICRSS Matrix Spiking Program**
Program Period	May 1998 - Dec. 1998	Jan. 1997 - Dec. 1998	Mar. 1999 - Feb. 2000
Method	ICR Method	ICR Method	Method 1622/23
Spiking Enumeration Technique	Hemacytometer (Region 10)	Hemacytometer (Contractor)	Flow cytometry (Wisconsin State Lab)
Spiking Method	Bulk, 100 L water sample spiked in line by central spiking laboratory, then filtered	Spiked directly onto filter (did not account for losses due to filtration)	Bulk, 10 L water sample spiked by analytical laboratory, then filtered
Volume Analyzed	8 L	47 L	10 L
Spiking Doses	5,000 - 10,000	200 - 6,100	80 - 200
***Cryptosporidium* Mean Recovery**	12%	41%	43%
Cryptosporidium* Spiking Relative Standard Error	Low Spike Period 10% - 22%	7% - 32%	1.0%
	High Spike Period 11% - 24%		
***Giardia* Mean Recovery**	26%	48%	53%
Giardia Spiking Relative Standard Error	Low Spike Period 14% - 22%	7% - 25%	1% - 2%
	High Spike Period 11% - 23%		

* The *Cryptosporidium* Spiking Relative Standard Error accounts for the uncertainty in the amount spiked.

3.3.3 Microbial Occurrence Data Analysis Techniques

This section describes the techniques used for analyzing observed data and for conducting the Bayesian analysis, which models occurrence using ICR and ICRSS data. These data were then used to estimate the exposure associated with *Cryptosporidium* and *Giardia* in the source water.

3.3.3.1 Observed Data Analysis

This section describes how the protozoans were counted when observed under a microscope, describes any special considerations for analyzing viruses and indicators, and describes how source water was taken into consideration.

Categorization of Cryptosporidium and Giardia

The protozoa data are divided into four categories for *Cryptosporidium* (total, empty, amorphous, and with internal structures) and five categories for *Giardia* (total, empty, amorphous, with one or more internal structures, and greater than one internal structure). These categories are described for *Cryptosporidium* and *Giardia* in this section.

Cryptosporidium

Cryptosporidium concentration data are divided into four differential interference contrast microscopy categories in the AUX1 ICR database and ICRSS database:

- Total: Empty oocysts, amorphous oocysts, and oocysts with internal structures combined. These are combined in data analysis as an indicator of viable or infectious oocysts to some degree.

- Empty: A wall is present; appears to be an oocyst. While these oocysts are empty and presumed to be unlikely to be viable or infectious (at the time of the laboratory analysis), they still are indicators of the presence of *Cryptosporidium*.

- Amorphous: Oocysts with internal material structures, but whose structures may be masked (e.g., because of their position on a slide).

- With Internal Structures: Oocysts that have a wall and recognizable internal structures consistent with *Cryptosporidium*.

For ICR data, the value for the total nearly always equals the sum of the other categories for each type of protozoa, but does not in a few cases, due to rounding and possibly to reporting errors. For instance, in a few cases, a plant appears to have reported a total number of oocysts, but not the numbers of oocysts in each category.

Because expert opinions vary regarding the data categories that best describe occurrence, three *Cryptosporidium* categories were used for analysis and graphical presentations in this document:

- Total

- Non-empty (oocysts with either amorphous or internal structures)

- With Internal Structures

Giardia

Giardia concentrations are divided into five categories in both the ICR and the ICRSS: total cysts, empty cysts, amorphous cysts, cysts with one or more internal structures, and cysts with more than one internal structure. The four categories used in data analysis are total cysts, non-empty cysts (amorphous plus cysts with one or more internal structures), cysts with one or more internal structures, and cysts with more than one structure.

Viruses

Virus data were analyzed as described earlier for ICR data only. Virus occurrence data were not collected as part of the ICRSS.

Indicators (Coliforms)

Three categories of coliforms were analyzed during these studies: total coliforms, fecal coliforms, and *E. coli*. These bacteria were monitored to determine whether any subsets of coliforms correlate with the presence of pathogens (see Chapter 2 for more on coliforms as indicators of contamination). Each is analyzed as described earlier. All plants monitored total coliform for the ICR and ICRSS. Each plant also monitored either fecal coliform or *E. coli*. A few monitored both fecal coliform and *E. coli*.

Analysis of Source Water Type

All microbial data were categorized according to source water type, which was reported by the treatment plant. All ICR plants sampling for protozoa and viruses were large systems (serving populations of 100,000 or more), so data are not categorized by system size.

- All–Includes all *filtered* source water types—surface, GWUDI, and mixed (plant uses both surface and groundwater sources)
- Flowing Stream (FS)
- Reservoir/Lake (RL)
- Unfiltered

Analysis of ICRSS Observed Data

For the ICRSS, microbial data are categorized according to source water type and system size. As in the ICR, the source water type for each plant was identified by the treatment system and includes the following categories:

- All–Includes all water source types (a few unfiltered sources were included in the ICRSS)
- Flowing Stream (FS)

- Reservoir/Lake (RL)

In the ICRSS, two system sizes were monitored for protozoa occurrence:

- Large systems (serving populations of 100,000 or more)
- Medium systems (serving populations of 10,000 - 99,999)

Observed data are graphically presented in Chapter 4 and in the appendices using cumulative distribution of plant means (for 80 plants).

3.3.3.2 Modeled Distributions

The ICR and ICRSS data collection efforts faced practical limitations including sample volume, the number of samples analyzed, and microbial measurement techniques. To account for these limitations and other sources of variability in the data, model-based occurrence estimates were chosen over observed data to predict national estimates of occurrence.

Using model-based estimates rather than observed data can also properly account for:

- Variability in the data, based on location, sampling technique, and other contextual variables.
- Low and variable recovery of *Cryptosporidium* by the analytical method.
- The volume assayed and its adequacy in representing the much larger volume of source water at the time of sampling.
- The small number of samples assayed at each location and their ability to represent the average concentration for a given source water during the periods of the survey.

Two hierarchical models (one for filtered plants and one for unfiltered plants) with Bayesian parameter estimation techniques were developed to help analyze and correct for the limitations of data collection and variability inherent in the data. Good statistical answers require models that are consistent with observed data, but flexibility within the model is also required to allow specification of realistic underlying features not adequately addressed by observed data. Markov Chain Monte Carlo models provide such flexibility with the following features:

- An ability to accommodate many parameters.
- Hierarchical structuring, which is the essential tool for achieving partial pooling of estimates and compromising in a scientific way between alternative sources of information.
- An emphasis on inference in the form of distributions or at least interval estimates, rather than simple point estimates.
- Parameter estimates that are robust over a wide range of model assumptions.

- Special statistical issues can be easily incorporated into the modeling framework; these include the probability of false-positive readings, differential recovery rates, and the ability to include as an input observed values of zero for the number of microbes and in the model the probability that a finding of zero microbes reflects the true number of microbes in the source water.

Model for Source Water Occurrence

In the process of developing the models for characterizing microbial source water occurrence, EPA developed a comprehensive model, also referred to here as the “full model”, for describing filtered systems, which comprise the majority of surface water systems in the U.S. EPA also developed a reduced-form model for the unfiltered systems to better accommodate the limited data available on those systems. The majority of the information presented in this occurrence document describe the inputs, assumptions, and methods used for the full model. Both the full model and the reduced-form model are discussed in detail in Appendix B. For consistency sake, EPA used reduced-form models for both filtered and unfiltered systems to support the economic impact analyses performed te evaluate regualtory alternatives for the LT2 rule.

The statistical model employed for this analysis is called "hierarchical," because it includes parameters at three tiers or levels. In the highest tier, three precision parameters describe how random effects vary around mean zero for locations, months, source water type. A fourth precision parameter describes additional variability. A number of global fixed effects (an overall median, a coefficient for turbidity, a false-positive rate, and a percentage of waters with zero occurrence) complete the highest tier. In the second tier are the effects for individual locations, months, and source water types. At the third, and lowest tier, are the observed counts, volumes assayed, and turbidity as well as the unobserved concentrations and measurement recoveries associated with those observations.

The oocyst or cyst count for a particular assay is modeled as a Poisson random variable, with expected value equal to the product of the concentration, the volume assayed, and the measurement method's recovery for that assay. A zero count is highly likely when this product is small. Because of false positives, nonzero counts are possible, even when the actual concentration is zero. Through the likelihood function (probability of observing what was observed, as a function of parameter values), the survey data inform us about the model parameters. Appendix B, Modeling Microbial Source Water Occurrence, provides greater detail about how this is accomplished.

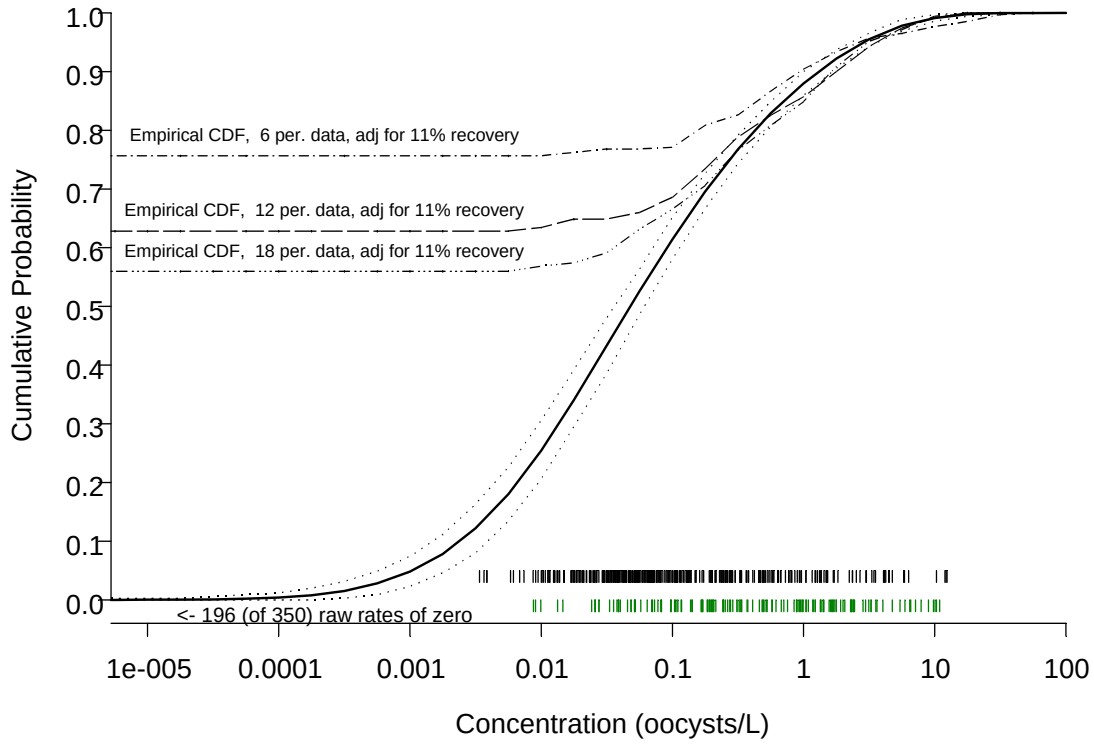
While the ICRSS data sets included oocyst counts, the ICR database contains only concentrations (concentration per 100L). Counts were derived from the reported concentrations using two methods: (1) dividing the concentration by the "detection limit" and (2) dividing the concentration by 100 times the volume assayed. Both methods will produce the same count value when data are reported to the same degree of accuracy, because "detection limit" is defined as 100 divided by volume assayed. The product of "detection limit" and volume assayed should be 100, but this is not always the case. Most discrepancies were explained by rounding (the volume assayed was reported to the nearest liter, while the "detection limit" had as many as five significant digits). The remaining discrepancies were resolved by reviewing other microbial data for the same sampling event and determining the appropriate volume assayed.

Graphs

Using the Bayesian model with MCMC sampling, 500 draws of the complete set of uncertain variables were produced to evaluate the occurrence distributions. For each of these draws, the model

calculates a plant-mean for each plant in the data set. The resulting set of plant-means provides a possible true distribution that is not inconsistent with the observed data. In the aggregate, the 500 occurrence distributions describe a range of true distributions that could have reasonably given rise to the ICR or ICRSS data set. From these 500 distributions, a central estimate of occurrence was generated with a 90-percent "credible interval" for the estimate. Exhibit 3.5 is an example of the cumulative distribution of *Cryptosporidium* concentrations from this approach. The solid curve represents the central estimate of the predicted occurrence of microbial pathogens in influent water based on plant-month data. The dotted curves on either side of the solid curve are the bounds of the 90-percent "credible interval."

Exhibit 3.5 Bayesian Cumulative Distribution of Source Water Occurrence



Note: The plant-means of the 18-month observed ICR data are depicted by the bottom row of hash marks in the lower right-hand corner of the graph. The top row of hash marks represents the plant-means of the 18-month modeled data.

The dashed lines in Exhibit 3.5 represent the cumulative distributions of the empirical, or observed (adjusted for recovery), data. They represent the plant-means of source water *Cryptosporidium* concentrations, calculated from the 6, 12, and 18 months of ICR data. These three empirical distributions show that as more data were collected, the closer the observed occurrence distribution came to the modeled distribution. Comparing the 18-month empirical CDF to the modeled data at the upper ends (>0.1 oocysts/L) indicates the model fits the observed data. At the lower end (<0.1 oocysts/L) the empirical CDF is driven by the large number of non-detects for which the model estimates low occurrence levels. Overall, the comparison supports the model's capability to predict source water occurrence.

The plant-means of the 18-month observed ICR data are depicted by the bottom row of hash marks in the lower right-hand corner of the graph. The top row of hash marks represents the plant-means of the 18-month modeled data. These give another visual perspective of the plant-mean concentrations that are represented by the distribution curves. The number of plants with non-detects for all months of sampling and the total number of samples reported in the ICR data set are given in the bottom left-hand corner of the graph.

Cryptosporidium

The Bayesian models contain parameters that are assigned values, allowing the data to be viewed in many ways. For *Cryptosporidium* analysis of filtered plants, the following parameters were assigned the following values:

- Data Subset — Total, Non-Empty, and With Internal Structure
- Source Water — *All*, Flowing Stream (*FS*), and Reservoir/Lake (*RL*)
- False Positives — =1 percent
- True Zero — =various small percentages (with little effect on results)
- Recovery Rate — r_{ij} =beta distribution based on spiking studies.

The distributions used in the Economic Analysis are based on total oocysts. The distribution of *Cryptosporidium* occurrence in unfiltered plants is derived using a slightly different model and is described in Appendix B.

Giardia

Giardia data were also run through the Bayesian models with ICR data. The analysis was performed using the following parameter values:

- Data Subset — Total, Non-Empty, With One Internal Structure, and Greater than One Internal Structure
- Source Water — *All*, Flowing Stream (*FS*), and Reservoir/Lake (*RL*)
- False Positives = 0 percent
- True Zero = 0 percent

3.4 Conclusion

The data analysis procedures outlined in this chapter and Appendix B were used to describe the ICR and ICRSS data pertaining to pathogen occurrence in source water, which are presented in Chapter 4. Analyzing samples for protozoa using the ICR Method and Methods 1622 and 1623 is a complex task that may lead to varied results. This variation is evident in the mean and range of recoveries between the two methods determined from spiked source water sample studies performed under the ICR and the ICRSS. The ICR Method was characterized by mean *Cryptosporidium* recoveries of 12 percent, with a range of 0 to 60 percent, while Methods 1622 and 1623 were characterized by mean *Cryptosporidium* recoveries of 43 percent, with a range of 0 to 100 percent. Similarly, the ICR Method was characterized by mean *Giardia* recoveries of 26 percent, with a range of 0 to 83 percent, while Methods 1622 and 1623 were characterized by mean *Giardia* recoveries of 53 percent, with a range of 0 to 105 percent. To account for variability in recovery and other variable factors among plants (e.g., geographical and temporal), microbial occurrence can be examined more appropriately with Bayesian analyses. This modeling approach was used to produce the modeled occurrence distributions from the ICR and ICRSS data.

4. Occurrence of Pathogens in Source Water

This chapter summarizes the source water microbial occurrence data used in the development of the LT2ESWTR rule. The primary sources of data are the observed data from the ICR Monitoring Program and ICRSS, along with modeled results using those data. Sections 4.1 through 4.4 present and briefly discuss the *Cryptosporidium*, *Giardia*, and virus data, as well as potential indicator data for these microbes. Section 4.5 presents data analyses pertaining to microbial co-occurrence. Section 4.6 discusses temporal effects and the possibility of seasonal bias in annual estimates of *Cryptosporidium* occurrence.

For each microbial contaminant (i.e., *Cryptosporidium*, *Giardia*, virus, and coliforms), summary tables contain statistics of observed and modeled ICR and ICRSS data. The statistics presented are means, medians, and 90th percentiles for each source water type. These statistics are of plant-mean data, that is, for each plant all monthly data are averaged together and statistics are calculated from those plant-means. The total number of plants and the number of plants with positive samples are also included in the summary tables. A discussion of results follows each summary table.

Additional information on the observed occurrence of pathogens in the ICR and ICRSS data are provided in the appendices. Appendix C contains statistics of all ICR observations by month for each source water type (as compared to the plant-means in this chapter). Appendix D contains bar charts of ICRSS data by month for each source water type.

4.1 *Cryptosporidium*

Cryptosporidium monitoring results are presented in this section and in Appendices C and D. Observed results are presented in sections 4.1.1.2, and 4.1.1.3 for the ICR and ICRSS, respectively. Note, due to the different mean *Cryptosporidium* recovery rates of the two monitoring programs – 12% for ICR, and 43% for ICRSS – raw data from the two monitoring programs are not directly comparable. Appendix B provides details of adjustments made in the model for differences in mean recovery rates between the ICR and ICRSS. *Cryptosporidium* was not detected in most of the samples (93 percent of ICR samples and 86 percent of ICRSS samples). *Cryptosporidium*, however, may have been present in many more source waters but simply not captured in samples as a result of small sample volumes and low concentrations. Moreover, even if captured in a sample, *Cryptosporidium* oocysts were recovered in the laboratory at an estimated rate of just 12 percent for ICR data (see section 3.3.2).

Another byproduct of small sample volumes is high "detection limits". The median volume analyzed in ICR samples was only 3 L. If a 3-L volume is analyzed, it cannot have a measured concentration below 0.33 oocysts/L, the concentration that would be reported if a single oocyst was detected in that sample (1 oocyst ÷ 3 L = 0.33 oocysts/L). Section 4.1.2 presents modeled occurrence estimates that are adjusted to compensate for these sampling and testing conditions.

4.1.1 Observed Results

As described in Chapter 3, oocyst observations were reported as falling into one of three categories: oocysts containing defined internal structures characteristic of *Cryptosporidium* ("oocysts with internal structures"); probable oocysts with characteristic walls and with internal material, but that material does not appear characteristic of a *Cryptosporidium* oocyst ("oocysts with amorphous structure"); and oocyst-type walls not presently containing internal material ("empty oocysts"). The

presence of internal structure increases the confidence that the observation is indeed a *Cryptosporidium* oocyst and not some other item or organism with similar gross morphology. On the other hand, an empty oocyst, if actually an oocyst, may have contained infectious *Cryptosporidium* at some time prior to analysis. For the following analyses, amorphous oocysts and oocysts with internal structures were combined into a category called "non-empty oocysts." Non-empty and empty oocysts counts were also combined for a count of "total oocysts."

4.1.1.1 ICR Monitoring Program Results

Exhibits 4.1 and 4.2 summarize the observed ICR *Cryptosporidium* data for filtered plants for the 18-month monitoring period in terms of selected oocyst categories and source water characteristics.

Exhibit 4.1 Summary of ICR *Cryptosporidium* Data for Filtered Plants

Source	Total Number of Plants	Number and Percentage of Plants with at Least One Positive Sample	Observed Plant-Mean Data (oocysts/L)		
			Mean	Median	90th Percentile
Total *Cryptosporidium* Oocysts					
All	338	147 (43%)	0.068	0	0.194
FS	128	81 (63%)	0.137	0.022	0.407
RL	206	62 (30%)	0.033	0	0.058
Non-Empty Oocysts					
All	338	118 (35%)	0.038	0	0.121
FS	128	61 (48%)	0.067	0	0.253
RL	206	52 (25%)	0.026	0	0.031
Oocysts with Internal Structures					
All	338	34 (10%)	0.011	0	0.001
FS	128	19 (15%)	0.024	0	0.049
RL	206	15 (7%)	0.005	0	0

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

The percentage of positive samples (7 percent of all total *Cryptosporidium* observations) and the percentage of plant positives (43 percent of all plants had one or more positive sample) demonstrate that *Cryptosporidium* is relatively rare in many source waters. The observed data, however, do not account for the ICR Method's low recovery efficiencies and the small sample volumes analyzed. Adjusting for these factors increases the estimated occurrence several-fold (see section 4.1.2).

As shown in Exhibit 4.1, the mean concentration of total *Cryptosporidium* plant-means for all filtered source waters is 0.068 oocysts/L, but the mean of non-empty (observations of amorphous and internal structures) is 0.038 oocysts/L, 56 percent of total *Cryptosporidium* levels. The mean plant-mean

concentration of oocysts with internal structures is 0.011 oocysts/L, 16 percent of total *Cryptosporidium* detections. Exhibit 4.2 displays the cumulative distributions of selected oocyst categories for all filtered source water types.

As is clear from Exhibit 4.1, the occurrence of *Cryptosporidium* in flowing stream sources was greater and more variable than the occurrence in reservoir/lake waters. A comparison of total *Cryptosporidium* plant-mean concentrations by source water category shows a substantial difference between plants with sources of flowing streams and those with reservoir/lake sources. The percentage of positive samples is also higher for flowing streams than for reservoir/lakes. Exhibit 4.3 displays the cumulative distributions of plant-mean oocyst concentrations for each filtered source water type for selected oocyst categories.

Exhibit 4.4 summarizes *Cryptosporidium* concentrations for unfiltered plants. Unfiltered plants had a much lower concentrations of total oocysts than filtered plants (0.002 oocysts/L as opposed to 0.068 oocysts/L). This difference is expected, since unfiltered plants must meet high source water quality standards to avoid the filtration requirements of the Surface Water Treatment Rule. However, the percentage of plants with one or more detections is higher for unfiltered plants (58 percent vs. 43 percent). This result may be influenced by sampling noise, since the number of unfiltered plants participating in the ICR is small (12 unfiltered vs. 338 filtered plants).

Exhibit 4.2 Cumulative Distribution of Plant-Mean *Cryptosporidium* Concentrations for Filtered Plants for All Source Water Types—ICR Observed Results

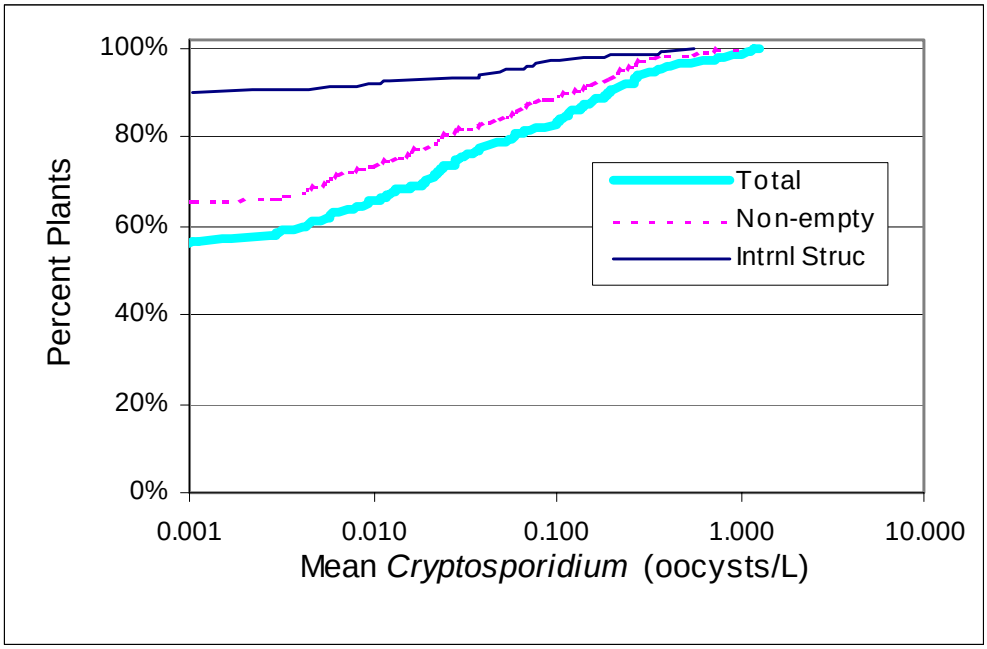
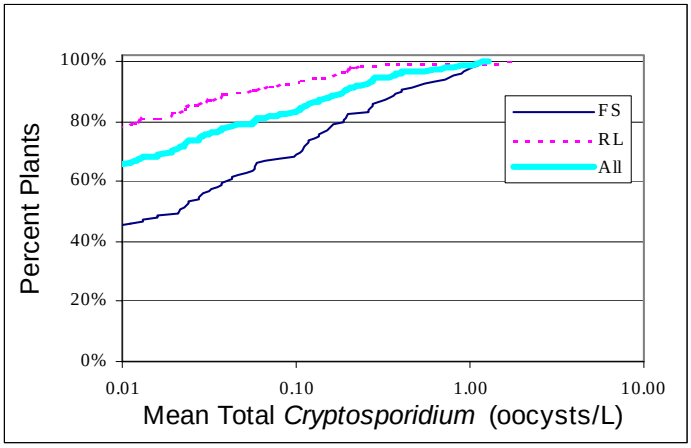
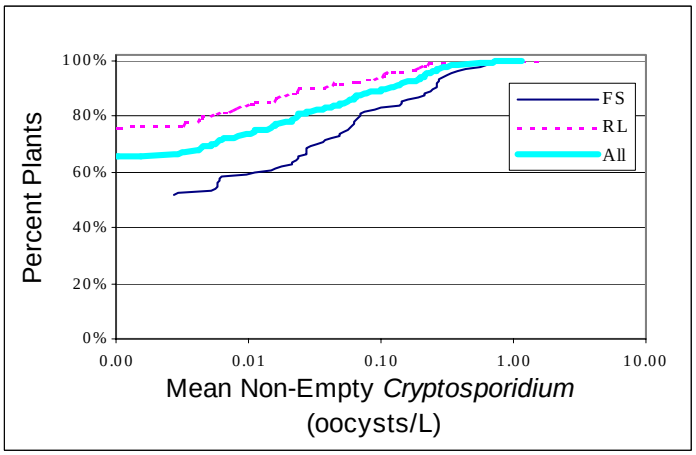


Exhibit 4.3 Cumulative Distribution of Plant-Mean *Cryptosporidium* Concentrations by Source Water Type—ICR Observed Results





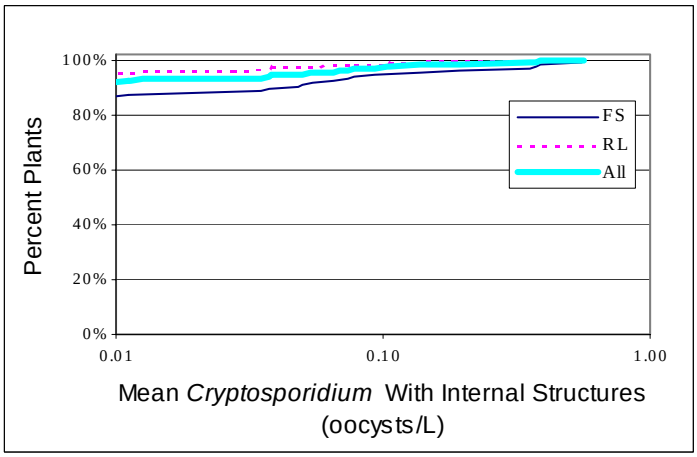


Exhibit 4.4 Summary of ICR *Cryptosporidium* Data for Unfiltered Plants

Total Number of Plants	Number and Percentage of Plants with at Least One Positive Sample	Observed Plant-Mean Data (oocysts/L)		
		Mean	Median	90th Percentile
Total *Cryptosporidium* Oocysts				
12	7 (58%)	0.002	0.001	0.005
Non-Empty Oocysts				
12	6 (50%)	0.002	0.001	0.005
Oocysts with Internal Structures				
12	2 (17%)	0.0002	0	0.001

4.1.1.2 ICRSS Results

Exhibit 4.5 summarizes the ICRSS *Cryptosporidium* data from large and medium filtered plants over the entire 12-month monitoring period, by oocyst structure and source water type. Exhibit 4.6 plots cumulative distributions for the same data without the breakout by system size and source water type.

Exhibit 4.5 Summary of ICRSS *Cryptosporidium* Data

Source	Total Number of Plants	Number and Percentage of Plants with at Least One Positive Sample	Observed Plant-Mean Data (oocysts/L)		
			Mean	Median	90th Percentile
Total *Cryptosporidium* Oocysts					
All	80	68 (85%)	0.06	0.02	0.10
Large	40	34 (85%)	0.04	0.02	0.10
Medium	40	34 (85%)	0.08	0.02	0.11
FS	33	32 (97%)	0.09	0.04	0.11
RL	41	32 (78%)	0.04	0.01	0.06

Source	Total Number of Plants	Number and Percentage of Plants with at Least One Positive Sample	Observed Plant-Mean Data (oocysts/L)		
			Mean	Median	90th Percentile
Non-Empty Oocysts					
All	80	66 (83%)	0.05	0.01	0.08
Large	40	34 (85%)	0.03	0.01	0.08
Medium	40	32 (80%)	0.06	0.01	0.09
FS	33	32 (97%)	0.07	0.03	0.08
RL	41	31 (76%)	0.03	0.01	0.06
Oocysts with Internal Structures					
All	80	41 (51%)	0.02	0.004	0.03
Large	40	20 (50%)	0.01	0.002	0.03
Medium	40	21 (53%)	0.03	0.006	0.06
FS	33	22 (67%)	0.03	0.04	0.04
RL	41	17 (42%)	0.02	0	0.03

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

Cryptosporidium total oocysts were detected in 14 percent of samples overall. *Cryptosporidium* results were highly variable, and plant-mean concentrations for total oocysts ranged from a low value of 0 oocysts/L to a high value of 12.1 oocysts/L, with a median concentration of 0.02 oocysts/L. Eighty-five percent of ICRSS plants detected *Cryptosporidium* in at least one sample, well beyond the equivalent 43 percent of plants detecting oocysts in the ICR. Approximately half of the plants detected *Cryptosporidium* oocysts with internal structures in at least one sample. Given the larger average sample volume analyzed in the ICRSS and the higher recovery rates of laboratory Method 1622/1623, one would expect a higher proportion of plants to detect *Cryptosporidium* in the ICRSS than in the ICR. Although more plants did detect *Cryptosporidium* in the ICRSS, it is also possible that the source waters sampled during the ICRSS contained higher concentrations of *Cryptosporidium*, leading to higher detection rates.

Exhibit 4.5 also shows that the mean of the plant-mean concentrations was approximately 0.06 oocysts/L for total *Cryptosporidium* oocysts and was only slightly lower at 0.05 oocysts/L for non-empty oocysts. This implies that most of the *Cryptosporidium* oocysts identified in the ICRSS were *not* empty shells. The mean of the plant-mean concentration of oocysts with internal structures was 0.02 oocysts/L. Plant-mean concentrations in the upper 10th percentile of concentrations for oocysts with internal structures ranged from 0.03 to 0.06 oocysts/L.

In general, as shown in Exhibits 4.5 and 4.7, the occurrence of *Cryptosporidium* oocysts in the ICRSS was greater in flowing stream plants than in reservoir/lake plants: 97 percent of flowing stream plants had at least one sample in which *Cryptosporidium* was detected, while *Cryptosporidium* was detected at 78 percent of reservoir/lake plants. The plant-mean concentration of 0.09 oocysts/L for flowing stream plants is more than double the plant-mean of 0.04 oocysts/L for the reservoir/lake plants.

The 90th percentile to the maximum plant-mean of total *Cryptosporidium* concentrations ranged from 0.11 to 1.18 oocysts/L for flowing stream plants and from 0.06 to 0.40 oocysts/L for reservoir/lake plants. Similar patterns were evident for non-empty oocysts and oocysts with internal structures. The 90th percentile to the maximum of plant-mean concentrations of oocysts with internal structures ranged from 0.04 to 0.38 oocysts/L for flowing stream plants and 0.03 to 0.26 oocysts/L for reservoir/lake plants.

Although *Cryptosporidium* oocysts were detected by a much larger percentage of plants in the ICRSS, the mean plant-mean concentrations and the plant-mean concentrations on the high end of the distributions are substantially lower in the ICRSS than in the ICR. For example, the 90th percentile for total *Cryptosporidium* for all sources in the ICRSS is 0.10 oocysts/L versus 0.194 oocysts/L for the same category in the ICR. It is not possible to determine whether this difference was driven by smaller sample size (only 80 plants participated in the ICRSS), more precise sampling and testing, or true concentration differences in the source waters at the time of sampling.

The distributions of plant-mean total *Cryptosporidium* concentrations were similar between large and medium plants, as shown in Exhibit 4.8, except at the upper end of the distribution. This difference was attributable to several high measurements at two medium plants, which also contributed to the difference in the means of plant-mean concentrations—the mean of 0.08 oocysts/L for medium plants is double that of 0.04 oocysts/L for large plants. The maximum plant mean concentrations (not shown in Exhibit 4.5) are approximately one order of magnitude greater than the 90th percentiles.

Exhibit 4.6 Cumulative Distributions of Plant-Mean *Cryptosporidium* Concentrations for Total, Non-Empty, and Internal Oocysts for All Sources—ICRSS Observed Data

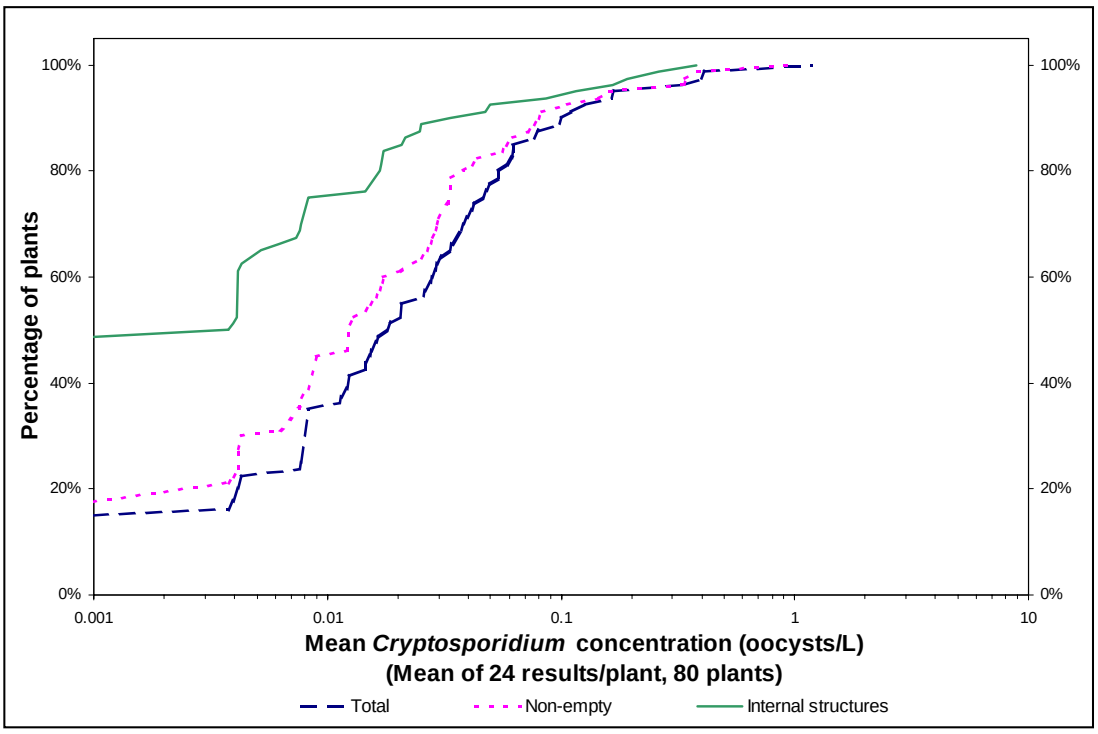
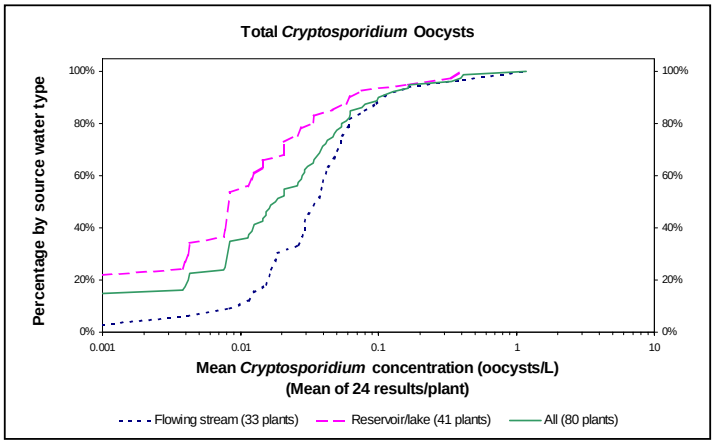
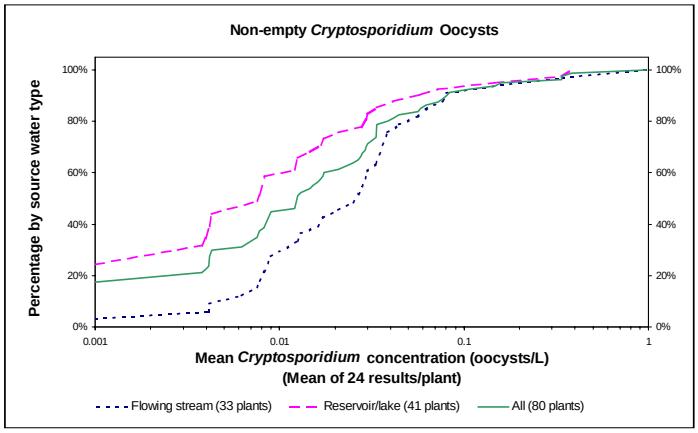


Exhibit 4.7 Cumulative Distribution of Plant-Mean *Cryptosporidium* Concentrations for Total, Non-Empty, and Internal Oocysts by Source Water Type—ICRSS Observed Data





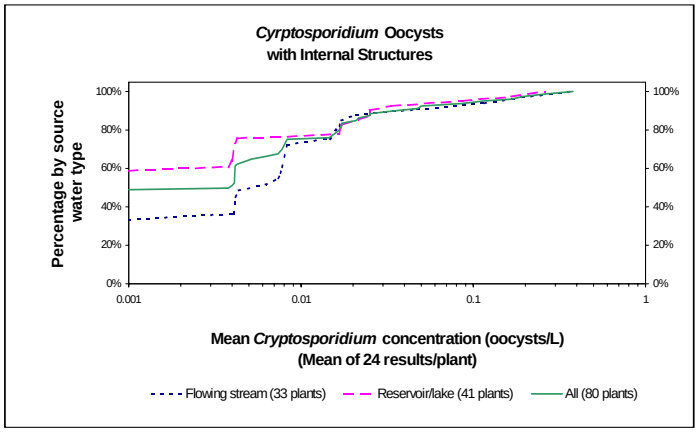
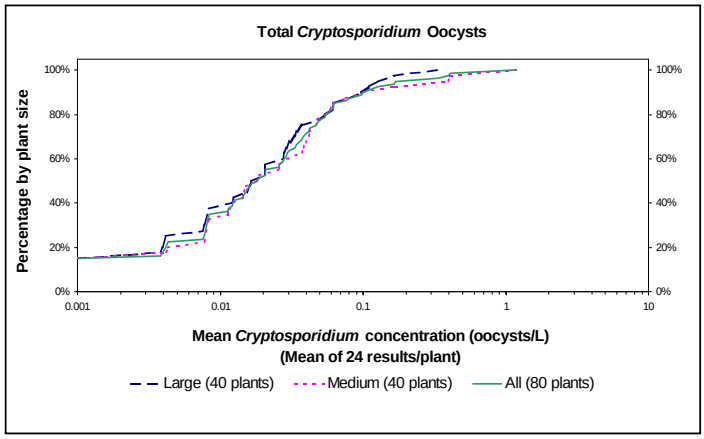
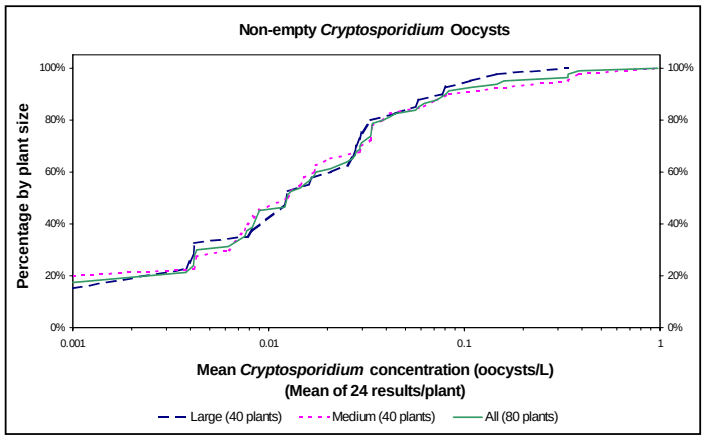
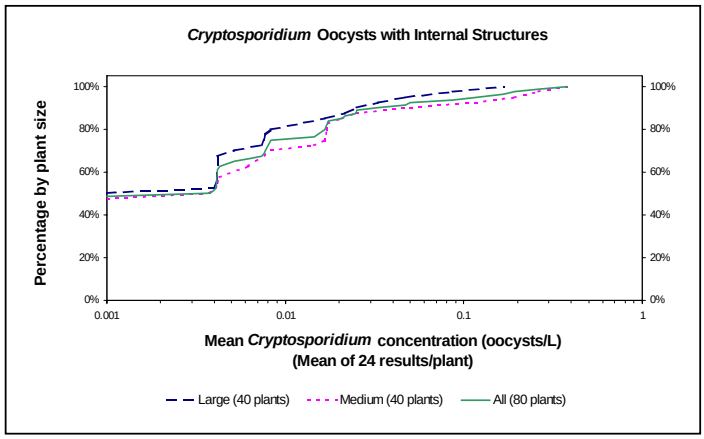


Exhibit 4.8 Cumulative Distribution of Plant-Mean *Cryptosporidium* Concentrations for Total, Non-Empty, and Internal Oocysts by Plant Size–ICRSS Observed Data







4.1.2 Modeled Results

A hierarchical model with Bayesian parameter estimation techniques was used to model the source water occurrence of *Cryptosporidium* and *Giardia* in an attempt to account for some of the variability and limitations in the data collection (previously discussed in Chapter 3). A summary of this model with a description of the graphical display is presented in Section 3.3.3.2. The model is described in further detail in Appendix B. As noted previously, EPA developed both a full and a reduced-form of the model. The majority of the description that follows here addresses the full form of the model for filtered systems and the reduced form for unfiltered systems.

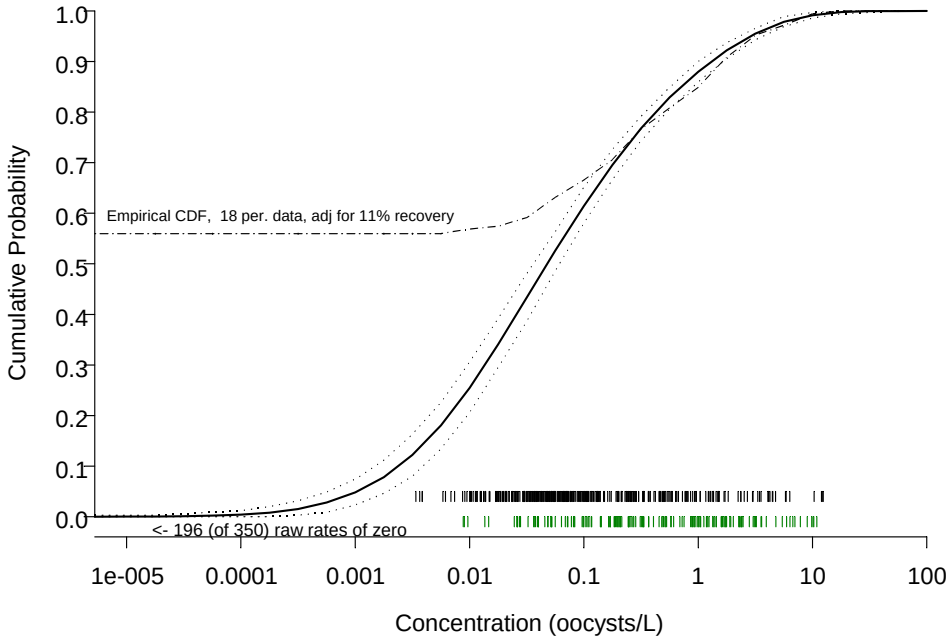
For the modeling effort, each of three separate data sets (ICR, ICRSS Large Systems, or ICRSS Medium Systems) were used to estimate occurrence of either Cryptosporidium or Giardia in drinking water sources. Each analysis produced a large sample of occurrence distributions. Each single occurrence distribution from that large set describes variability in the average concentration among the nation's drinking water sources. The large "sample" of such distributions, taken as a whole, reveals uncertainty that is due to limited data and limitations in the analysis methods. This section summarizes the modeled results, reporting summary statistics and graphics that depict both variability and uncertainty. Note, as with many modeling efforts of this type, the scope of the uncertainty analysis is constrained by the specific distributional assumptions adopted in performing the hierarchical modeling, and therefore results obtained from the analysis represent a lower bound on the overall uncertainty.

4.1.2.1 Modeled Results for ICR Data

Exhibit 4.9 displays the estimated cumulative distribution of total *Cryptosporidium* for all source waters (including unfiltered plants). Appendix E contains the graphs of modeled occurrence for all source water and microbial categories.

The lower part of the curve is built on the model's assumptions, since all observations below 0.01 oocysts/L were zero counts. The model is not limited by the observations of zero below that level and predicts a positive concentration for nearly all the observed zero counts. The observed data consisted of 196 plant-means (out of 350) with zero concentration. For *Cryptosporidium*, the model assigned a very small percentage of true zeroes for the zero count observations, causing the 196 plant-means to have a positive mean concentration. This also slightly increased the modeled plant-means for some of the low observed plant-mean concentrations that had zero count observations in the 18-month period.

Exhibit 4.9 Cumulative Distributions of Total *Cryptosporidium* in All Source Water Types—ICR Modeled Data



At the upper portion of the curve the empirical cumulative distribution function converges with the predicted "true" distribution. The median of reported "detection limits" was 0.2 oocysts/L, which is approximately where the empirical distribution begins to follow the modeled distribution. Above 0.2 oocysts/L there are slightly more high concentration source waters of observed data than the predicted data.

The modeled results shown in Exhibit B.9 reflect the median (solid line) and 90% confidence bounds (dotted lines) of the model output. These were obtained by ordering the results from the 1,000 iterations at each concentration value and using the median of those for the solid line and the 5th and 95th percentile values for the lower and upper bounds.

From these distributions of probable source water occurrence, statistics were calculated for all source water types and microbial categories. Exhibits 4.10 and 4.11 summarize the results of the ICR modeled data; the discussion following the exhibit refers to those results.

Exhibit 4.10 Summary of ICR *Cryptosporidium* Modeled Data for All Plants

Source	Modeled Plant-Mean Data (oocysts/L)		
	Mean	Median	90th Percentile
Total *Cryptosporidium*			
All	0.57	0.048	1.3
FS	1.2	0.20	3.2
RL	0.25	0.021	0.46
Non-Empty Oocysts			
All	0.31	0.024	0.63
FS	0.60	0.081	1.4
RL	0.16	0.014	0.28
Oocysts with Internal Structures			
All	0.10	0.00062	0.069
FS	0.23	0.0025	0.27
RL	0.025	0.00068	0.023

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake
Note: Modeled values in Exhibit 4.10 account for low recovery rate and should not be compared directly to similar statistics presented elsewhere for observed results, which were produced using observations without adjustments for recovery.

Exhibit 4.11 Summary of ICR *Cryptosporidium* Modeled Data for Filtered and Unfiltered Plants

Source	Modeled Plant-Mean Data (oocysts/L)		
	Mean	Median	90th Percentile
Total *Cryptosporidium*			
Filtered	0.59	0.052	1.4
Unfiltered	0.014	0.0079	0.033

For all plants the median and 90th percentile values for total *Cryptosporidium* were 0.048 and 1.3 oocysts/L, respectively. The mean concentration was 0.57 oocysts/L. (This value is not marked on the graph.) The mean was considerably greater than the median concentration, as expected, because of the predicted large number of low concentrations and the presence of a few very high data points.

The median concentrations of non-empty *Cryptosporidium* oocysts (amorphous plus oocysts with internal structures) (Exhibits E.3, E.6, and E.11) were 50, 40, and 67 percent, respectively, of the median concentration of total *Cryptosporidium* oocysts for the three source water categories (all, flowing stream,

and reservoir/lakes). The difference between the total and non-empty categories was greatest for flowing streams and least for reservoir/lake plants (i.e., a higher percentage of empty oocysts occurred in flowing stream plants). The median concentrations of oocysts with internal structures (Exhibits E.4, E.7, and E.10) were about 1 to 3 percent of the total *Cryptosporidium* concentration for flowing streams and reservoirs/lakes, respectively.

Cryptosporidium concentrations in flowing stream plants were much higher than in reservoir/lake plants. The median concentration of total *Cryptosporidium* was 0.2 oocysts/L for flowing streams, compared to 0.021 oocysts/L for reservoir/lake plants. The 90th percentile values were 3.16 and 0.46 oocysts/L, respectively, for flowing stream and reservoir/lake plants.

Because unfiltered sources have higher water quality than most reservoir/lake and flowing stream sources, occurrence was modeled for the subsets of filtered sources and unfiltered sources (see Exhibit 4.11). The occurrence of *Cryptosporidium* was estimated to be substantially lower for unfiltered sources. Unfiltered systems must have watershed protection programs and source waters that meet very low turbidity (5 NTU) and low coliform limits.

4.1.2.2 Modeled Results for ICRSS Data

Exhibits 4.12 and 4.13 display the predicted "true" cumulative distribution of total *Cryptosporidium* for ICRSS large and medium plants. Appendix E contains the cumulative distributions of each source water category and *Cryptosporidium* category. The gap between the empirical ICRSS data and modeled ICRSS data (seen by comparing Exhibits 4.5 and 4.14) is less than that for the ICR because the ICRSS observed data had fewer plant-means with zero concentrations.

Exhibit 4.12 Cumulative Distribution of Modeled Data—ICRSS Large Plants

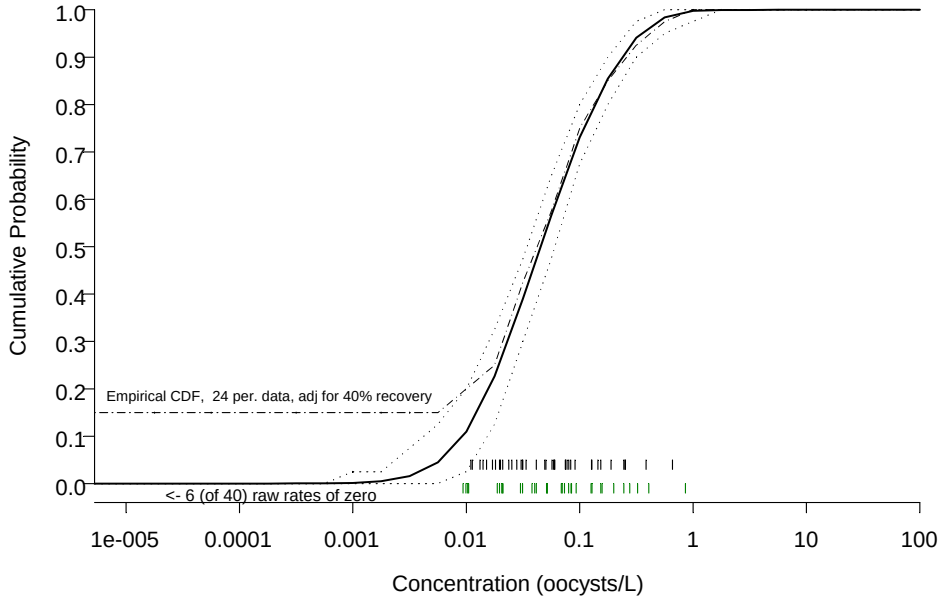
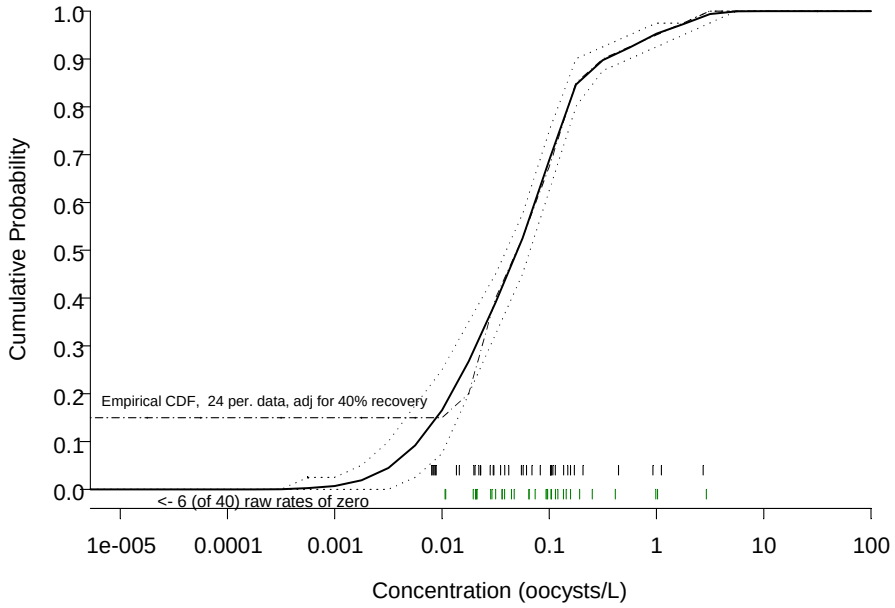


Exhibit 4.13 Cumulative Distribution of Modeled Data—ICRSS Medium Plants



A comparison of the large and medium plant distributions in Exhibits 4.12 and 4.13 indicates the medium plants have a slightly wider distribution. However, the medium central estimate curve falls within the 90 percent credible limits of the large plants, except in the approximate 90-95 percent cumulative probability range, where the medium plants have higher concentrations.

Exhibit 4.14 summarizes the results of the Bayesian analysis using ICRSS data, categorized by plant size and source type.

Exhibit 4.14 Summary of ICRSS *Cryptosporidium* Modeled Data

Source	Plant Size	Modeled Plant-Mean Data (oocysts/L)		
		Mean	Median	90th Percentile
Total *Cryptosporidium*				
All	Large	0.094	0.045	0.24
All	Medium	0.19	0.050	0.33
FS	Large	0.10	0.077	0.22
FS	Medium	0.32	0.095	0.88
RL	Large	0.087	0.030	0.19
RL	Medium	0.083	0.023	0.16
Non-Empty Oocysts				
All	Large	0.077	0.036	0.18
All	Medium	0.16	0.031	0.31
FS	Large	0.071	0.055	0.15
FS	Medium	0.25	0.060	0.72
RL	Large	0.084	0.026	0.18
RL	Medium	0.073	0.016	0.14
Oocysts with Internal Structures				
All	Large	0.030	0.0079	0.067
All	Medium	0.072	0.0072	0.18
FS	Large	0.018	0.0099	0.042
FS	Medium	0.11	0.019	0.43
RL	Large	0.041	0.0070	0.11
RL	Medium	0.038	0.0048	0.049

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

The median and 90th percentiles for total *Cryptosporidium* were 0.045 and 0.24 oocysts/L for large plants and 0.05 and 0.33 oocysts/L for medium plants. Although medium and large plants had similar median values, the mean value for medium plants is much higher than that for large plants, because the values at the upper end of the medium plant distribution are higher. The medium plants had greater concentrations than the large plants for flowing streams, with medians of 0.095 and 0.077 oocysts/L and 90th percentiles of 0.88 and 0.22 oocysts/L, for medium and large plants, respectively. This pattern was not evident for reservoir/lake—the median and 90th percentiles were slightly greater with large plants.

The median concentrations of non-empty oocysts in all source water types were approximately 80 and 60 percent of the total *Cryptosporidium* median for large and medium plants, respectively. Oocysts with internal structures were a small portion of the non-empty oocysts, 18 and 14 percent for large and medium plants calculated with the medians of all source water types.

4.2 *Giardia*

4.2.1 Observed Results

Observed *Giardia* monitoring results are presented below for plant-mean data. In general, *Giardia* cysts occurred in almost three times as many samples and in higher concentrations than *Cryptosporidium* oocysts did. Unlike the data for *Cryptosporidium*, *Giardia* concentrations between 0 and 0.01 cysts/L were reported.

The ICR *Giardia* data analyses in Appendix C describe statistics for all monthly observations (as compared to plant-mean data) for each source water category.

4.2.1.1 ICR Monitoring Program Results

Exhibits 4.15 and 4.16 summarize the ICR *Giardia* results for the entire monitoring period, categorized by cyst structure, source water type, and treatment.

Giardia was found in 18 percent of all laboratory samples and in 64 percent of all plants (filtered and unfiltered). Detected *Giardia* concentrations were highly dependent on the volume assayed in the laboratory, with sample concentrations ranging from 0.01 to more than 25 cysts/L. Laboratory tests showed that mean recovery of *Giardia* cysts was approximately 25 percent and varied from sample to sample. The recovery factor also caused *Giardia* concentrations and detects to be under-reported in the ICR survey.

Mean concentration of total *Giardia* (which includes empty, amorphous, cysts with internal structures, and cysts with more than one internal structure) is 0.28 cysts/L for filtered source waters. The mean concentrations of non-empty cysts are 50 percent of the mean total *Giardia*. Cysts detected with internal structures are 11 percent of the total *Giardia*, and cysts with more than one internal structure are 7 percent of total *Giardia*, calculated from the means. Exhibit 4.17 displays the cumulative distributions of each cyst category for all filtered source water types.

Exhibit 4.15 Summary of ICR *Giardia* Data for Filtered Plants

Source	Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Data (cysts/L)		
			Mean	Median	90th Percentile
Total *Giardia*					
All	338	218 (64%)	0.282	0.024	0.815
FS	128	110 (86%)	0.625	0.328	1.633
RL	206	105 (51%)	0.054	0.002	0.144
Non-Empty Cysts					
All	338	179 (53%)	0.141	0.004	0.423
FS	128	97 (76%)	0.305	0.085	0.638
RL	206	79 (38%)	0.034	0	0.064
Cysts with Internal Structures					
All	338	58 (17%)	0.031	0	0.023
FS	128	36 (28%)	0.070	0	0.118
RL	207	19 (9%)	0.006	0	0
Cysts with More Than One Internal Structure					
All	338	43 (13%)	0.020	0	0.007
FS	128	27 (21%)	0.046	0	0.087
RL	206	14 (7%)	0.004	0	0

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

Exhibit 4.16 Summary of ICR *Giardia* Data for Unfiltered Plants

Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Data (cysts/L)		
		Mean	Median	90th Percentile
Total *Giardia*				
12	10 (83%)	0.026	0.014	0.054
Non-Empty Cysts				
12	10 (83%)	0.019	0.012	0.034
Cysts with Internal Structures				
12	5 (42%)	0.002	0	0.005
Cysts with More Than One Internal Structure				
12	3 (25%)	0.001	0	0.002

In general, *Giardia* was detected more often and in larger concentrations in flowing stream plants than in reservoir/lake plants. Based on total *Giardia* results, 86 percent of flowing stream plants detected *Giardia* compared to 51 percent of reservoir/lake plants. The concentrations were also greater for flowing stream plants, as plant mean concentrations were more than 10 times as high (mean of 0.625 as compared to 0.054 cysts/L). The 50 highest reported *Giardia* concentrations were all from flowing stream plants, with a maximum value greater than 25 cysts/L. The highest reported concentration for a reservoir/lake plant was approximately 5.4 cysts/L (Exhibit C.6). Exhibit 4.18 displays the cumulative distributions of each cyst category for each source water type.

Exhibit 4.17 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for All Source Water Types—ICR Observed Results

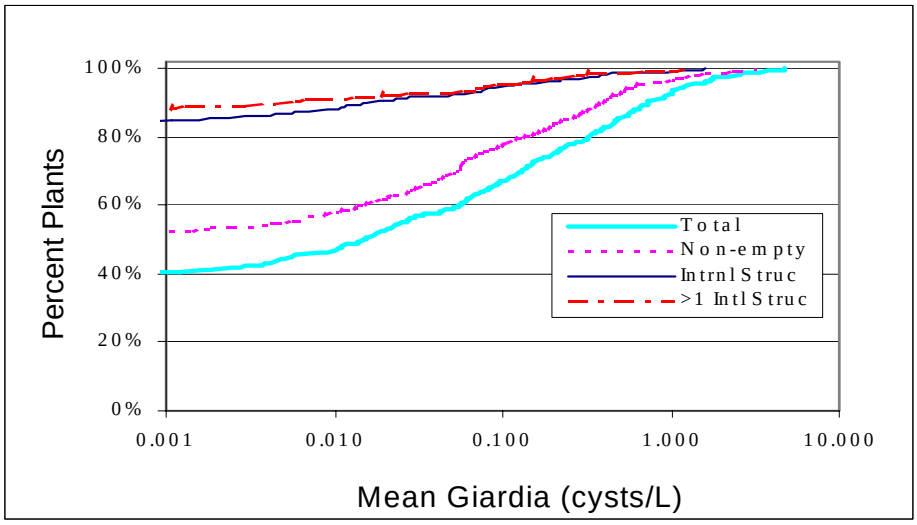
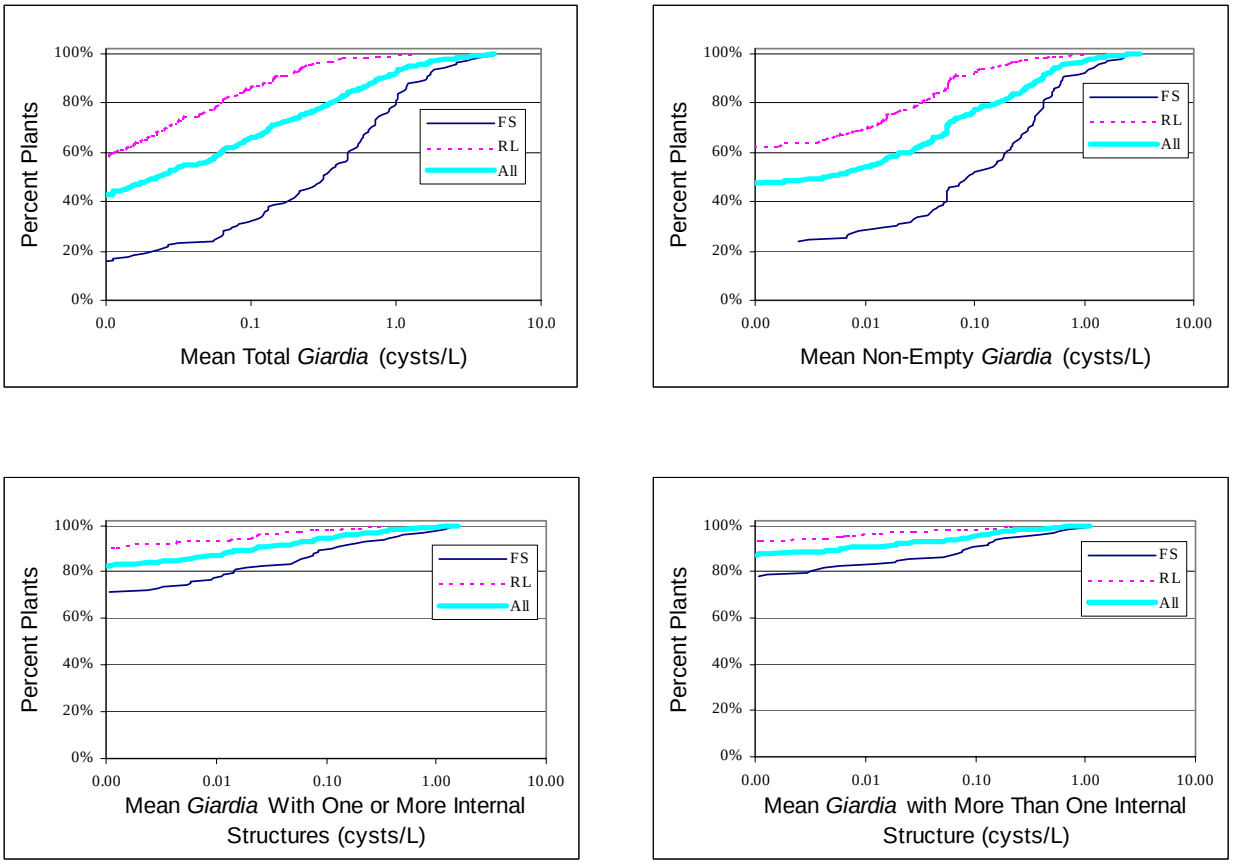


Exhibit 4.18 Cumulative Distribution of Plant-Mean *Giardia* Concentrations by Source Water Types—ICR Observed Results



4.2.1.2 ICRSS Results

Exhibit 4.19 summarizes the *Giardia* data from the ICRSS by source water type and cyst structure. Note that *Giardia* data were not collected for the first 4 months of the ICRSS monitoring period. All ICRSS *Giardia* data analyses included data from both large and medium plants.

Exhibit 4.19 Summary of ICRSS *Giardia* Data

Source	Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Data (cysts/L)		
			Mean	Median	90th Percentile
Total *Giardia*					
All	80	66 (83%)	0.27	0.06	0.74
Large	40	33 (83%)	0.30	0.07	0.72
Medium	40	33 (83%)	0.24	0.06	0.90
FS	33	31 (94%)	0.55	0.29	1.30
RL	41	30 (73%)	0.07	0.02	0.13
Non-Empty Cysts					
All	80	65 (81%)	0.21	0.05	0.49
Large	40	32 (80%)	0.23	0.05	0.42
Medium	40	33 (83%)	0.18	0.04	0.74
FS	33	31 (94%)	0.43	0.21	0.98
RL	41	29 (71%)	0.05	0.01	0.09
Cysts with One or More Internal Structures					
All	80	55 (69%)	0.08	0.02	0.24
Large	40	28 (70%)	0.08	0.02	0.20
Medium	40	27 (68%)	0.09	0.02	0.37
FS	33	31 (94%)	0.18	0.06	0.47
RL	41	20 (49%)	0.02	0	0.03
Cysts with More Than One Internal Structure					
All	80	45 (56%)	0.03	0.01	0.08
Large	40	22 (55%)	0.03	0.01	0.05
Medium	40	23 (58%)	0.04	0.01	0.12
FS	33	29 (88%)	0.07	0.02	0.24
RL	41	14 (34%)	0.01	0	0.01

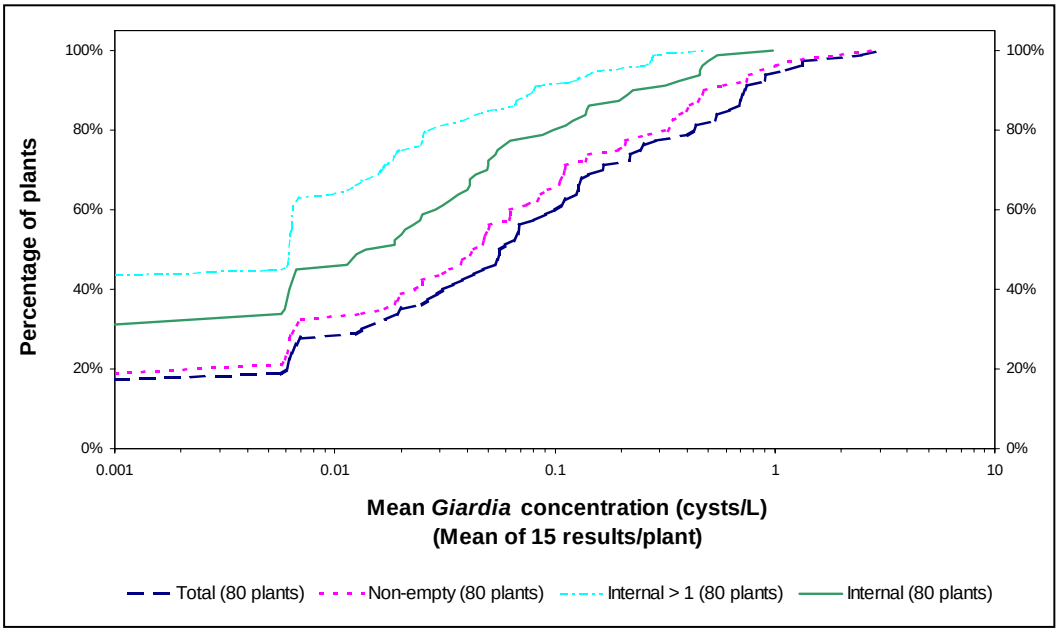
All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

Exhibit 4.20 shows the distribution of plant-mean *Giardia* concentrations for total cysts, non-empty cysts, cysts with one or more internal structures (henceforth referred to as "with internal structures"), and cysts with more than one internal structure (henceforth referred to as "with internal structures >1"). *Giardia* cysts were detected in 33 percent of all samples. In addition, 83 percent of the

utilities that participated in the surveys detected *Giardia* in at least one sample, and approximately two-thirds detected *Giardia* cysts with internal structures in at least one sample.

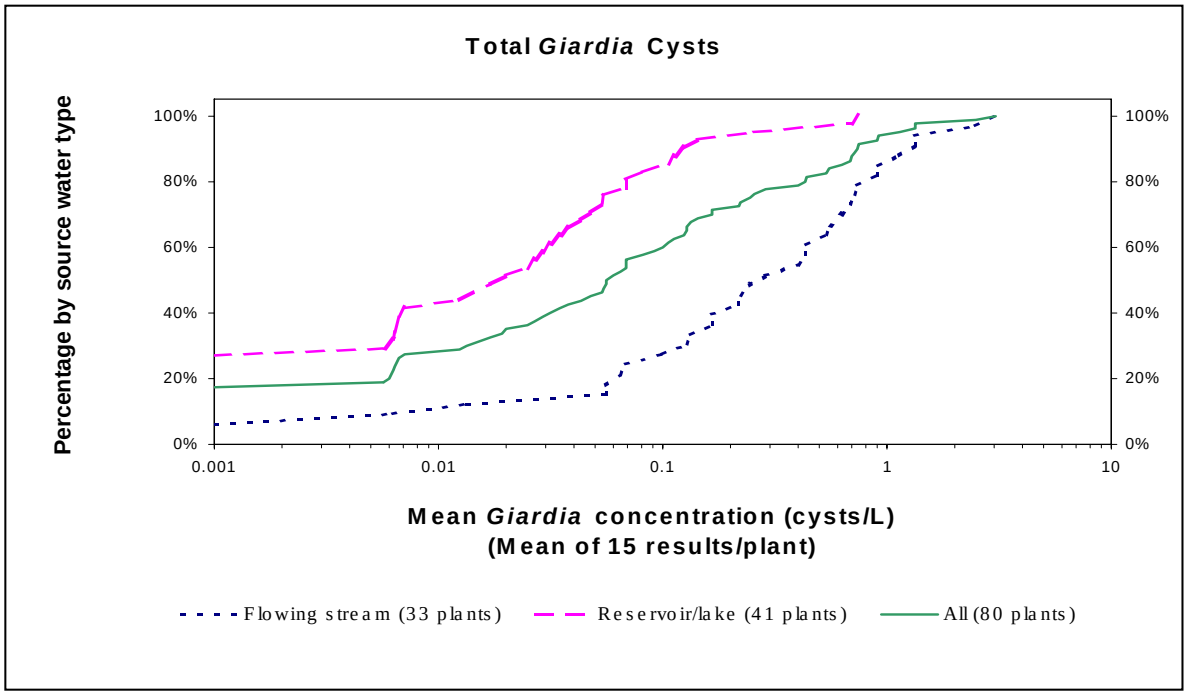
The mean of the plant-mean concentrations of total *Giardia* cysts was approximately 0.27 cysts/L. The mean of the plant-mean concentrations of non-empty cysts was only slightly lower at 0.21 cysts/L. This means that most of the *Giardia* cysts identified in the ICRSS were *not* empty shells. The mean of the plant-mean concentrations of cysts with one or more internal structures was 0.08 cysts/L. The upper 10th percentile plant-mean concentrations of cysts with internal structures ranged from 0.24 to 0.98 cysts/L.

Exhibit 4.20 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for Total, Non-Empty, Internal, and Internal >1 Cysts—ICRSS Data



In general, as seen in Exhibits 4.19, 4.21, and 4.22, the occurrence of *Giardia* cysts was greater in flowing streams than in reservoir/lake plants; cysts were detected in at least one sample for 94 percent of flowing-stream plants, compared to 73 percent for reservoir/lake plants. The mean of the plant-mean concentrations for flowing stream plants was almost an order of magnitude greater than that of reservoir/lake plants, with means of 0.55 and 0.07 cysts/L, respectively. The upper 10th percentile plant-mean concentrations of total cysts ranged from 1.30 to 1.69 cysts/L for flowing stream plants and from 0.13 to 0.48 cysts/L for reservoir/lake plants. Similar patterns were observed for plant-mean concentrations of non-empty cysts, internal cysts, and cysts with internal structures >1. For example, the upper 10th percentile plant-mean concentrations of internal cysts ranged from 0.47 to 0.99 cysts/L for flowing stream plants and 0.03 to 0.20 cysts/L for reservoir/lake plants.

Exhibit 4.21 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for Total and Non-Empty Cysts by Source Water Type—ICRSS Observed Data



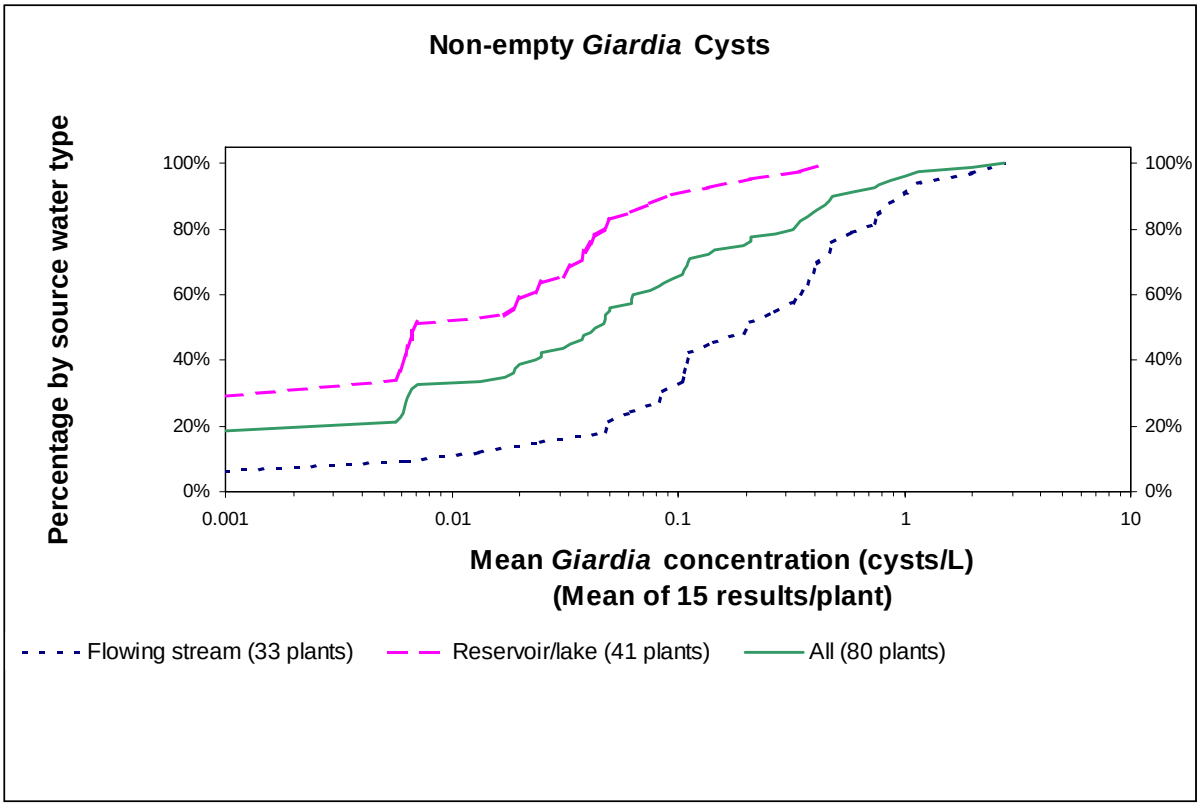
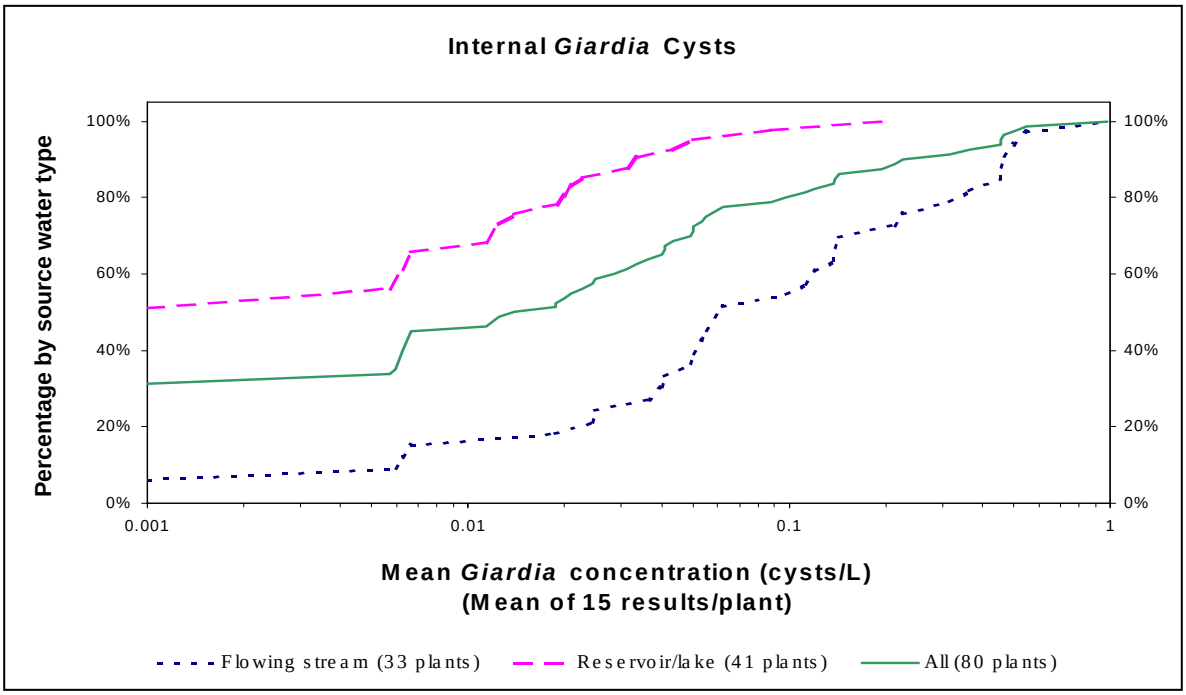
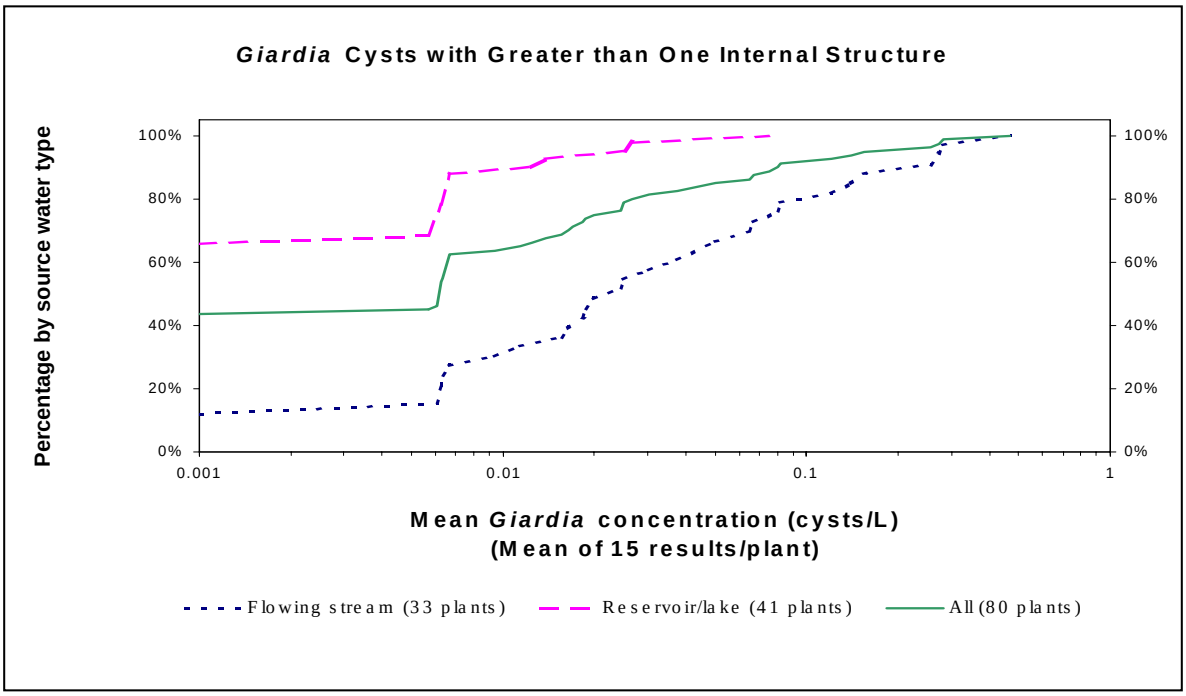


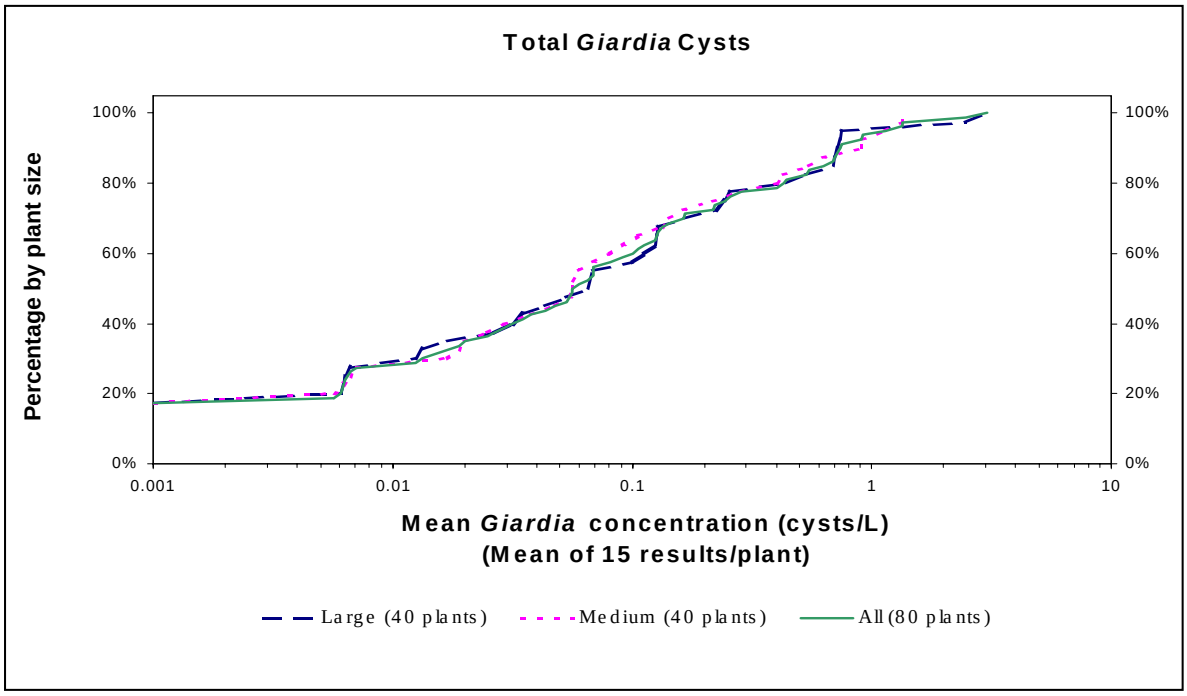
Exhibit 4.22 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for Internal and >1 Internal Structure Cysts by Source Water Type—ICRSS Observed Data





Very little difference was observed between the distribution of plant-mean *Giardia* concentrations for large and medium plants, as shown in Exhibits 4.19, 4.23, and 4.24. The means of the plant-mean total cyst concentrations for large and medium plants were 0.30 and 0.24 cysts/L, respectively. The range of the 90th percentile for large plants was wider than the range of values from medium plants, ranging from 0.72 to 3.03 cysts/L for large plants and from 0.90 to 1.34 cysts/L for medium plants. Distribution trends of *Giardia* by plant size were similar for non-empty cysts and cysts with internal structures. For instance, the 90th percentile of plant-mean concentrations of cysts with internal structures for large and medium plants ranged from 0.20 to 0.98 cysts/L and 0.37 to 0.55 cysts/L, respectively.

Exhibit 4.23 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for Total and Non-Empty Cysts by Plant Size—ICRSS Observed Data



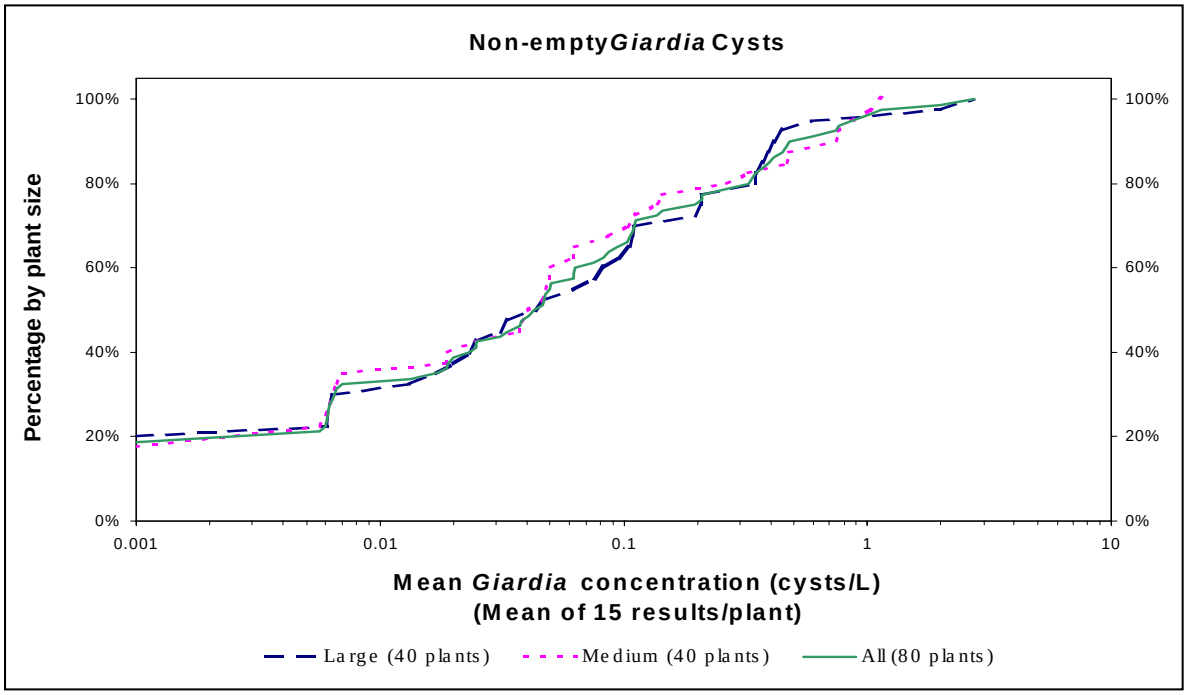
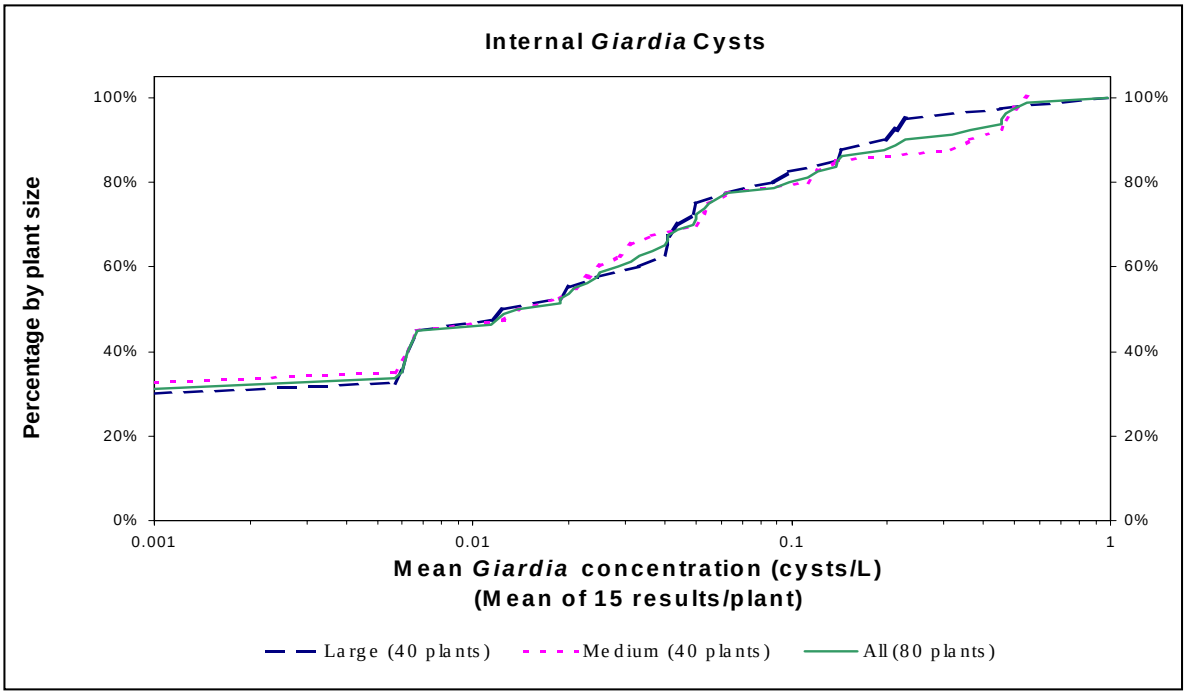
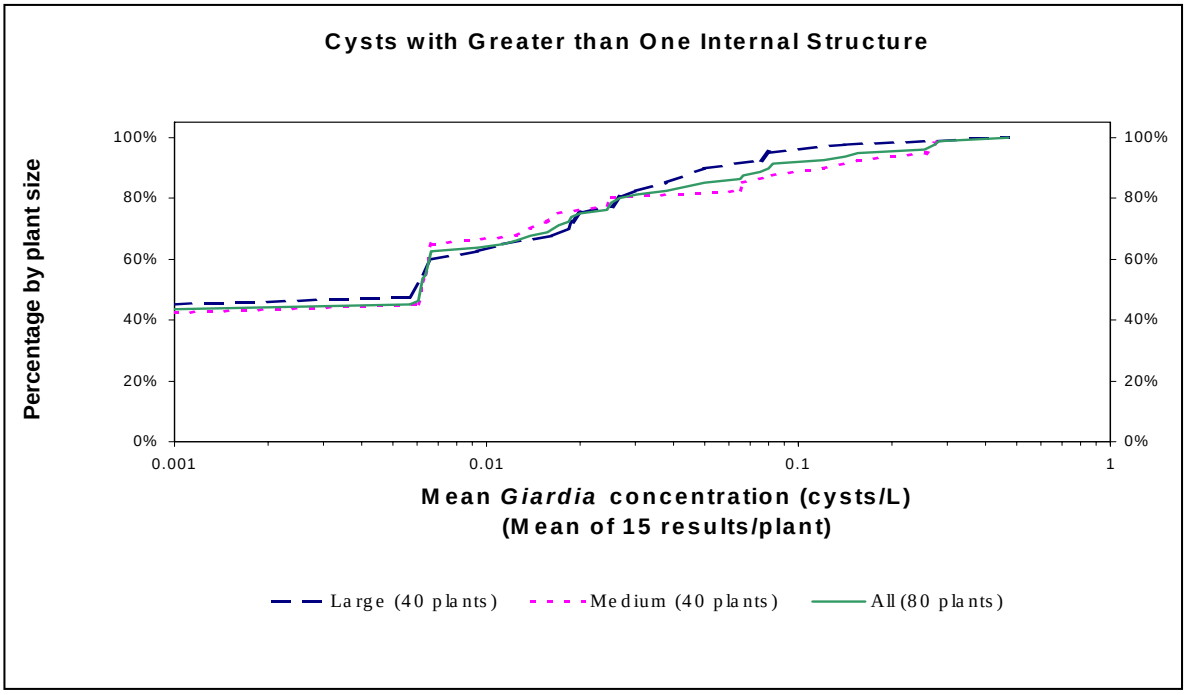


Exhibit 4.24 Cumulative Distribution of Plant-Mean *Giardia* Concentrations for Internal and >1 Internal Structure Cysts by Plant Size—ICRSS Observed Data





4.2.2 Modeled Results for ICR Data

Exhibit 4.25 displays the predicted "true" cumulative distribution of total *Giardia* for all source waters.

Exhibit 4.25 Cumulative Distribution of Total *Giardia* in All Source Water Types—ICR Modeled Data

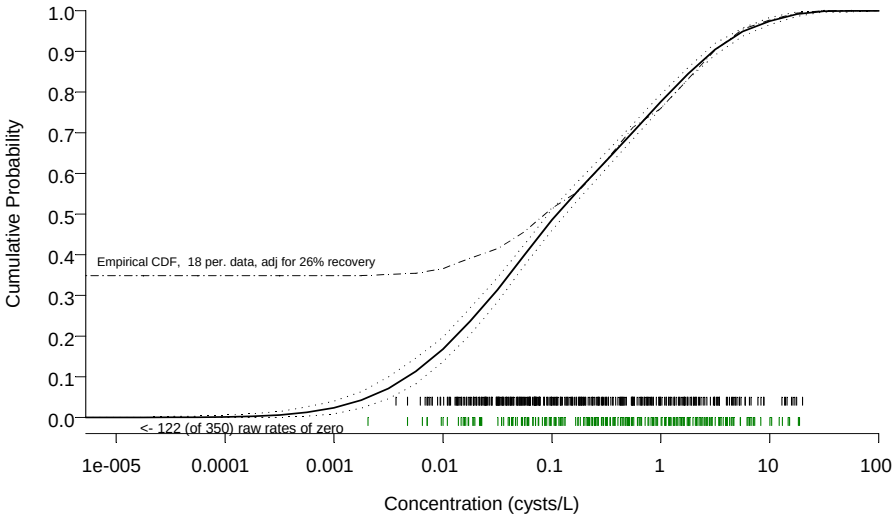


Exhibit 4.26 summarizes the results of the ICR *Giardia* modeled data; the following discussion refers to these results. Graphics of the modeled data are presented in Appendix E. Note that data modeled for *Giardia* have only one category for internal structures, whereas the ICR data collection also included the greater than one internal structure category.

Exhibit 4.26 Summary of ICR Modeled *Giardia* Data

Source	Modeled Results (cysts/L)		
	Mean	Median	90th Percentile
Total *Giardia*			
All	1.2	0.11	3.0
FS	2.5	0.72	6.5
RL	0.35	0.039	0.65
Non-Empty Cysts			
All	0.59	0.045	1.41
FS	1.1	0.22	2.8
RL	0.21	0.015	0.35
Cysts with Internal Structures			
All	0.13	0.00090	0.096
FS	0.24	0.0045	0.32
RL	0.044	0.00038	0.024

All = FS + RL+ other categories, FS = Flowing Stream, RL = Reservoir/Lake

Exhibit 4.27 Summary of ICR *Giardia* Modeled Data for Filtered and Unfiltered Plants

Source	Modeled Plant-Mean Data (cysts/L)		
	Mean	Median	90th Percentile
Total *Giardia*			
Filtered	1.2	0.11	3.1
Unfiltered	0.14	0.06	0.40

The median and 90th percentile values for total *Giardia* were 0.11 and 3.0 oocysts/L, respectively. The mean concentration was 1.2 cysts/L. The mean was considerably greater than the median concentration, as expected, because of the predicted large number of low concentrations and the presence of a few very high data points.

The median concentrations of non-empty *Giardia* cysts (amorphous plus cysts with internal structures) (Exhibits E.12, E.15, and E.18) were 41, 31, and 38 percent, respectively, of the median concentration of total *Giardia* cysts for the three source water categories (all, flowing stream, and

reservoir/lakes). The difference between the total and non-empty categories is greater for flowing streams than for reservoir/lake plants (i.e., a higher percentage of empty oocysts occurs in flowing stream plants).

Giardia concentrations in flowing stream sources were much higher than in reservoir/lake sources. The median concentration of total *Giardia* was 0.72 cysts/L for flowing streams, compared to 0.039 cysts/L for reservoir/lake plants. The 90th percentile values were 6.5 and 0.65 cysts/L, respectively, for flowing stream and reservoir/lake plants. This same pattern was evident for the non-empty and with internal structure *Giardia* categories.

Exhibit 4.27 shows the difference between predicted *Giardia* occurrence in filtered and unfiltered sources.

4.3 Viruses

Observed results for virus monitoring are shown in Exhibit 4.28 and 4.29. Fewer samples were taken for viruses than for *Cryptosporidium* and *Giardia,* because many plants were eligible for a monitoring waiver if they could demonstrate that their total coliform density was consistently less than 100 per 100 ml or that their fecal coliform/*E. coli* density was consistently less than 20 per ml during 6 consecutive months of monitoring. Although viruses were found more frequently than total *Cryptosporidium* and *Giardia* (occurring in 24 percent of samples rather than 7 and 19 percent, respectively), their concentrations were relatively low compared to concentrations of total oocysts and cysts. Total *Cryptosporidium* and *Giardia* concentrations, however, included empty oocysts and cysts, which are unlikely to be infectious. In contrast, the virus concentrations represented only those viruses capable of forming plaques (i.e., those that are infectious).

Exhibits 4.28 and 4.29 summarize the ICR virus detection data for the entire monitoring period, by source water type and treatment. Virus concentrations are expressed as most probable numbers of plaque-forming units per liter (MPN/L) (see Section 3.1.3). Appendix C contains results of all monthly observations (as compared to plant-mean data).

Exhibit 4.28 Summary of ICR Virus Results, Filtered Plants

Source	Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Plant-Mean Results (MPN/L)		
			Mean	Median	90th Percentile
All	215	181 (84%)	0.036	0.004	0.047
FS	113	104 (92%)	0.048	0.011	0.12
RL	94	70 (74%)	0.013	0.0031	0.016

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake

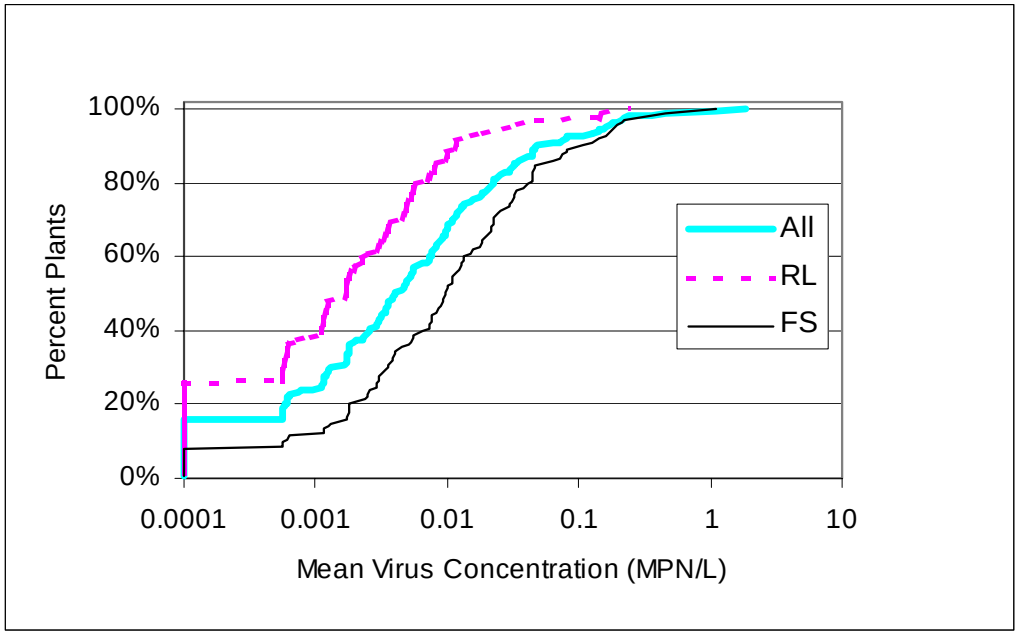
Exhibit 4.29 Summary of ICR Virus Results, Unfiltered Plants

Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Plant-Mean Results (MPN/L)	
		Observation #1	Observation #2
2	2 (100%)	0.0009	0.0012

Approximately 24 percent of all influent samples contained viruses, and 84 percent of filtered plants had virus detections (Exhibit C.10). The median of the plant-mean virus data at filtered plants was 0.0047 MPN/L for all source water types.

Flowing stream filtered plants had greater virus occurrence and higher virus concentrations than reservoir/lake plants. About 92 percent of flowing stream plants had positive plant-means, as opposed to about 74 percent in reservoir/lake plants. The mean for flowing stream plants was 0.048 MPN/L, more than three times higher than the mean for reservoir/lakes. Exhibit 4.30 displays the filtered plant-mean cumulative distributions for each source water type. Only two unfiltered plants reported virus sampling data and they were considerably lower than filtered plants.

Exhibit 4.30 Cumulative Distribution of Plant-Mean Virus Concentrations for All Source Water Types—ICR Observed Results



4.4 Indicators

4.4.1 Observed Results

Because of their low concentrations in source water, *Cryptosporidium* and *Giardia* are difficult to monitor. Technical limitations in current detection methods also contribute to the difficulties in assessing the occurrence of *Cryptosporidium* and *Giardia*. Therefore, organisms such as coliforms are commonly used as indicators of fecal contamination in drinking water. Most coliforms are relatively harmless; however, coliforms are often found in water that contains disease-producing organisms (see Section 2.5). Fecal coliforms are a subset of total coliform bacteria commonly found in the feces of warm-blooded animals. *E. coli* is considered a better indicator of fecal contamination and can be differentiated from other fecal coliforms by the production of certain enzymes, specifically beta-galactosidase and glucuronidase.

4.4.1.1 ICR Monitoring Program Results

Exhibits 4.31 and 4.32 summarize the ICR data collected for each type of indicator, by water source and treatment. Statistics are calculated from plant-mean data.

Exhibit 4.31 Summary of ICR Coliform Data for Filtered Plants

Source	Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Plant-Mean Results (CFU/100mL)		
			Mean	Median	90th Percentile
Total Coliforms					
All	340	339 (100%)	2633	276	6738
FS	125	125 (100%)	4480	1174	7140
RL	199	199 (100%)	1599	83	4540
Fecal Coliforms					
All	241	216 (90%)	276	14	417
FS	87	83 (95%)	605	101	789
RL	141	125 (89%)	118	8	87
E. coli					
All	233	209 (90%)	256	10	353
FS	83	75 (90%)	428	138	761
RL	141	127 (90%)	215	4	44

All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake, CFU = colony-forming unit

Exhibit 4.32 Summary of ICR Coliform Data for Unfiltered Plants

Number of Plants	Number of Plants with Positive Samples (Percent)	Observed Plant-Mean Results (CFU/100mL)		
		Mean	Median	90th Percentile
Total Coliforms				
12	12 (100%)	137	36	482
Fecal Coliforms				
12	12 (100%)	2	2	4
E. coli				
5	4 (80%)	1	0.33	2

Nearly all plants had a positive sample for total coliform, fecal coliform, and *E. coli* during the data collection period. Total coliform bacteria were detected in 88 percent of all filtered plant samples (Exhibit C.11a), with fecal coliforms and *E. coli* detected in approximately 66 and 63 percent of the total samples (Exhibits C.12a and C.13a).

Coliform bacteria generally were detected more often and at higher concentrations in flowing stream plants than in reservoir/lakes. Filtered plant-mean values for total coliforms in flowing streams were 180 percent higher than reservoir/lake plants (based on means). The difference between flowing streams and reservoir/lake plants was greatest with fecal coliform concentrations, which were 413 percent higher for flowing stream plants than for reservoir/lake plants. The source water concentration of coliforms from unfiltered plants was substantially lower than filtered plants.

4.4.1.2 ICRSS Results

Exhibit 4.33 summarizes the ICRSS data collected for each type of indicator and source water type.

Exhibit 4.33 Summary of ICRSS Coliform Data

Source	Number of Plants	Number of Plants with a Positive Sample (Percent)	Mean (CFU/100mL)	Median (CFU/100mL)	90th Percentile (CFU/100mL)
Total Coliform					
All	80	79 (99%)	1897	417	5848
Large	40	40 (100%)	1840	530	5848
Medium	40	39 (98%)	1955	331	4491
FS	33	33 (100%)	4051	1751	11,006
RL	41	40 (98%)	366	77	718
Fecal Coliform					
All	42	40 (95%)	432	18	795
Large	19	19 (100%)	315	9	611
Medium	23	21 (91%)	528	40	1074
FS	19	19 (100%)	937	146	2408
RL	22	20 (91%)	15	3	46
E. coli					
All	57	52 (91%)	118	9	294
Large	30	29 (97%)	95	18	227
Medium	27	23 (85%)	144	7	369
FS	22	22 (100%)	278	83	740
RL	30	26 (87%)	14	3	25

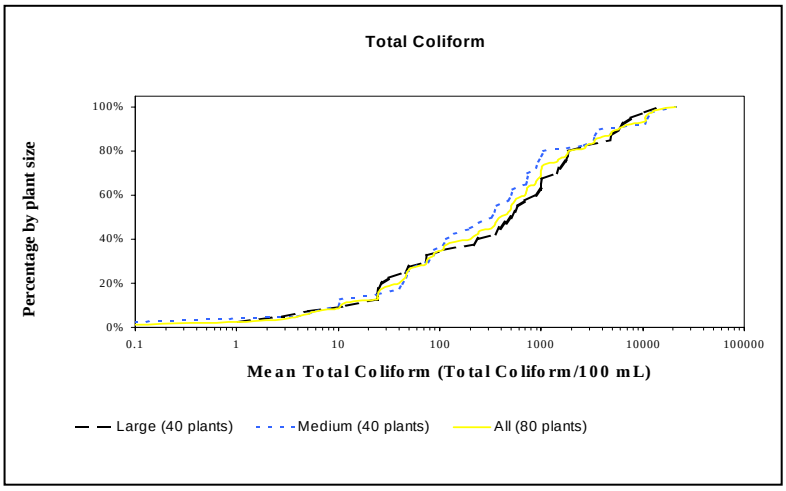
All = FS + RL + other categories, FS = Flowing Stream, RL = Reservoir/Lake, CFU = colony-forming unit

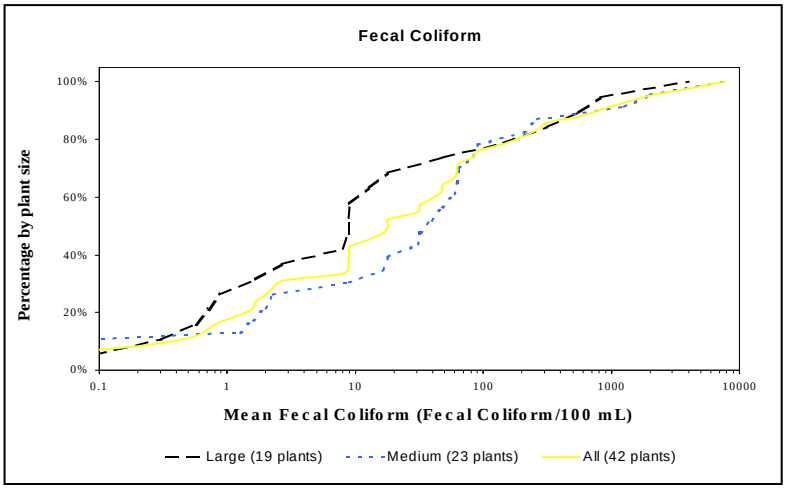
Exhibits 4.34 and 4.35 plot the distributions of plant-mean concentrations for total coliforms, fecal coliforms, *E. coli*, and the percentage of plants with a given concentration. Total coliforms were detected in 91 percent of all samples analyzed. Approximately two-thirds of the samples analyzed for fecal coliforms and *E. coli* were positive. For all three coliform categories, more than 90 percent of the plants had at least one positive sample. Coliform results in general are highly variable and, for the

ICRSS, ranged several orders of magnitude. Often, a few very high results had a strong impact on the mean values.

Coliform bacteria generally were detected more often and at higher concentrations in flowing stream plants than in reservoir/lake plants. The means of the total coliform plant-mean concentrations were 4,051 colony forming units (CFU)/100 mL for flowing stream plants and 366 CFU/100 mL for reservoir/lake plants. The means of the fecal coliform plant-mean concentrations for flowing stream and reservoir/lake plants were 937 CFU/100 mL and 15 CFU/100 mL, respectively. The means of the *E. coli* plant-mean concentrations for flowing stream and reservoir/lake plants were 278 and 14 CFU/100 mL, respectively.

Exhibit 4.34 Cumulative Distribution of Plant-Mean Coliform Concentrations by Plant Size—ICRSS Observed Data





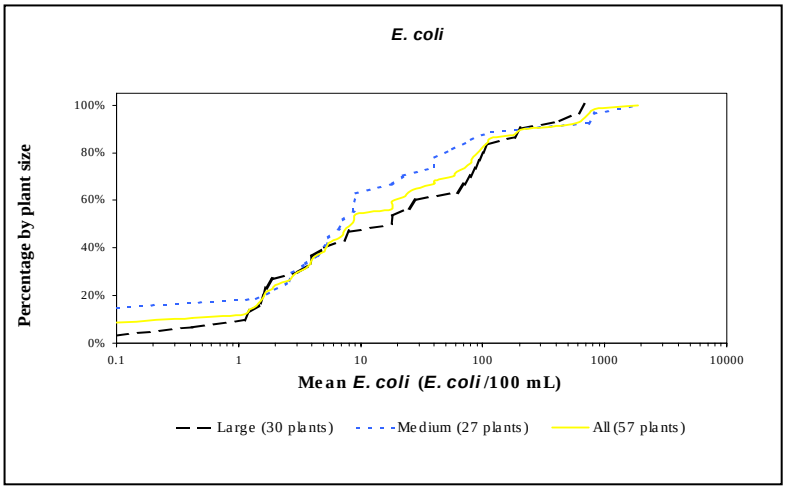
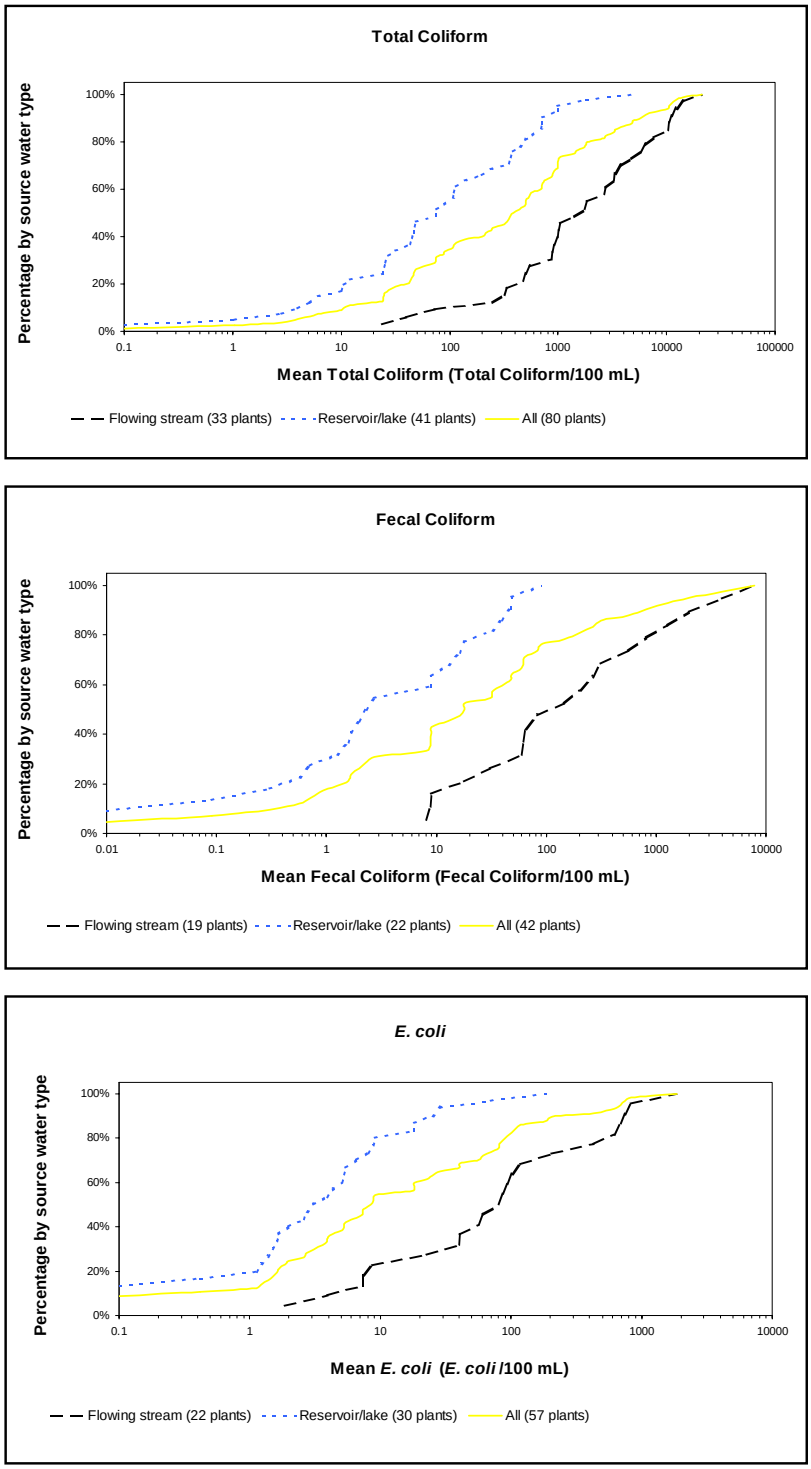


Exhibit 4.35 Cumulative Distribution of Plant-Mean Coliform Concentrations by Source Water Type—ICRSS Observed Data



The distributions of large and medium plant-mean concentrations were very similar for total coliforms, fecal coliforms, and *E. coli*, with the exception of a small difference around the medians for fecal coliforms and *E. coli*. The mean of the total coliform plant-mean concentrations for large plants was 1,840 CFU/100 mL; the mean of the medium plant-mean concentrations was 1,955 CFU/100 mL. Fecal coliform plant-means for large and medium plants were 315 and 528 CFU/100 mL, respectively. For *E. coli*, the mean of the plant-mean concentrations was 95 CFU/100 mL for large plants and 144 CFU/100 mL for medium plants.

EPA asked utilities participating in the ICRSS to analyze samples for total coliforms and *E. coli*, but permitted them to analyze samples for fecal coliforms rather than *E. coli* if they did not have *E. coli* analysis capability. (Several utilities submitted results for both parameters.) Because not all plants provided data on *E. coli* or fecal coliforms, caution should be used when analyzing data for these parameters, because differences between *E. coli*, fecal coliform, and total coliform concentrations could be the result of utility-specific effects.

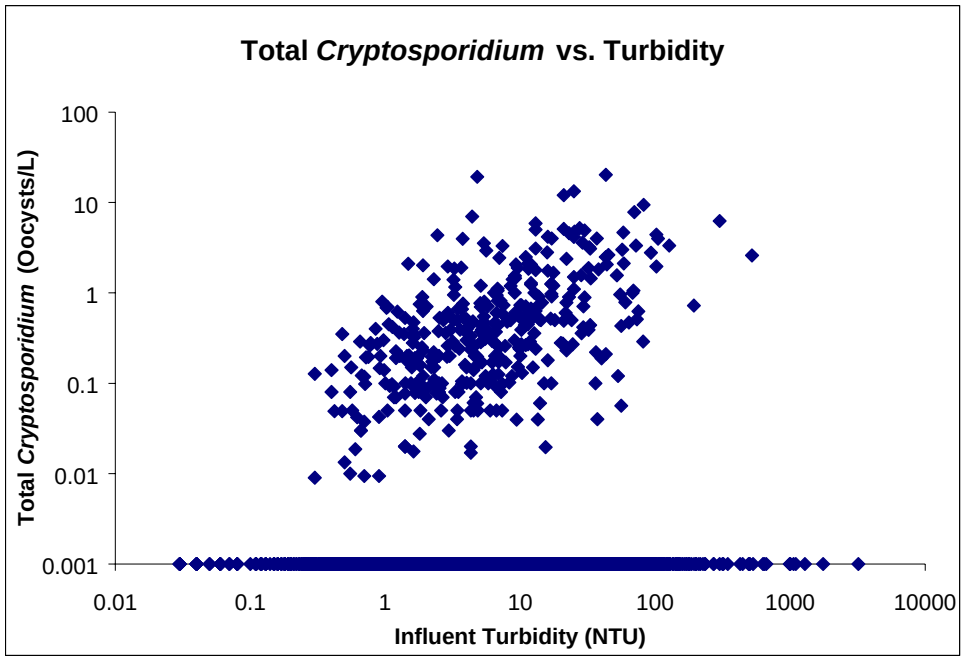
4.5 Co-Occurrence Data Analyses

Identifying relationships between source water pathogen concentrations and other source water characteristics could be helpful for controlling pathogens in the water treatment processes. Because *Cryptosporidium* and *Giardia* occur in low concentrations and are difficult to detect, it might be beneficial to use coliform or turbidity as indicators for pathogen presence if a correlation can be shown between pathogen and indicator levels. Sections 4.5.1 and 4.5.2 present the ICR and ICRSS data analyses of *Cryptosporidium, Giardia, E. coli*, coliforms, and viruses relative to indicators. To further assess the use of indicators, a "microbial index" was developed from the ICR and ICRSS data and is described in Section 4.5.3.

4.5.1 Turbidity Related to Occurrence of *Cryptosporidium, Giardia, E. coli,* and Viruses

Turbidity measurements indicate the clarity of water. Material suspended in water increases turbidity. Periodic or continuous monitoring of turbidity is commonly used in process control for water treatment. Because protozoa are particulates and often float in water, and because they often adsorb to suspended material in the water, many have hoped that turbidity can be used to indicate the presence of protozoa. Exhibits 4.36 and 4.37 contain the plots of source water turbidity compared to observed *Cryptosporidium* (total), *Giardia* (total), *E. coli*, and viruses (Exhibit 4.36 only) using ICR and ICRSS data on a log-log scale for individual water samples. No strong correlations are evident for any of the pathogens. However, *Cryptosporidium*, and to a lesser extent *Giardia* concentrations, show a weak increasing trend with increased turbidity. Because these graphs are plotted on logarithmic scales, values of zero cannot be shown. Therefore, where protozoans were not present in a sample, their concentrations were assigned a value of 0.001 oocysts or cysts/L (for Exhibit 4.36) or 0.01 oocysts or cysts/L (for Exhibit 4.37). Zero values for viruses and *E. coli* concentrations were also assigned low values so they could be shown on the graphs.

Exhibit 4.36 ICR Total *Cryptosporidium*, Total *Giardia*, Viruses, and *E. coli* vs. Turbidity



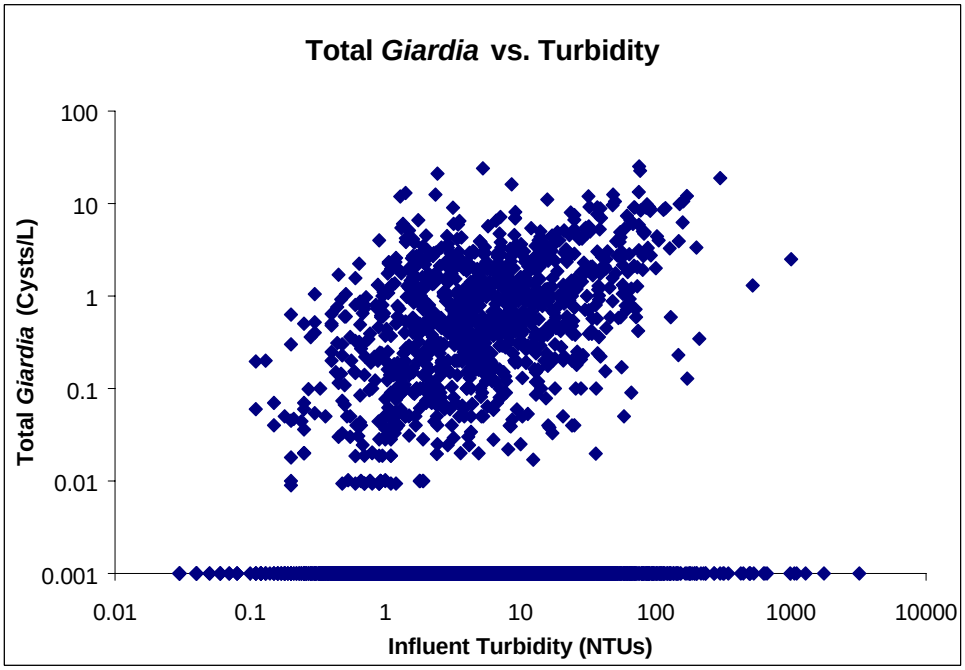
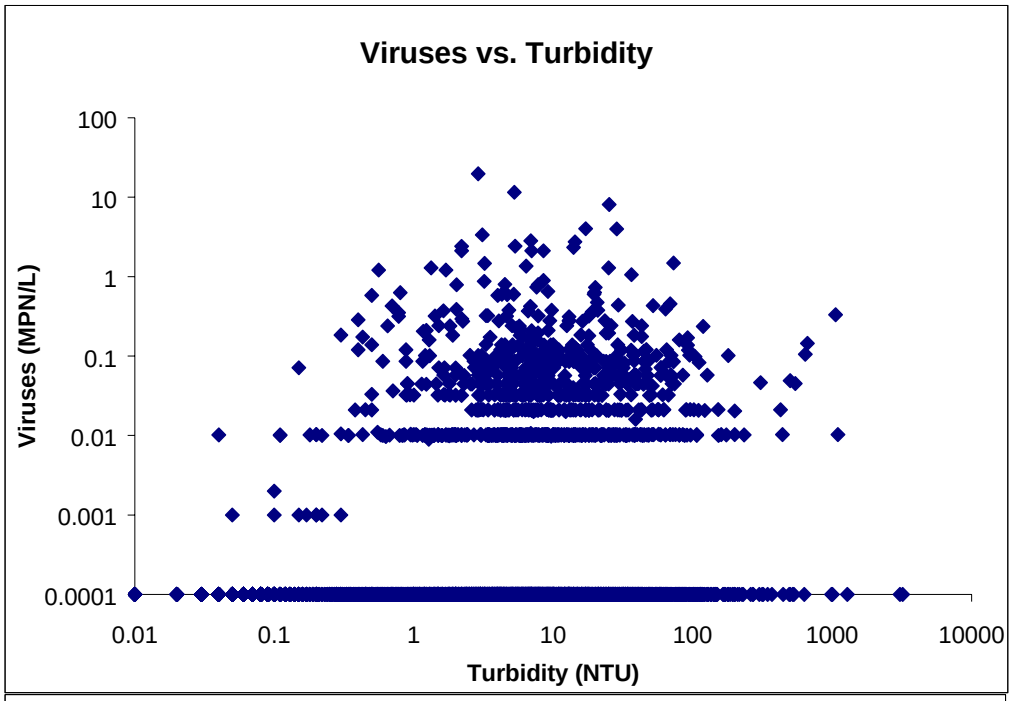


Exhibit 4.36 ICR Total *Cryptosporidium*, Total *Giardia*, Viruses, and *E. coli* vs. Turbidity (continued)



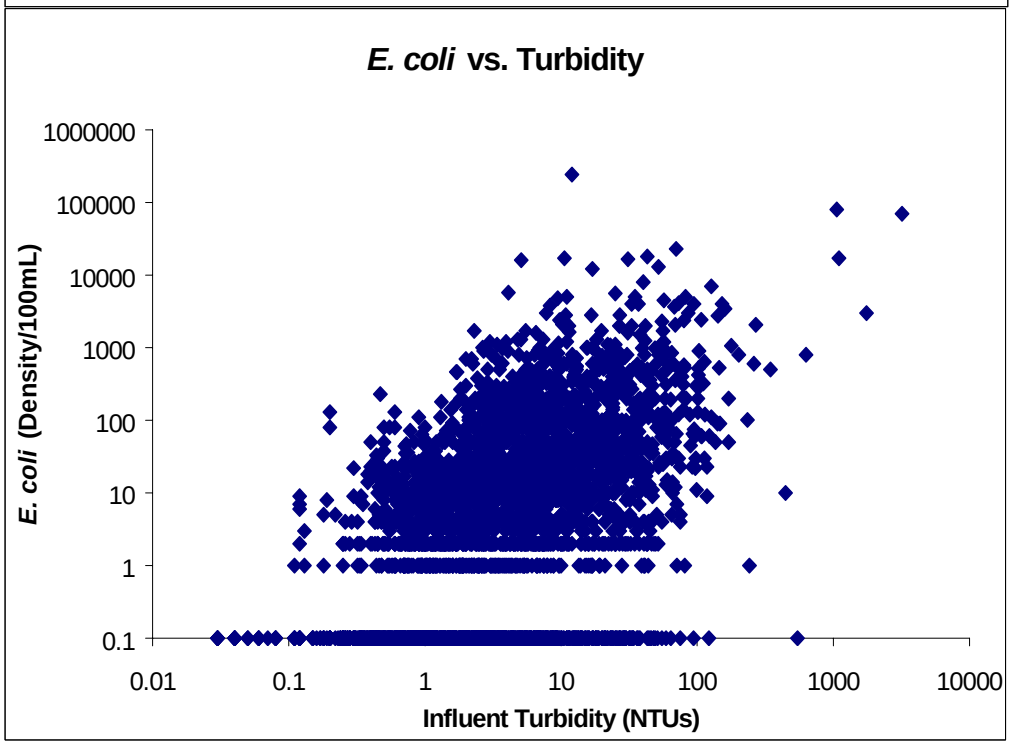
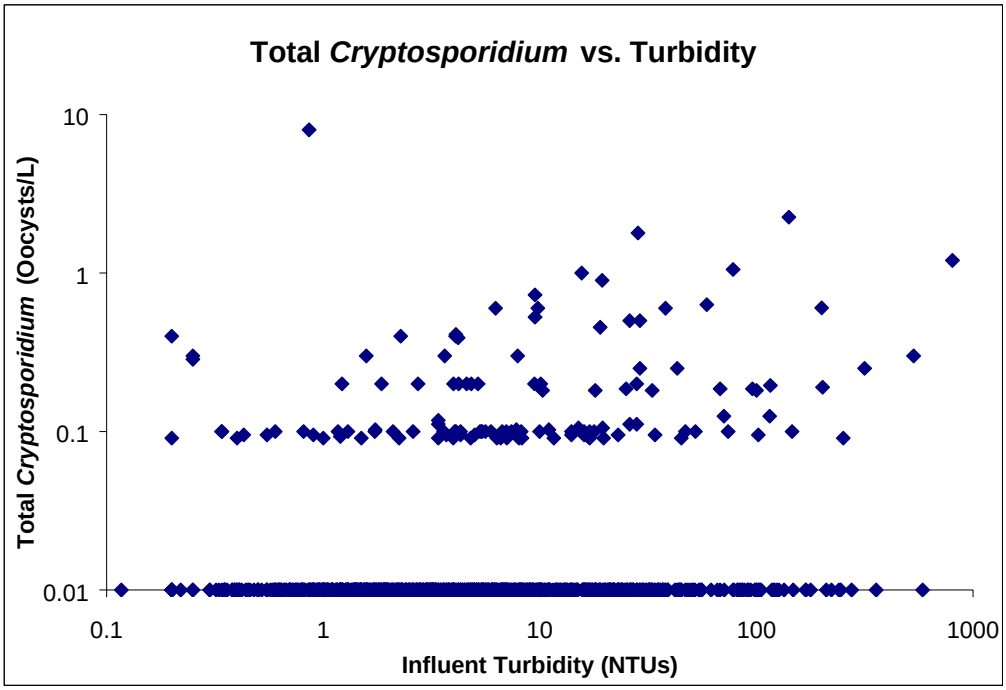
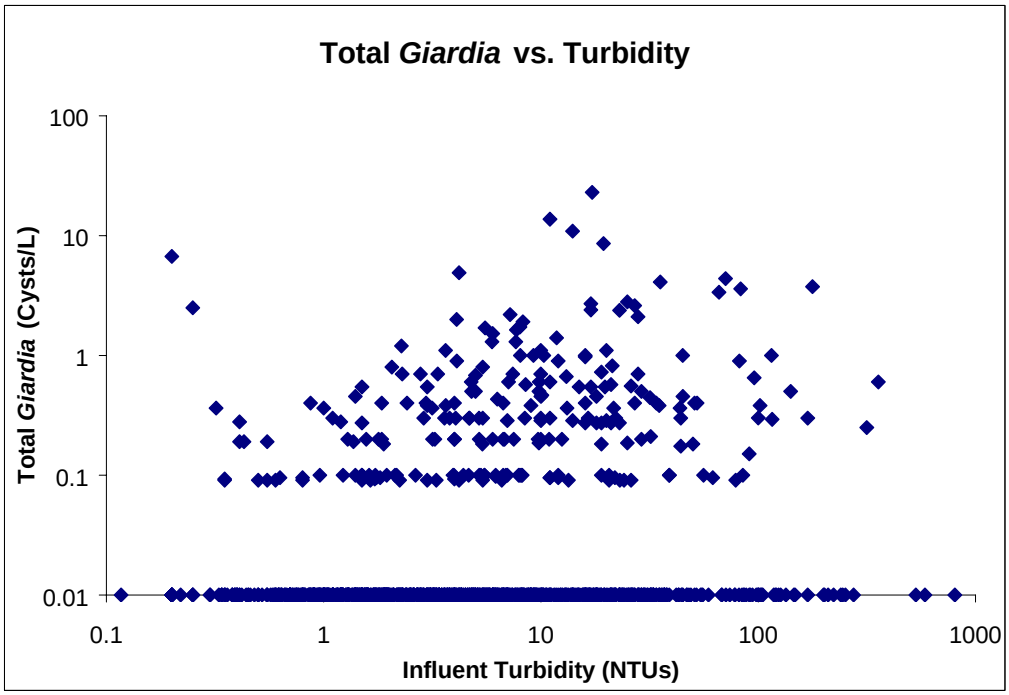


Exhibit 4.37 ICRSS Total *Cryptosporidium* and Total *Giardia* vs. Turbidity





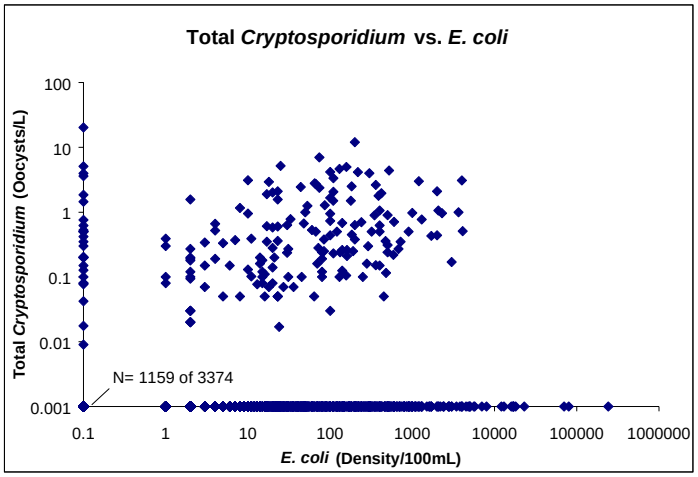
4.5.2 Indicators Related to Occurrence of *Cryptosporidium, Giardia,* and Viruses

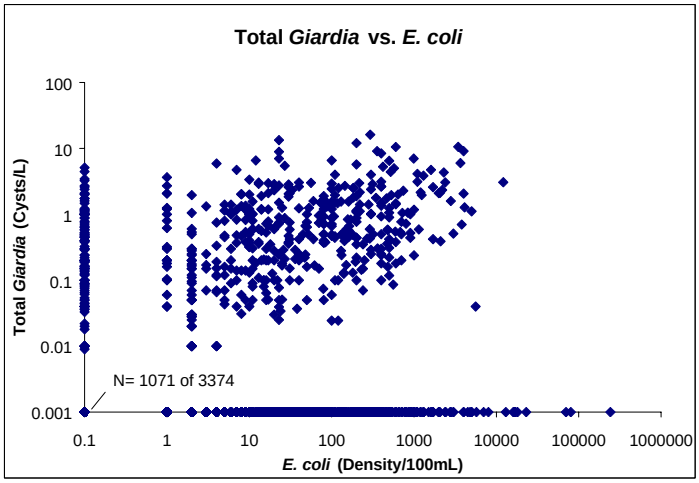
E. coli is commonly used to indicate the presence of other waterborne pathogens and is thought to be the best indicator of the three types of coliforms used for this purpose. Exhibits 4.38 and 4.39 display *E. coli* concentrations compared to *Cryptosporidium, Giardia,* and virus (Exhibit 4.38 only) concentrations using ICR and ICRSS data from individual water samples on a log-log scale. No correlations with *E. coli* are visually evident for *Cryptosporidium, Giardia,* or viruses using these data. Because these graphs are plotted on logarithmic scales, values of zero cannot be shown. Therefore, where protozoans were not present in a sample, their concentrations were assigned a value of 0.001 oocysts or cysts/L (for Exhibit 4.38) or 0.01 oocysts or cysts/L (for Exhibit 4.39). Zero values for viruses and *E. coli* concentrations were also assigned low values so they could be shown on the graphs. The same was done in Exhibits 4.40 through 4.43.

Further analysis of correlation between *Cryptosporidium* and *E. coli*, based on comparison of annual average concentrations of these organisms for each plant, is presented in section 4.5.3.

Exhibits 4.40 and 4.41 show ICR and ICRSS *Cryptosporidium, Giardia,* and virus (Exhibit 4.40 only) concentrations vs. fecal coliform concentrations for individual water samples, and Exhibits 4.42 and 4.43 compare ICR and ICRSS protozoa and virus (Exhibit 4.42 only) levels to total coliform concentrations for individual samples. No correlation is apparent in these exhibits.

Exhibit 4.38 ICR Total *Cryptosporidium*, Total *Giardia*, and Viruses vs. *E. coli*





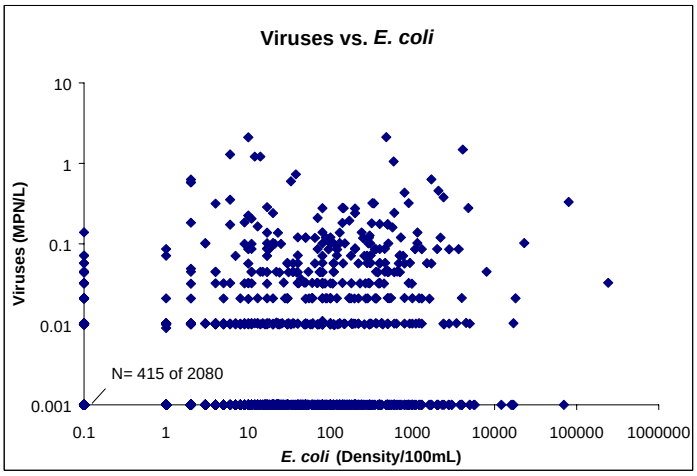
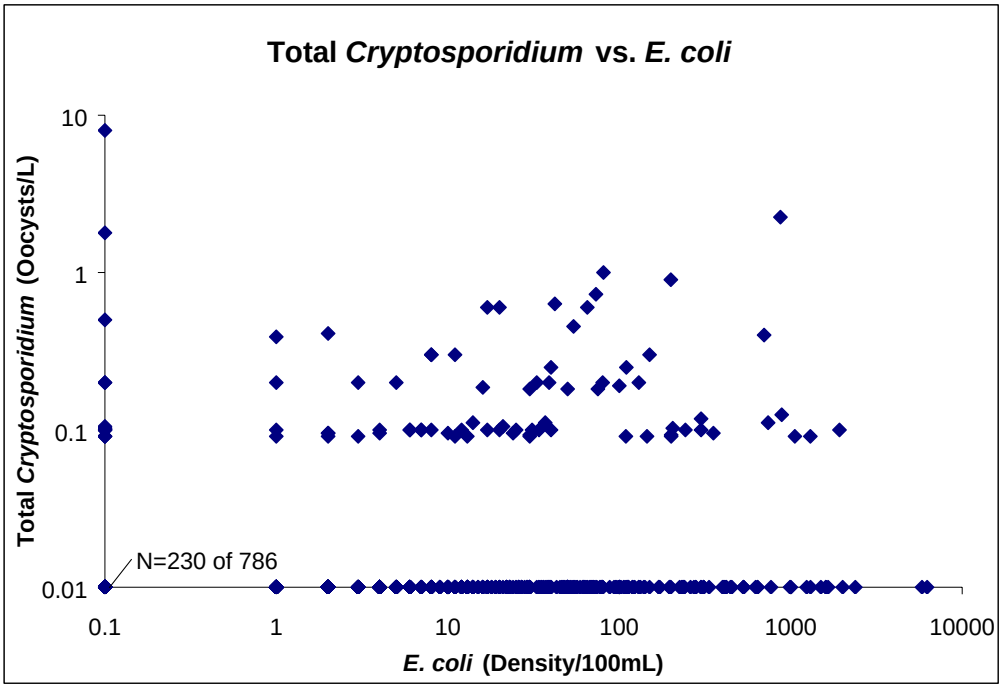


Exhibit 4.39 ICRSS Total *Cryptosporidium* and Total *Giardia* vs. *E. coli*



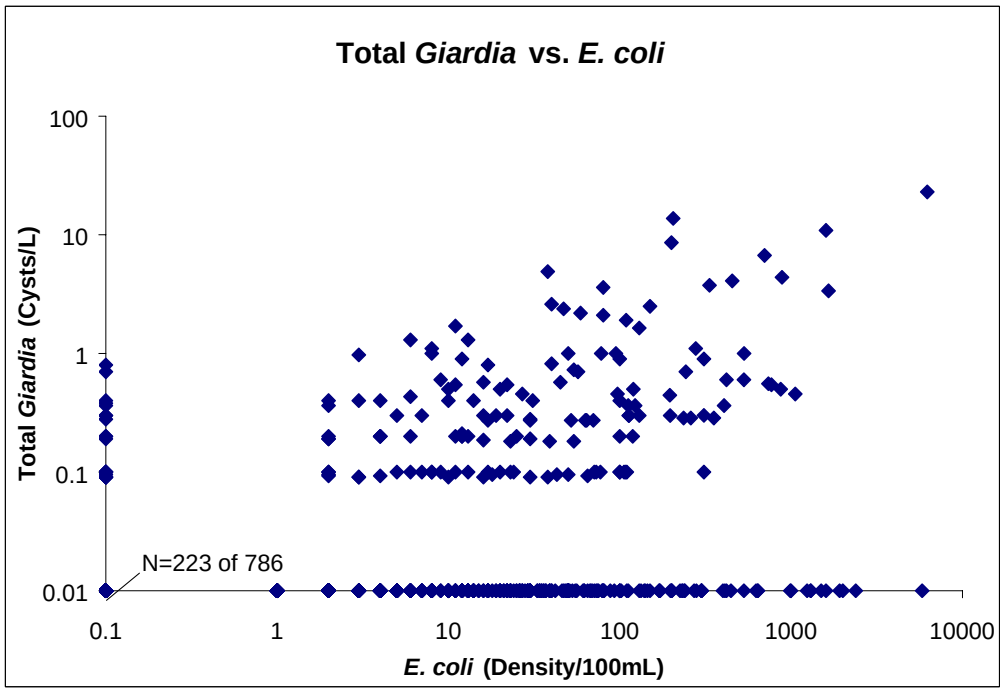
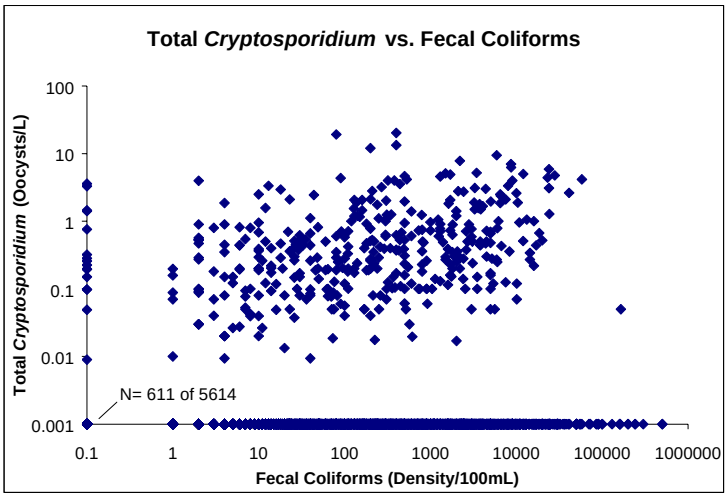
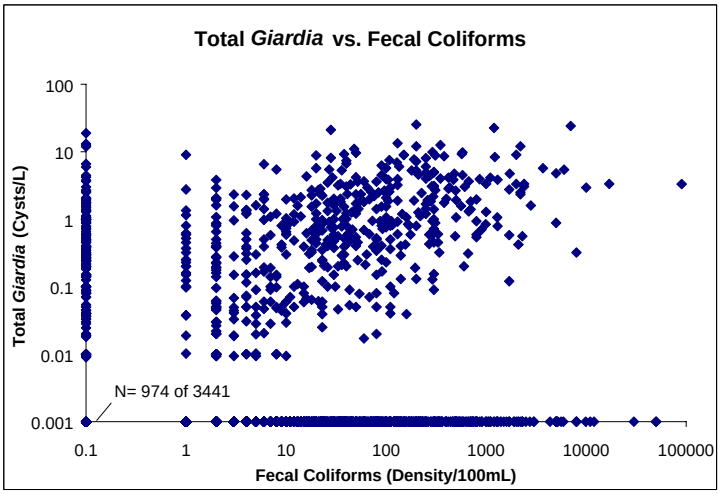


Exhibit 4.40 ICR Total *Cryptosporidium*, Total *Giardia*, and Viruses vs. Fecal Coliform





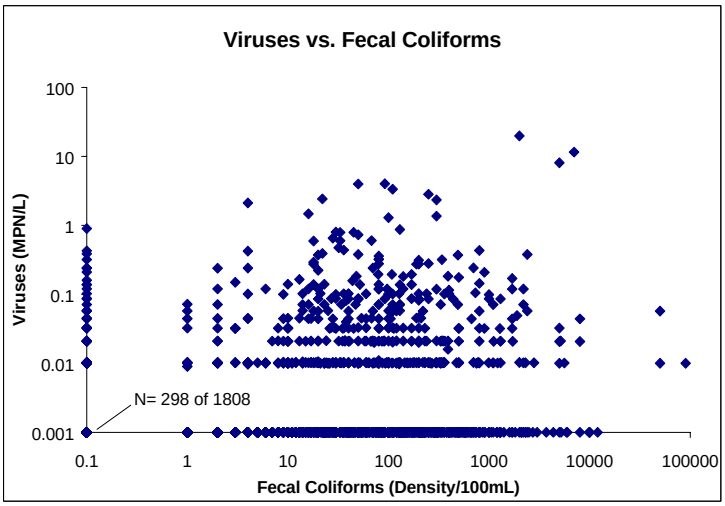
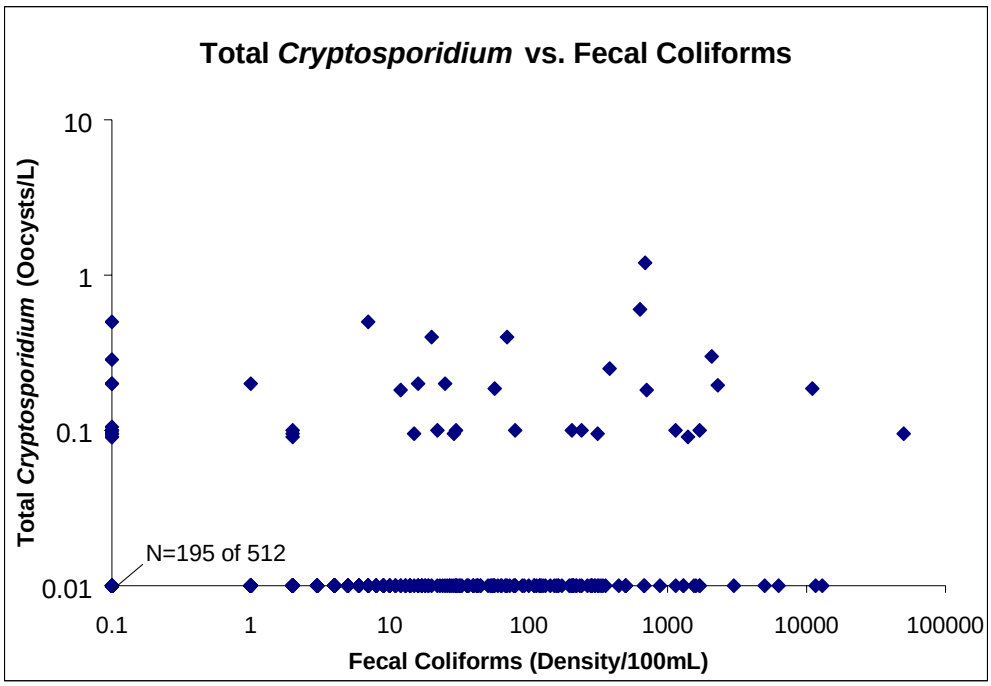


Exhibit 4.41 ICRSS Total *Cryptosporidium* and Total *Giardia* vs. Fecal Coliform



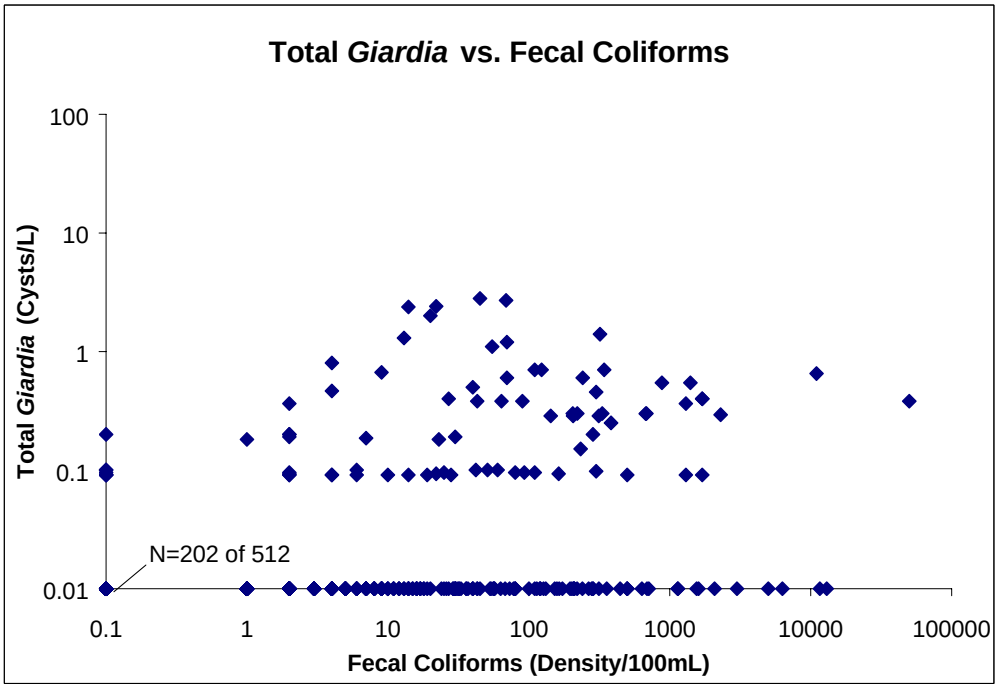
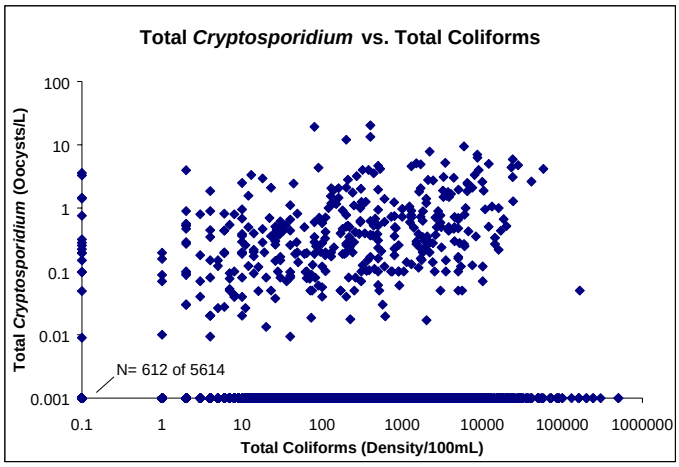
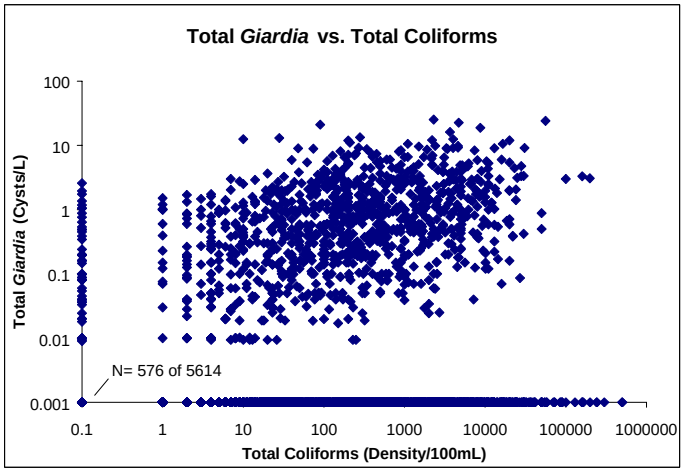


Exhibit 4.42 ICR Total *Cryptosporidium*, Total *Giardia*, and Viruses vs. Total Coliform





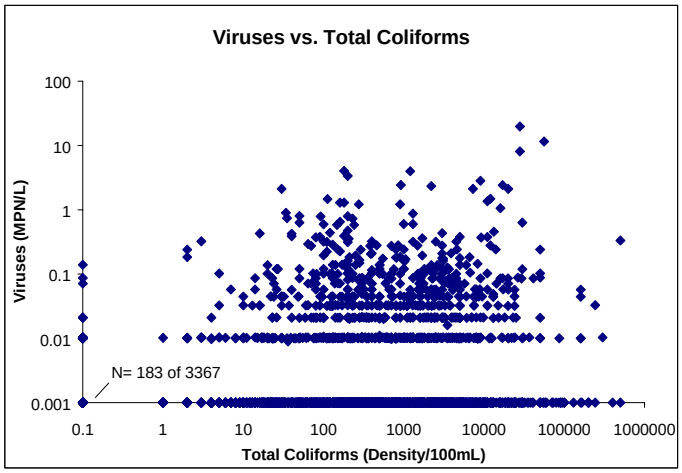
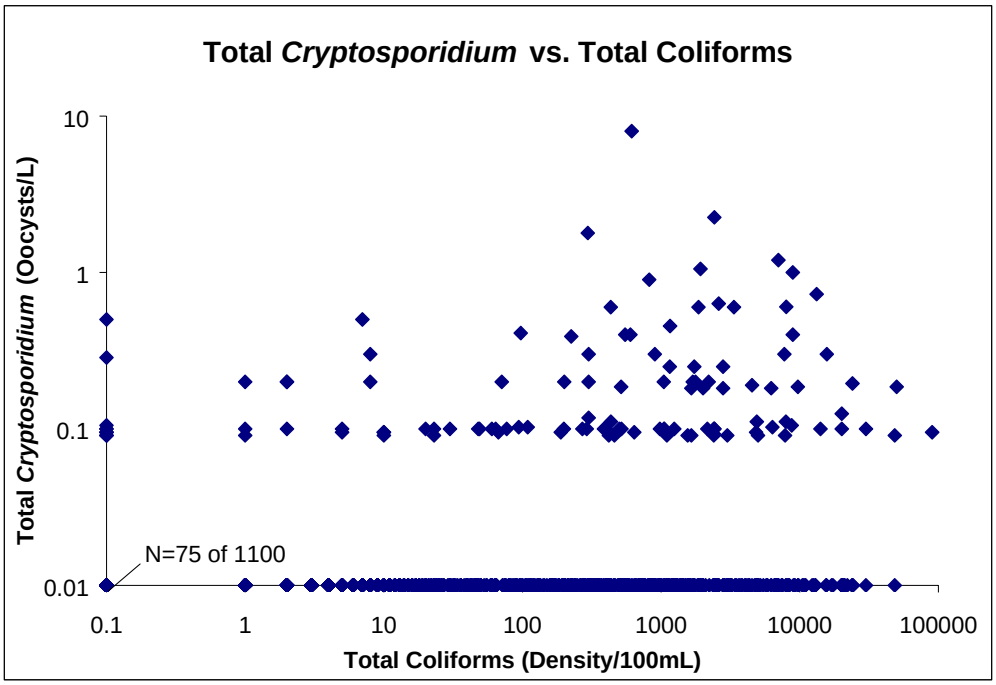
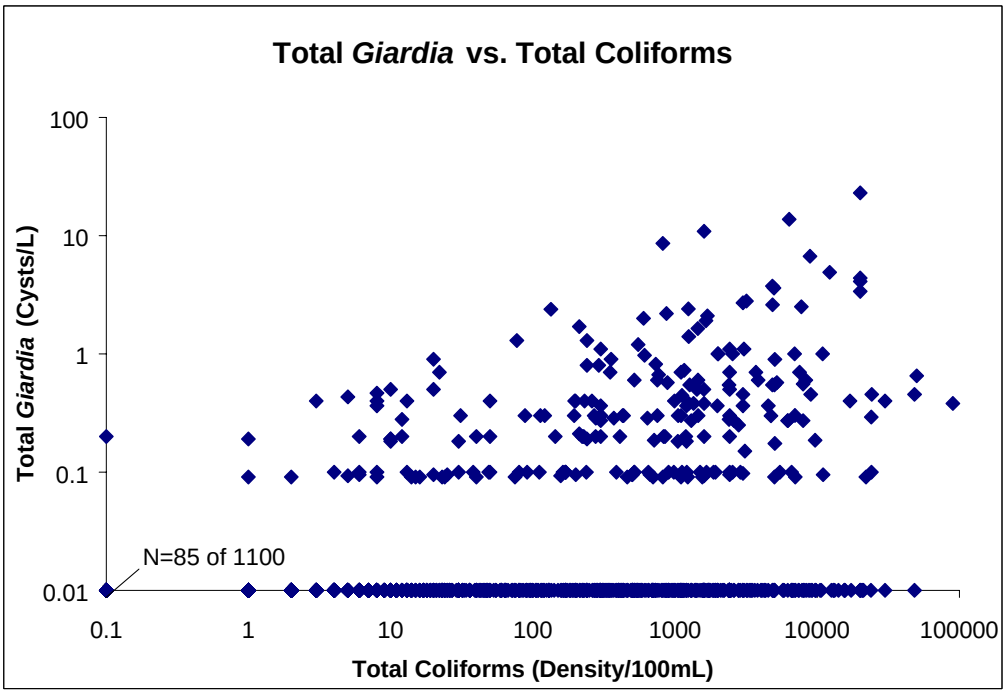


Exhibit 4.43 Total *Cryptosporidium* and Total *Giardia* vs. Total Coliform





4.5.3 Microbial Index

An alternate approach to evaluating the relationships between *Cryptosporidium* concentrations and potential indicator concentrations in *individual* samples is to evaluate the average or maximum *Cryptosporidium* concentrations and potential indicator concentrations from multiple samples at a plant over time. This analysis tests the ability of an indicator to identify plants susceptible to *Cryptosporidium* levels of concern. That is, in contrast to considering only co-occurrence of pathogens and indicators in each individual sample presented in the previous sections, this approach considers whether the occurrence of an indicator in one or more samples from a given location over some period of time is predictive of occurrence of pathogens at that location regardless of whether the indicator and the pathogen occur together in any individual samples. Such an approach was applied to ICR and ICRSS data to develop a "microbial index," which could be used as a screening tool to identify plants that are more likely to be susceptible to *Cryptosporidium* levels of concern in their source waters.

In developing the index, the ICR and ICRSS data were evaluated to determine parameters that could be used to assess a water source's susceptibility to high *Cryptosporidium* occurrence. Initially, fecal coliforms, total coliforms, *E. coli*, viruses, and turbidity were compared to concentrations of total *Cryptosporidium* oocysts and oocysts with internal structures. Although preliminary analyses indicated that total coliform, fecal coliform, and *E. coli* might be useful screening tools, analyses placed greater emphasis on *E. coli* and fecal coliforms because of the direct relationship between these parameters and fecal contamination. The microbial index approach is based on the assumption that waters exposed to greater levels of fecal contamination have a greater probability of *Cryptosporidium* occurrence. After the microbial index screening protocol was developed using *E. coli*, turbidity was evaluated in an effort to improve the screening tool. However, turbidity was found not to improve the screening tool.

4.5.3.1 Expressing Plant Summary Data

Several approaches for presenting plant data were evaluated, including a maximum rolling annual average, a maximum rolling 6-month average, a plant maximum, and a simple mean. Plant data were not summarized using plant maxima because of concerns that a utility might be unfairly classified as a result of analytical error in a single sample. This concern stemmed from the results of the *Cryptosporidium* occurrence data collected during the ICR, which may have included overestimates of *Cryptosporidium* concentrations in some samples as a result of false positives and extrapolation errors from the analysis of small sample volumes. As discussed below, overestimates in the ICRSS data set were not considered to be a significant issue, as a result of steps taken during that study to mitigate false positives and to standardize the volumes analyzed for each sample.

For the ICR plant data, *Cryptosporidium* concentrations were summarized as maximum rolling annual averages and *E. coli* concentrations were summarized as simple means. The maximum rolling annual average for *Cryptosporidium* ICR plant data was used in an effort to characterize the maximum numbers of *Cryptosporidium* in a specific source without overestimating *Cryptosporidium* concentrations, as mentioned above. The *Cryptosporidium* maximum rolling annual averages were calculated for each plant as follows:

1) The average concentration of 12 monthly samples was calculated for each of the seven, 12-month periods within the ICR sample collection period (i.e., for July 1997–June 1998, August 1997–July 1998, September 1997–August 1998, etc.). The analysis used data from plants that had at least nine *Cryptosporidium* records and nine *E. coli* records.

2) The maximum of the seven 12-month-period averages was considered the maximum rolling annual average.

Because all ICRSS samples were collected semimonthly within a single year, simple plant means were calculated for both *Cryptosporidium* and *E. coli* for ICRSS data. A minimum of six *E. coli* results was required. ICRSSM and ICRSSL results were combined for the microbial index analysis.

4.5.3.2 Plant Source Water Designations

As the microbial index was being developed, it became apparent that it would be necessary to evaluate plants based on source water type, due to significantly lower *E. coli* concentrations in reservoirs/lakes as compared to flowing streams. Source water categories were somewhat ambiguous in the ICR database because of the use of multiple water sources and ambiguity in how the source water categories were defined. The data sets used in the index analyses were constructed based on the best available data from multiple tables in the ICR AUX 1 database. Only data that could be clearly associated with a source water category were used in these analyses. Only samples from one source type were used when calculating plant means. This classification approach could result in difficulty in duplicating these results for the ICR data. For the ICRSS, each plant's source water type was designated as "flowing stream," "reservoir/lake" or "both." Plants with a source water designation of "both," used both flowing streams and reservoir/lakes during the ICRSS. Plants using both sources were not included in the index development.

4.5.3.3 Microbial Index Design

The microbial index is an approach for categorizing source water data in a way that allows the effectiveness of the indicator to be evaluated. The format is based on two values:

- The *Cryptosporidium* concentration that is considered to be the level of concern.
- The potential indicator "trigger" level that would be used to identify potentially high-*Cryptosporidium* source waters in the absence of direct *Cryptosporidium* measurements.

These values were used to sort plants into four categories presented in Exhibit 4.44, based on *Cryptosporidium* and indicator concentrations.

- Plants with high *Cryptosporidium* concentrations that exceeded the indicator trigger level (Exhibit 4.44, box D).
- Plants with high *Cryptosporidium* concentrations that did not exceed the indicator trigger level (Exhibit 4.44, box C).
- Plants with low *Cryptosporidium* concentrations that exceeded the indicator trigger level (Exhibit 4.44, box B).
- Plants with low *Cryptosporidium* concentrations that did not exceed the indicator trigger level (Exhibit 4.44, box A).

E. coli average trigger values presented in this document range from 5 *E. coli* per 100 mL to 100 *E. coli* per 100 mL. The *Cryptosporidium* "level of concern" was 0.075 oocysts/L, the average concentration above which additional water treatment might be necessary under the LT2ESWTR.

Cryptosporidium concentrations below this level were considered low; concentrations above this level were considered high.

Exhibit 4.44 Summary of Microbial Index Approach

***Cryptosporidium* Indicator Index Table Template**

Breakdown of Plants into *Cryptosporidium* "bin"	Breakdown of Plants Using "Trigger": Did not exceed trigger value	Breakdown of Plants Using "Trigger": Exceed trigger value
"Low"	**A**	**B**
"High"	**C**	**D**

Calculations Used to Assess Index Performance

What percent of plants with "high" *Cryptosporidium* exceed the *E. coli* trigger? (**Sensitivity** of indicator)	**D / (C+D)**
What percent of plants would be required to monitor?	**(B+D) / (A+B+C+D)**

4.5.3.4 Censored Coliform Data

The ICR and ICRSS data sets contain censored *E. coli* data ("greater than" or "less than" results for individual samples). The Long Term 2 Enhanced Surface Water Treatment Rule's Microbial Occurrence Subgroup felt that retaining the maximum amount of data on *E. coli* concentrations in each plant's source water was important for developing the microbial index. The Subgroup also wanted to ensure that censored data that might inaccurately characterize *E. coli* for a particular plant was not used in development of the microbial index. The following approach to censored *E. coli* data was agreed upon by the Subgroup as the most appropriate approach to censored data and was applied to *E. coli* data during development of the microbial index:

Low-censored data

- Results below the "detection limit" were replaced with zero if it was ≤10. (For example, <1 *E. coli* /100 mL would be replaced by zero).

  After reviewing the ICR and ICRSS data, the Microbial Occurrence Subgroup noted that many of the censored *E. coli* data points are reflective of *E. coli* levels below the "detection limits" (i.e., *E. coli* <1/100 mL) and agreed that it was appropriate to include this data in plant source water characterization. In the absence of a more accurate measure of the actual *E. coli* concentration, and in recognition that the concentration was very low, relative to other samples, a value of zero *E. coli*/100 mL was considered most appropriate.

- Results below the "detection limit" were not used if it was >10. (For example, <100 *E. coli* /100 mL was not used).

  The Microbial Occurrence Subgroup wanted to ensure that a plant's source water was not inaccurately characterized as having high *E. coli* concentrations in cases where no *E. coli*

were detected but the "detection limit" was very high. For example, some utilities, in an effort to quantify high total coliform concentrations, diluted samples, resulting in *E. coli* results with very high "detection limits" (*i.e.* *E. coli* <1,000/100 mL). After reviewing the ICR and ICRSS data, the Subgroup agreed that samples with high "detection limits" should not be included in the development of the microbial index.

High-censored data

- Censored high results were replaced by the censor limit if the limit was >500 (for example, >1,600 *E. coli* /100 mL is replaced by 1,600 *E. coli* /100 mL).

  Some plants did not consistently analyze additional dilutions, as is appropriate to quantify *E. coli* levels, despite numerous requests for the analysis of additional dilutions. After reviewing the ICR and ICRSS data available for each plant, the Microbial Occurrence Subgroup agreed that retaining high censored data, when the censor limit was high, was the most appropriate approach to characterizing a plant's source water.

- Censored high results were not used if the censor limit was ≤500 (for example, >350 *E. coli*/100 mL was not used).

  After reviewing the ICR and ICRSS data available for each plant, the Microbial Occurrence Subgroup agreed that using high censored data when the censor limit was low would result in inaccurate plant source water characterization, and as a result, deemed the use of such data inappropriate.

4.5.3.5 Assessment of Microbial Index Performance

The usefulness of the microbial index was evaluated based on sensitivity and the percentage of plants required to monitor for *Cryptosporidium* (see Exhibit 4.45). Sensitivity was defined as the percentage of plants with "high" *Cryptosporidium* concentrations that would be triggered into monitoring based on a specific *E. coli* trigger level. Sensitivity was considered critical because it evaluated the ability of the index to require *Cryptosporidium* monitoring when a plant had a source water with "high" *Cryptosporidium* concentrations. A high sensitivity value is preferred and can be achieved by lowering the *E. coli* trigger value. However, if *E. coli* trigger values are lowered, more plants would be required to monitor. A balance between sensitivity and the number of plants required to monitor was considered an optimum result.

Exhibit 4.45 Summary of Microbial Index Results for ICR and ICRSS Data at a *Cryptosporidium* Level of Concern of 0.075 Oocysts/L, Using Triggers of 5, 10, 50, and 100 *E. coli* per 100 mL

Reservoirs/Lakes

E. coli trigger level:	5 *E. coli*/100 mL				10 *E. coli*/100 mL				50 *E. coli*/100 mL				100 *E. coli*/100 mL			
Study:	ICR		SS		ICR		SS		ICR		SS		ICR		SS	
Did plant exceed *E. coli* trigger level?	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
<=0.075 *Cryptosporidium* per L	70	44	17	13	87	27	24	6	107	7	28	2	108	6	29	1
>0.075 *Cryptosporidium* per L	1	9	1	2	2	8	2	1	8	2	3	0	9	1	3	0
What percent of plants with "high" *Cryptosporidium* exceed the *E. coli* trigger? (**Sensitivity** of indicator)	90%		67%		80%		33%		20%		0%		10%		0%	
What percent of plants would be required to monitor?	43%		45%		28%		21%		7%		6%		6%		3%	

Flowing Streams

E. coli trigger level:	5 *E. coli*/100 mL				10 *E. coli*/100 mL				50 *E. coli*/100 mL				100 *E. coli*/100 mL			
Study:	ICR		SS		ICR		SS		ICR		SS		ICR		SS	
Did plant exceed *E. coli* trigger level?	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
<=0.075 *Cryptosporidium* per L	6	36	2	15	7	35	5	12	18	24	8	9	24	18	11	6
>0.075 *Cryptosporidium* per L	0	24	0	4	0	24	0	4	0	24	0	4	3	21	2	2
What percent of plants with "high" *Cryptosporidium* exceed the *E. coli* trigger? (**Sensitivity** of indicator)	100%		100%		100%		100%		100%		100%		88%		50%	
What percent of plants would be required to monitor?	91%		90%		89%		76%		73%		62%		59%		38%	

Notes:

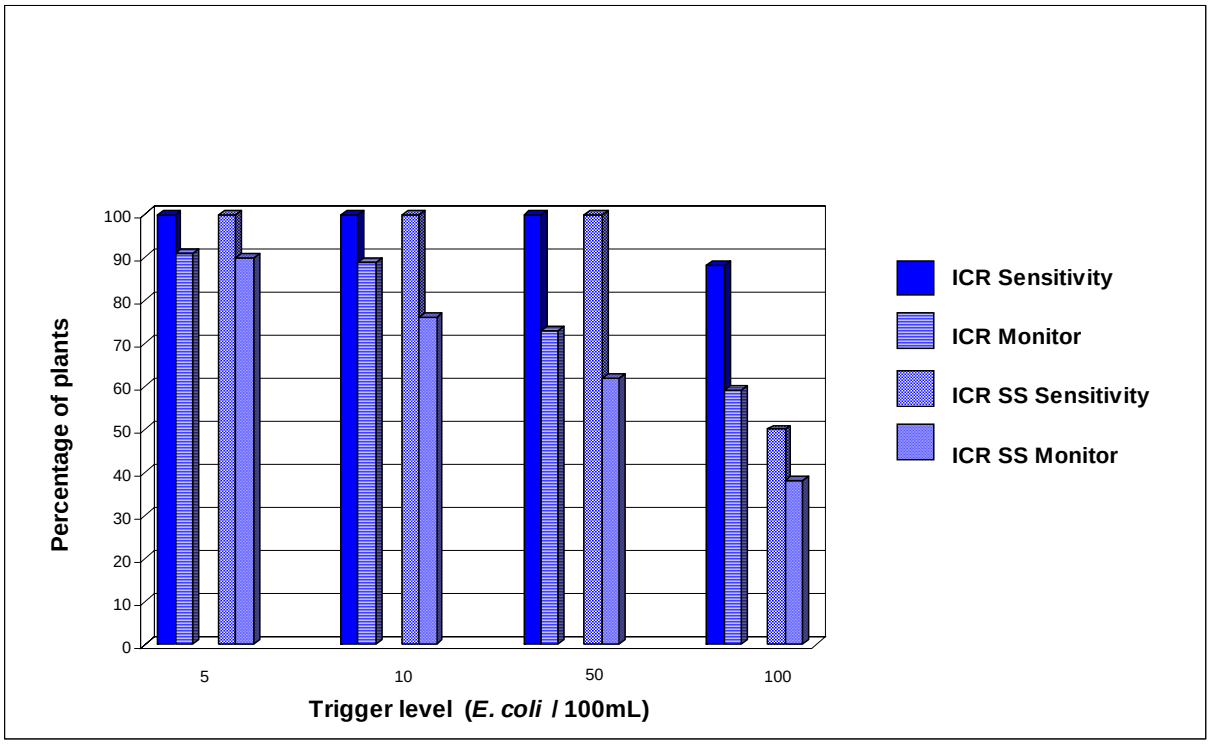
ICR approach: Index based on *Cryptosporidium* maximum rolling annual average and *E. coli* simple means (50% *E. coli* results minimum)
ICR sampling period: 18 months (July 1997 through December 1998)

SS approach: Index based on *Cryptosporidium* and *E. coli* simple means (25% *E. coli* results minimum)
SS sampling period: 12 months (March 1999 through February 2000)

4.5.3.6 Flowing Stream Index Results

The results of the *E. coli* index for flowing stream plants are shown in Exhibit 4.45 and Exhibit 4.46. A trigger of 50 *E. coli* per 100 mL is favorable with regard to sensitivity, producing 100-percent sensitivity for both data sets but resulting in 73 percent of ICR plants, and 62 percent of ICRSS plants, being required to monitor for *Cryptosporidium.*

Exhibit 4.46 Effect of Different Trigger Levels on Microbial Index Sensitivity (Flowing Streams)



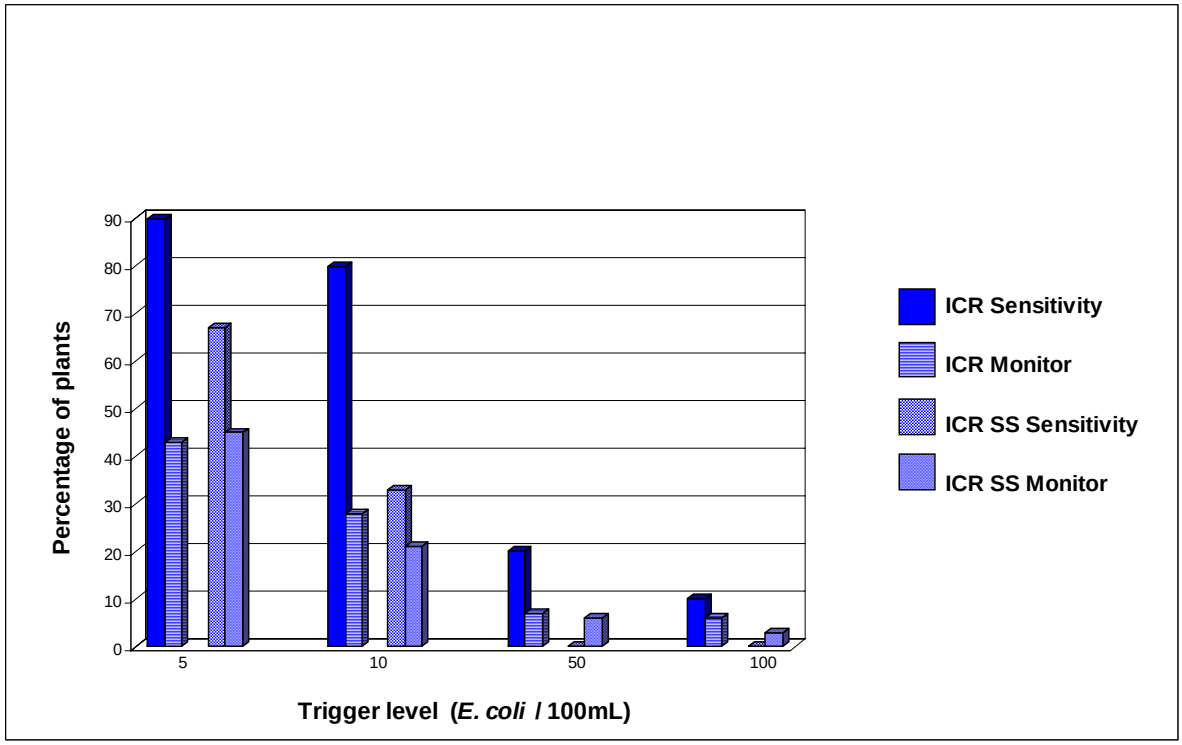
4.5.3.7 Reservoir/Lake Index Results

The results of the *E. coli* index for reservoir/lake plants are shown in Exhibit 4.45 and Exhibit 4.47. For the ICR, a trigger of 10 *E. coli* per 100 mL results in a sensitivity of 80-percent and would require 28-percent of plants to monitor. Lowering the trigger to 5 *E. coli* per 100 mL for the ICR data increased the sensitivity to 90 percent but the number of plants required to monitor increased to 43 percent. A greater difference in sensitivities between the triggers is observed for the ICRSS data set, with sensitivities increasing from 33 percent to 67 percent using triggers of 10 and 5 *E. coli* per 100 mL, respectively. The percentage of plants required to monitor based on the ICRSS data increased from 21 percent to 45 percent using triggers of 10 and 5 *E. coli* per 100 mL, respectively. Although an *E. coli* trigger of 5 per 100 mL would be more conservative for identifying source waters with high concentrations of *Cryptosporidium*, it would increase the number of reservoir/lake plants required to monitor for *Cryptosporidium* from approximately one-quarter to almost one-half.

4.5.3.8 Conclusions of Microbial Index Analysis

The results of the analyses indicate that the microbial index could potentially be used to screen drinking water plant sources for susceptibility to high occurrences of *Cryptosporidium*. However, because of ICR and ICRSS data set limitations—including analytical methodology, limited *E. coli* data, variations in *E. coli* method, number of plants, sample volumes analyzed, data reporting anomalies, and false positive results—verification of the microbial index results with additional, larger *Cryptosporidium* and *E. coli* data sets is imperative.

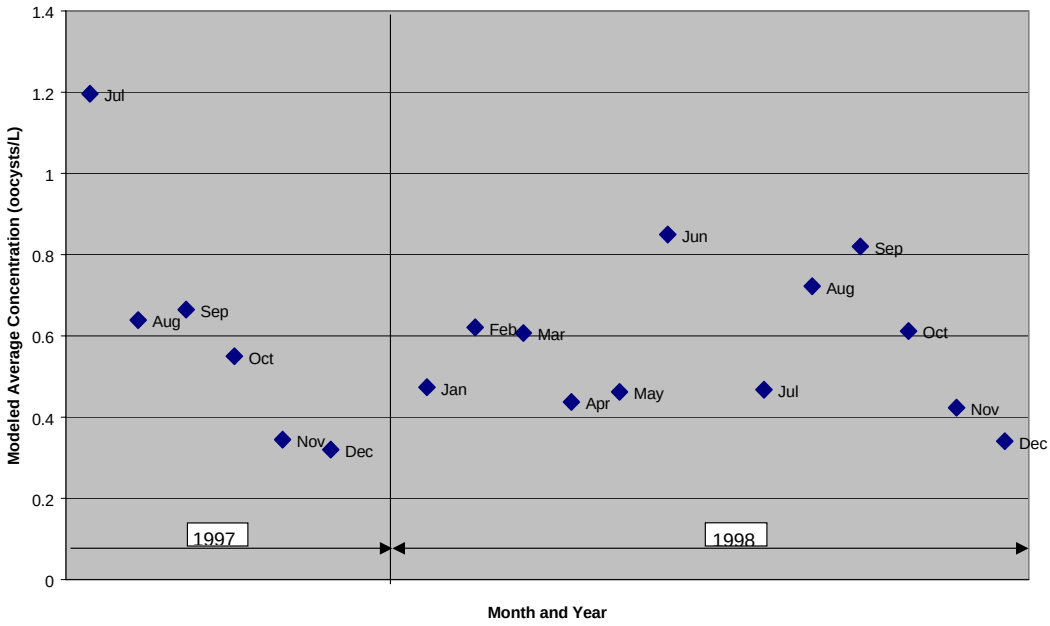
Exhibit 4.47 Effect of Different Trigger Levels on Microbial Index Sensitivity (Reservoir/Lake)



4.6 *Cryptosporidium* and *Giardia* Seasonal Patterns

Temporal events such as spring rains, which increase runoff from farming activities, are thought to affect the occurrence of protozoa. Analysis of observed data did not allow for geographical considerations and other factors that substantially affect temporal changes. Analysis of modeled data allowed comparison of more non-zero concentrations. The modeled total *Cryptosporidium* concentrations at all plants were averaged together for each month to provide a monthly mean concentration, as shown in Exhibit 4.48. Mean concentrations during November and December were lower than in other months, and the months of June, July (1997 only), August, and September had the highest values. Values during other months do not show a clear seasonal trend. At the same time, the existence of such a trend cannot be ruled out.

Exhibit 4.48 ICR Monthly Mean *Cryptosporidium* Concentrations—Modeled Data



4.7 Source Water Occurrence—Summary

The source water occurrence of *Cryptosporidium* and *Giardia*, based on ICR and ICRSS data, was low but highly variable, due to difficulties with sampling for low occurrence events and the sample analysis methods. To account for these factors and other uncertainties, a hierarchical model with Bayesian parameter estimating techniques was used with the ICR and ICRSS data to provide a more probable national distribution of *Cryptosporidium* and *Giardia* occurrence in source water. The *Cryptosporidium* modeled distributions for source water, described in this chapter, were used to estimate a Pre-LT2ESWTR finished water occurrence (described in Chapter 5) and were used to determine the extent to which systems would be affected by the LT2ESWTR and the benefits consumers would gain as a result of the rule (described in the Economic Analysis for the LT2ESWTR).

Based on the observed ICR and ICRSS data, flowing stream sources proved to be more susceptible to *Cryptosporidium* and *Giardia* occurrence than reservoir or lake sources. The ICRSS data showed little to no difference between occurrence in large and medium plants.

The percentages of unfiltered ICR plants with samples positive for *Cryptosporidium*, *Giardia*, viruses, and coliforms were higher than those for filtered ICR plants, but the overall number of unfiltered ICR plants was very small. For viruses and coliforms, 80 to 100 percent of unfiltered plants had positive samples. However, as expected, the unfiltered sources had very low concentrations of pathogens and coliforms, due to the high source water quality standards unfiltered systems must meet.

The modeled data show most plants having low *Cryptosporidium* occurrence; 50 percent of plants had plant-mean levels below 0.048 oocysts/L based on ICR data and 90 percent of plant-means fell below 0.24 oocysts/L and 0.33 oocysts/L, based on ICRSS large system and medium system data, respectively.

No direct correlation was found between *Cryptosporidium*, *Giardia*, and virus occurrence and coliform or turbidity levels in the observed data at the individual sample level. However, a microbial index developed to find a connection between selected pathogen and indicator levels suggests that higher *E. coli* levels can indicate an increased likelihood that pathogens are present.

5. Treatment by Physical Removal

Treatment systems remove *Cryptosporidium* oocysts, *Giardia* cysts, other pathogens, and particles to protect consumers from drinking contaminated water. Because *Cryptosporidium* oocysts are resistant to inactivation by chlorine (the most commonly used disinfectant in the United States) and because of the health concerns associated with high concentrations of disinfection byproducts, significant attention has been focused on the efficacy of filtration for removing pathogens. This chapter summarizes information on the removal of *Cryptosporidium*, *Giardia*, and viruses using filtration technologies, including bench-scale and pilot-scale studies of removal efficiencies. Treatment via disinfection is discussed in Chapter 2.

5.1 Removal of *Cryptosporidium* and *Giardia*

Many studies have been conducted to evaluate the level of *Cryptosporidium* and *Giardia* removal achieved by filtration and the conditions under which optimal removal occurs. Aspects of *Cryptosporidium* and *Giardia* removal and the results of those studies are discussed in this section and summarized in Exhibit 5.1. Results from studies using conventional and direct filtration treatment trains are discussed in sections 5.1.1 to 5.1.3.

Exhibit 5.1 *Cryptosporidium* and *Giardia* Removal Efficiencies

Type of Treatment Plant	Log Removal		Experimental Design	Reference
Conventional filtration plants	*Cryptosporidium*	0.2–>5.3	Pilot plant	Dugan et al. 2001
	Cryptosporidium *Giardia*	4.2–5.2 4.1–5.1	Pilot plants	Patania et al. 1995
	Cryptosporidium *Giardia*	1.9–4.0 2.2–3.9	Pilot plants	Nieminski and Ongerth 1995
	Cryptosporidium *Giardia*	1.9–2.8 2.8–3.7	Full-scale plants	Nieminski and Ongerth 1995
	Cryptosporidium *Giardia*	2–2.5 2–2.5	Full-scale plants	LeChevallier et al. 1991
	Cryptosporidium *Giardia*	2.7–3.1 2.2–2.8	Full-scale plants	LeChevallier and Norton 1992
	Cryptosporidium *Giardia*	3–5 3–5	Pilot plant	McTigue et al. 1998

Type of Treatment Plant	Log Removal		Experimental Design	Reference
Direct-filtration plants	*Cryptosporidium* *Giardia*	2.7–5.9 3.4–5.0	Pilot plants	Patania et al. 1995
	Cryptosporidium *Giardia*	2.7–3.1 3.05–3.6	Pilot plants	Ongerth and Pecoraro 1995
	Cryptosporidium *Giardia*	1.9–3.8 2.9–4.0	Pilot plants	Nieminski and Ongerth 1995
	Cryptosporidium	3.9–5.4	Pilot plant	Patania et al. 1999
	Cryptosporidium	2–3	Pilot plant	West et al. 1994
	Cryptosporidium	3.7–4.5	Pilot plant	Yates et al. 1997
	Cryptosporidium	0.25–5.6	Pilot plants	Huck et al. 2000
	Cryptosporidium	1.3–5.8	Pilot plant	Emelko et al. 2000
	Cryptosporidium	3.3–4.2	Bench-scale	Emelko et al. 1999
Slow sand	*Cryptosporidium* *Giardia*	3.9-6.1 2.8->6.5	Pilot plant	Schuler and Ghosh 1991
	Cryptosporidium	>3.6	Pilot plant	Timms et al. 1995
	Cryptosporidium *Giardia*	0.1–0.5 0.9–1.4	Full-scale plant at 0.5 to 1.0°C	Fogel et al. 1993
	Cryptosporidium	2.8–>6.4	Pilot plant	Hall et al. 1994
Diatomaceous earth	*Cryptosporidium* *Giardia*	4.6->6.4 4.3->6.7	Pilot plant	Schuler and Ghosh 1990
	Cryptosporidium	5.0-6.6	Pilot plant	Ongerth and Hutton 2001
Microfiltration and Ultrafiltration membranes	*Cryptosporidium* *Giardia*	>6.1–>7.0 >6.4–>7.0	Pilot-scale plant	Jacangelo et al. 1995
Nanofiltration membrane	*Cryptosporidium* *Giardia*	5-6 5-6	Pilot plant	Seyde et al. 1998
Cartridge filtration	*Cryptosporidium*	1.1->3.5	Bench-scale	Schaub et al. 1993
Cartridge filtration	*Cryptosporidium*	3.2-3.6	Full-scale filter	Roessler 1998

5.1.1 Conventional and Direct Filtration

The typical treatment train for removing particles, including *Cryptosporidium*, involves addition of coagulating chemicals (e.g., alum) to the raw water, causing the particles to coalesce into larger particles called floc. The floc either settles to the bottom of a sedimentation basin or is removed through subsequent filtration. Conventional filtration refers to a treatment process that includes coagulation, flocculation, sedimentation, and filtration. Direct filtration systems do not have a sedimentation process; coagulation and flocculation are followed by filtration.

Water systems monitor turbidity and in some cases, particle count, before and after filtration for process control purposes. (Current regulations require systems to monitor turbidity after filtration.) Because of the delay between the time of sampling and the receipt of test results for *Cryptosporidium* and *Giardia*, and the challenge of quantifying low concentrations of these pathogens, measuring protozoa concentrations is not a practical method for on-line process control. Instead, identifying a relationship

between turbidity or particle counts and protozoa is desirable. This is the focus of many studies discussed in this section.

Dugan et al. (2001) evaluated the ability of conventional treatment to control *Cryptosporidium* under varying water quality and treatment conditions, and assessed turbidity, particle counts, and aerobic endospores as indicators of *Cryptosporidium* removal. Fourteen runs were conducted on a small pilot scale plant that had been determined to provide equivalent performance to a larger plant. Under optimal coagulation conditions, oocyst removal across the sedimentation basin ranged from 0.6 to 1.8 log, averaging 1.3 log, and removal across the filters ranged from 2.9 to greater than 4.4 log, averaging greater than 3.7 log. Removal of aerobic spores and reduction in particle counts and turbidity all correlated with removal of *Cryptosporidium* by sedimentation, and these parameters were conservative indicators of *Cryptosporidium* removal across filtration. Sedimentation performance under optimal conditions related to raw water quality, with the lowest *Cryptosporidium* removals observed when raw water turbidity was low. Suboptimal coagulation conditions (underdosed relative to jar test predictions) significantly reduced plant performance. Oocyst removal in the sedimentation basin averaged 0.2 log, and removal by filtration averaged 1.5 log. Under suboptimal coagulation conditions, low sedimentation removals of *Cryptosporidium* were observed in four of the five runs, regardless of raw water turbidity.

A comprehensive study of *Cryptosporidium* and *Giardia* removal, turbidity, and particle counts under various treatment conditions was conducted by Patania et al. (1995). These variables were studied using four pilot plants at multiple study sites (and hence, different raw water qualities) with the objective of providing practical information on *Cryptosporidium* and *Giardia* removal over a wide range of treatment conditions and water qualities. *Cryptosporidium* and *Giardia* removal ranged from 1.4 to 6.2 log, with a median of 4.2 log for all studies. A comparison of two different types of treatment processes used—conventional treatment (including sedimentation) and direct filtration—demonstrated that *Cryptosporidium* removal was 1.4 to 1.8 log higher with conventional treatment than with direct filtration. When treatment conditions were optimized for turbidity removal at four different sites, *Cryptosporidium* removal ranged from 2.7 to 5.9 log, and *Giardia* removal ranged from 3.4 to 5.1 log.

The investigators noted that the log removal values would have been greater than reported if the raw water oocyst concentrations had been sufficiently high to allow oocyst detection in the filtered water, or if the detection method for finished water had a higher recovery efficiency. They also noted that removal of *Cryptosporidium* was 0.4 to 0.9 log lower during filter maturation than during filter operation, and *Giardia* removal was generally 0.4 to 0.5 log lower during maturation (Patania et al. 1995).

As part of the same study, the authors looked for a correlation between log removal of turbidity and log removal of protozoa. They did not observe a one-to-one correlation, but noted that maintaining effluent turbidity levels at 0.1 NTU was required to achieve 5.0-log removal of *Cryptosporidium* and *Giardia* 90 percent of the time, and a filter effluent goal of 0.2 NTU resulted in removals of 4.0 log or less for *Cryptosporidium* and *Giardia* 90 percent of the time. They also checked protozoa removal against particle removal and found that, again, there was no direct correlation. Particle removal, recommended as an indicator for *Giardia* removal (USEPA 1989c), tended to underestimate protozoa removal under some raw water conditions, such as high protozoa concentrations and low turbidity and particle counts. The authors felt that the one-to-one correlation between protozoa removal and particle removal reported by others (Nieminski 1994) was due to the combination of higher turbidity raw waters, a slightly higher range of particle removal, and lower overall protozoa removal compared to the results of their study.

Nieminski and Ongerth (1995) evaluated performance in a pilot plant and in a full-scale plant (not in operation during the time of the study) and examined two treatment modes: direct filtration and

conventional treatment. The turbidity of the raw water for the pilot plant typically was 4 NTU, with a maximum of 23 NTU. The pilot plant achieved filtered water turbidity levels between 0.1 and 0.2 NTU, and *Cryptosporidium* removals averaged 3.0 log (range = 1.9 to 4.0 log) for conventional treatment and 3.0 log (range = 1.9 to 3.8 log) for direct filtration, while the respective *Giardia* removals averaged 3.4 log and 3.3 log (ranges = 2.2 to 3.9 and 2.9 to 4.0 log, respectively). The turbidity of the raw water for the full-scale plant typically was between 2.5 and 11 NTU, with a peak level of 28 NTU. The full-scale plant achieved similar filtered water turbidity levels, and *Cryptosporidium* removal averaged 2.25 log for conventional treatment and 2.8 log for direct filtration, while the respective *Giardia* removals averaged 3.3 log for conventional treatment and 3.9 log for direct filtration. The authors reported log removals only for data with detections in the effluent samples; thus, reported removal efficiency is likely lower than the actual. The differences in performance noted between direct filtration and conventional treatment in the full-scale plant were attributed to differences in raw water quality during the respective filter runs.

A pilot plant study by Patania et al. (1999) evaluated removal of *Cryptosporidium* at varied raw and filter effluent turbidity levels using direct filtration. Raw water turbidity was less than 2 NTU (low) and 10 NTU (high). Targeted filtered water turbidity was 0.02 NTU at both low and high raw water turbidity and 0.05 NTU for one run at the low raw water turbidity. *Cryptosporidium* removal was slightly higher when the raw water turbidity was higher at equivalent levels of filtered water turbidity. Also, *Cryptosporidium* removal was a mean of 1.5 log higher when steady-state filtered water turbidity was 0.02 NTU compared to 0.05 NTU.

LeChevallier and Norton (1992) evaluated protozoa removal at raw water turbidity levels ranging from less than 1 to 120 NTU. Removals of *Giardia* (2.2 to 2.8 log) and *Cryptosporidium* (2.3 to 2.5 log) were slightly less than those reported by other researchers, possibly because the full-scale plants studied were operated under less ideal conditions than the pilot plants. The participating treatment plants were in varying stages of treatment optimization.

West et al. (1994) used pilot-scale direct filtration with anthracite monomedia at filtration rates of 6 and 14 gallons per minute per square foot (gpm/ft^2). Raw water turbidity was 0.3 to 0.7 NTU. Removal efficiencies for *Cryptosporidium* at both filtration rates were 2 log during filter ripening (despite filter water turbidity exceeding 0.2 NTU) and 2 to 3 log for the stable filter run, declining significantly during particle breakthrough. When effluent turbidity was less than 0.1 NTU, removal typically exceeded 2 log. Log removal of *Cryptosporidium* generally exceeded that of particle removal.

Nieminski and Bellamy (2000) studied full-scale treatment plants to determine whether water quality parameters could be used as indicators of protozoa and virus removal. These parameters included turbidity, particle count, bacteria, bacterial spores, and bacterial phages (viruses that infect bacteria). Spores are the bacterial equivalent of cysts; they are formed in conditions of environmental stress to protect the contents of a cell. In order for a parameter to be a good surrogate, it had to be detectable in raw and filtered water. With unseeded raw water, however, the initial levels were relatively low, and most parameters were undetectable in a large percentage of finished water samples. Nieminski and Bellamy found spores of aerobic bacteria to be good indicators of treatment effectiveness, since they were detectable in 85 percent of finished water samples. However, aerobic spore removal levels did not correlate with protozoa removal. The authors also examined turbidity removal along with protozoa removal and found that less turbid raw waters, where the concentration of protozoa was low, had slightly lower protozoa removals than more turbid raw waters.

In a study of particle counts and their relationship to *Cryptosporidium* and *Giardia* removal at 100 plants, McTigue et al. (1998) found that particle count data were normally distributed. The median log removal of particles larger than 2 micrometers (µm) after filtration was 2.8 log; removal depended to

some extent on the raw water particle concentration. There was not a significant correlation between particle count and turbidity in raw or finished water, although correlation was better in raw water. There was little correlation between particle counts and protozoa levels in finished water. In an analysis of previously collected data, the authors reported that median log removal for particles larger than 3 µm was 2.4 log; removal also correlated with raw water particle concentration. The authors also conducted pilot plant studies, in water seeded with protozoa, and found that *Cryptosporidium* and *Giardia* removal after filtration through dual media averaged 4 log and did not vary with initial concentration. They evaluated pathogen removal under stressed filter conditions (the filtration rate was doubled) and found both *Cryptosporidium* and *Giardia* removal decreased by approximately 2 log. When they compared pilot plant particle removal to log removal for protozoa, there appeared to be no correlation, but when they compared a subset of these data taken at similar temperatures and coagulation conditions, they determined that particle and protozoa removal correlated significantly.

Ongerth and Pecoraro (1995) studied the effect of coagulation on protozoa removal by direct filtration with low turbidity raw waters (0.35 to 0.58 NTU). With optimal coagulation, effluent turbidity averaged less than 0.1 NTU, *Cryptosporidium* removal ranged from 2.7 to 3.1 log, and *Giardia* removal ranged from 3.05 to 3.6 log. For one filter run, the coagulant dose was decreased by 50 percent to test suboptimal operation conditions. Effluent turbidity increased to a mean of 0.36 NTU and *Cryptosporidium* and *Giardia* removals decreased to 1.5 and 1.3 log, respectively.

LeChevallier et al. (1991) evaluated removal efficiencies for *Giardia* and *Cryptosporidium* at 66 surface water treatment plants in 14 States and one Canadian province. Most of the utilities achieved between 2 and 2.5 log removals for both *Giardia* and *Cryptosporidium*. When no cysts or oocysts were detected in the finished water, protozoan levels were set at the "detection limit" for calculating removal efficiencies.

5.1.2 Other Filtration Technologies

5.1.2.1 Slow Sand

Slow sand filtration plants are commonly associated with smaller systems. They operate at very low filtration rates without the use of coagulation in pretreatment and have a compacted media of smaller grain size sand. Most of the particulate removal occurs in a thin layer on top of the sand bed called the schmutzdecke layer. This layer not only traps particles but also provides biological treatment. EPA (1988) lists research studies indicating that a well-designed and operated plant using these technologies is capable of removing 3 log of *Giardia* and viruses. Results from more recent studies are presented below.

Schuler and Ghosh (1991) studied the removal of *Cryptosporidium* and *Giardia* by slow sand filtration with a pilot filter. *Cryptosporidium* removals ranged from 3.9 to 6.1 log, with a mean of 5.4 log. *Giardia* removals ranged from 2.8 to greater than 6.5 log, with a mean of 5.2 log. Lower *Giardia* removals occurred during the winter months and before the filter had matured (analysis of the top layer showed no biological activity). The 1-year experiment began in January 1988; after February 1988 the *Giardia* removals were all greater than 5.5 log. The sample collection for *Cryptosporidium* started in March.

Timms et al. (1995) conducted a pilot-scale slow sand filtration study of *Cryptosporidium* removal. No oocysts were detected in the filtered samples, indicating greater than 3.6 log removal of *Cryptosporidium*.

Fogel et al. (1993) evaluated the removal efficiencies for *Cryptosporidium* and *Giardia* with a full-scale slow sand filtration plant. The log removals for *Cryptosporidium* ranged from 0.1 to 0.5 log and 0.9 to 1.4 log for *Giardia*. Raw water turbidity ranged from 1.3 to 1.6 NTU and decreased to 0.35 and 0.31 NTU respectively, after filtration. The authors attributed the low *Cryptosporidium* and *Giardia* removals to the relatively poor grade of filter media and lower water temperature. The sand had a higher uniformity coefficient than recommended, which creates larger pore spaces within the filter bed subsequently retarding its biological removal capacity. Lower water temperatures (1 degree Celsius (1°C)) also decreased biological activity in the filter media.

Hall et al. (1994) researched the removal of *Cryptosporidium* with a pilot-scale slow sand filtration plant. *Cryptosporidium* removals ranged from 2.8 to 4.3 log after filter maturation, with a mean of 3.8 log (at least 1 week after filter scraping). Raw water turbidity ranged from 3.0 NTU to 7.5 NTU for three of the four runs and 15.0 NTU for the fourth run. Filtered water turbidity was 0.2 to 0.4 NTU, except for the fourth run with 2.5 NTU filtered water turbidity. For the fourth run, the *Cryptosporidium* removal (3.9 log) did not decrease with the surge in raw and filtered water turbidity.

Hall et al. (1994) also investigated *Cryptosporidium* removal during start-up of the pilot-scale slow sand filter. After scraping the sand, the filtration rate was slowly increased by steps of 100 liters per hour (l/hour) (0.05 m/h) over a 4-day period. The results indicated high *Cryptosporidium* removals, ranging from 4.3 log to greater than 6.4 log. From these results, filter ripening did not appear to affect *Cryptosporidium* removal, as there was high removals and no consistent increase in removal.

5.1.2.2 Diatomaceous Earth (DE)

DE filters, or precoat filters, use a thin layer of very fine material (usually diatomaceous earth) as a filter medium. During the filter cycle, additional filter media is metered into the influent water in proportion to the solids being removed. The dirt particles intermingle with the additional filter media, which maintains the permeability of the cake layer and allows for longer filter runs. DE filters can handle only lower flow rates and therefore are used by smaller systems.

Schuler and Ghosh (1990) also investigated the removal of *Cryptosporidium* and *Giardia* with a pilot-scale DE filter. *Cryptosporidium* removals ranged from 4.6 to greater than 6.4 log, with a mean of 5.3 log. The authors noted that finer grades of DE did not enhance *Cryptosporidium* removal and suggested that mechanical straining may not be a significant mechanism of removal. *Giardia* cysts were detected in the effluent from only one of five filter runs where a malfunctioning valve allowed air to enter and the filter cake was cracked, although *Giardia* removals for this run were still relatively high at 4.3 and 4.6 log. Overall, *Giardia* removal ranged from 4.3 to greater than 6.7 log and averaged 5.5 log.

Ongerth and Hutton (2001) investigated *Cryptosporidium* removal capabilities of DE filtration using a pilot-scale DE filter. Under normal operating conditions *Cryptosporidium* removals ranged from 6.1 to 6.6 log and effluent turbidity for most runs were 0.10 NTU or less. The authors also investigated filter performance under undesirable operating conditions. They used an undamped peristaltic feed pump that caused pressure fluctuations in the influent. (The authors noted this operating condition was unlikely to occur in a full-scale plant). *Cryptosporidium* removals dropped by roughly 1 log, ranging from 5.0 to 5.8 log compared to normal operating conditions; however, effluent turbidity increased to a range of 0.14 to 0.40 NTU.

5.1.2.3 Membranes

Membrane technology can achieve high levels of protozoa removal. The membranes commonly used in municipal-scale plants are composed of cellulose acetate or various types of polymers and have a spiral-wound or hollow-fiber structure. The process operates by forcing water through a membrane at low to moderate pressure. Spiral-wound membranes are composed of two flat sheets rolled up like a carpet; water passes through them into a hollow tube in the center of the roll. Hollow-fiber membranes are bundles of aligned fibers; water flows from the outside of the bundles to a hollow core in the center (Conlon 1990). Membrane technologies are categorized into three types by the membrane pore size: microfiltration (largest pore size), ultrafiltration (medium pore size), and nanofiltration (smallest pore size).

Microfiltration is becoming more prevalent in the water treatment industry due to several factors, including increased regulation of disinfection byproducts (DBPs) and microbial contaminants. If raw water quality is high, microfiltration can be applied without pretreatment. Other advantages of microfiltration over conventional filtration are that less space is needed, less time is required for filter runs, less operator expertise and labor are needed, less sludge is generated, and the cost of replacing membranes is comparable to that of buying chemicals for conventional treatment (de Haas 1997).

A few studies relate the experiences of public water systems (PWSs) that have installed microfiltration. A small PWS installed a pilot microfiltration unit and found that it produced turbidity levels of 0.06 NTU, which was comparable with the levels achieved by the system's conventional treatment plant (de Haas 1997). Another system built a small microfiltration plant for seasonal use and found that, even with occasional raw water turbidities of 100 NTU, finished water turbidities of 0.05 NTU could be obtained, while the system's direct filtration plant had to be shut down at 30 NTU (Gere 1997). One significant cost associated with membrane filtration, especially at small systems, is electricity, particularly that needed to provide pressure to force water through the membranes and to backwash the filters (Gere 1997).

The primary disadvantage of membrane filtration is the potential for membrane fouling (clogging of membranes by particulates and precipitates), especially since a common pore size for microfiltration is 0.2 μm. For this reason, microfiltration and ultrafiltration should be used on pretreated water or high-quality raw water. Additionally, membranes should be chemically cleaned in place every few weeks (Gere 1997). Some water systems are beginning to use microfiltration in combination with nanofiltration to remove particulates and DBP precursors. The first system to install such an integrated membrane system, a small system in Alaska, achieves mean finished water turbidities of 0.05 NTU (Lozier et al. 1997).

Membrane processes such as microfiltration have achieved greater than 4.8 log removal of both *Cryptosporidium* and *Giardia* under bench-scale worst-case operating conditions and 6 to 7 log removal under normal pilot-plant operating conditions (Jacangelo et al. 1995) (Exhibit 5.1). These removals are much greater than the log removals observed by other filtration technologies such as slow-sand and DE filtration. Seyde et al. (1998) reported 5- to 6-log removals of *Cryptosporidium* and *Giardia* using nanofiltration.

5.1.2.4 Bag and Cartridge Filtration

Bag and cartridge filters treat lower flow rates and thus are mostly used by smaller systems. The water is treated by passing it through a bag or porous cartridge that retains the particulate matter. The nature of the filter material and the direction of flow are two features that differentiate bag filtration from

cartridge filtration. Bag filters are typically felt or woven with materials such as polypropylene, polyester, or nylon. Cartridge filters are typically composed of fiberglass or ceramic membranes supported by a rigid core or are made from strings of polypropylene, acrylics, nylon, or cotton wrapped around a filter element. The direction of flow in bag filtration is through the inside of the bag to the outside, whereas for cartridge filtration systems the direction of flow is outside to inside.

Schaub et al. (1993) evaluated the removal of *Cryptosporidium* with two cartridge filters, one a melt blown polypropylene depth filter and the second a polypropylene pleated filter (both 3-micron pore size). Both filters were tested with "general" water and "worst case" water. For both types of influent water and flow rates of 1 gallon per minute (gpm) and 2 gpm, there were no detects of *Cryptosporidium* oocysts in the filtered water from the depth filter. Results indicated greater than 3.5 log-removal of *Cryptosporidium* and no impact of increased flow on removal efficiency. The pleated filter demonstrated lower removals, with *Cryptosporidium* removals ranging from 1.1 to 2.1 log and a mean of 1.6 log. The worse case influent caused the lowest *Cryptosporidium* removal of 1.1 log.

Goodrich and Lykins (1995) evaluated three different bag filters and one cartridge filter for the removal of *Cryptosporidium* surrogates, 4.5-micron polystyrene beads. The bag filters were tested at 25, 50, and 100 percent of their maximum flow rate (not indicated), at an inlet pressure of 50 pounds per square inch (psi), and at various pressure drops (from 0 to 25 psi). The mean removal of the polystyrene beads for the three bag filters were 0.5, 1.3, and 2.0 log. Changing the flow rate into the bag filters did not make a statistically significant difference in removal rates. The cartridge filter was tested at 25 gpm (50 percent of maximum flow rating) at an inlet pressure of 50 psi. The mean log removal of two runs through the cartridge filter was 3.6 log.

Roessler (1998) tested the ability of a cartridge filter to remove *Cryptosporidium*. The test consisted of three runs seeded with *Cryptosporidium*. The flow through the filter was constant at 2.4 gpm for approximately 2 hours for each run. The cartridge filter provided 3.2 to 3.6 log removal of *Cryptosporidium*.

5.1.3 Prefiltration Optimization and Filtration Characteristics

To achieve good removal efficiency, it is advantageous to optimize the water treatment process prior to filtration. Particulate removal prior to the filtration increases the length of the filtration cycle, reducing the need for backwashing, thereby reducing process fluctuations. Forming the oocyst/particulate complex by flocculation/coagulation is also a key element of filtration optimization. Several studies investigating prefiltration processes are reviewed in this section.

5.1.3.1 Coagulation Effects

In coagulation, chemicals are added to water to cause particles to coalesce and make the particles easier to remove. Yates et al. (1997) conducted pilot-scale studies to optimize coagulation/filtration processes for the removal of *Cryptosporidium* oocysts during direct filtration. Either liquid aluminum sulfate (alum) or ferric chloride ($FeCl_3$) was used as the primary coagulant, in combination with cationic, anionic, and/or nonionic polymers, to arrive at the optimal coagulation conditions for turbidity and particle removal. Each coagulant/polymer combination was evaluated only after stable, consistent filter operation had been demonstrated, usually after two or three complete filtration cycles. In the pilot-scale testing, $FeCl_3$-treated water generally provided slightly greater removal of turbidity, particles, and aerobic spores than alum-treated water; the filter runs were 32 hours, as opposed to 21 hours for alum-treated

water. Coagulation with $FeCl_3$ provided greater *Cryptosporidium* oocyst removal than alum at ambient pH. Oocyst removals of 4.5 log were found during filter challenges when $FeCl_3$ was used in conjunction with prechlorination and addition of filter aid (polyDADMAC). Similar conditions using alum yielded about a 3.7-log removal (Yates et al. 1997).

Huck et al. (2000) studied filtration efficiency during optimal and sub-optimal coagulation conditions at two direct filtration pilot-scale plants. One plant employed a high coagulation dose (38 milligrams per liter (mg/L) alum and 2 mg/L silicon dioxide (SiO_2)) for both total organic carbon (TOC) and particle removal, and the second plant used a low dose (5 mg/L alum) for particle removal only. Both plants were operated to maintain turbidity below 0.1 NTU during optimal conditions. The high coagulation dose plant achieved a median (mean was not reported) of 5.6 log removal of *Cryptosporidium*, while the low dose plant achieved a median of 3 log. The authors noted that the difference between the two plants' results was not fully understood but may be due to the difference in coagulation doses. When sub-optimal conditions were induced by decreasing the coagulation dose 40 to 60 percent for a target turbidity of 0.2 to 0.3 NTU, the median *Cryptosporidium* removals dropped to 3.2 log and 1 log at the high dose and low dose plants, respectively. However, the high coagulation dose plant showed a much wider range of *Cryptosporidium* removals, with the 25^{th} and 75^{th} percentiles approximately 1.7 log and 5.2 log, respectively. The authors also tested conditions of total coagulation failure, and results indicated minimal *Cryptosporidium* removal; from two runs the median was approximately 0.25 log.

5.1.3.2 Filter Breakthrough Effects

A 1996 pilot-plant study by Hall and Croll assessed aspects of rapid gravity filter operations that can influence the risk of breakthrough (in which particles and microbes pass through filters) into filtered water. They observed quality changes that can occur during a filter run. Initial peaks in particle counts, turbidity, and oocyst concentrations (mean = 6.3 oocysts/liter (L)) occurred in the first hour of the filter run. Stopping and restarting after backwashing the filters also produced peaks in particle counts and turbidity, but these were less significant than the peaks at the beginning. The initial peaks were a consistent feature of all filter runs monitored at the plant and were attributed to backwashing and filter ripening. Hall's and Croll's observations demonstrated that higher oocyst concentrations are detected in finished water during the first hour of a filter run, consistent with turbidity and particle count data, indicating that breakthrough of particles gives a good indication of potential exposure to *Cryptosporidium* (Hall and Croll 1996).

Emelko et al. (2000) conducted further investigations of *Cryptosporidium* removals during vulnerable filtration periods using a pilot-scale direct filtration system. The authors investigated four different operational conditions: stable; early breakthrough—when filter effluent turbidities were approximately between 0.1 and 0.3 NTU; late breakthrough—when filter effluent turbidity reached 0.3 NTU; and end of run—from when subtle changes in effluent turbidity and particle counts were noticed to approximately 0.1 NTU. The authors presented their results for stable operation in combination with data from previous work they conducted at the same facility (Coffey et al. 1999). *Cryptosporidium* removals during stable operation ranged from 4.7 to 5.8 log, with a mean of 5.5 log. At these times, turbidity was consistently low, approximately 0.04 NTU. The early breakthrough period started with effluent turbidities from 0.04 to 0.08 NTU and increased to approximately 0.2 NTU. *Cryptosporidium* removals diminished during this period; results from three experiments ranged from 1.7 to 2.8 log removal, with a mean of 2.1 log. For the late breakthrough period, turbidities were consistently 0.25 to 0.3 NTU at the start of seeding periods. (The authors provided ending effluent turbidity data for one of two experiments—0.35 NTU where the initial effluent turbidity was 0.25 NTU). *Cryptosporidium* removals

further decreased to a mean of 1.4 log and a range of 1.3 to 1.8 log. Two experiments tested *Cryptosporidium* removal during the end-of-run operation, when effluent turbidities generally start increasing. Turbidity started at about 0.04 NTU for both experiments and ended at 0.06 NTU for the first experiment and 0.13 NTU for the second experiment. The authors reported a range of 1.8 to 3.3 log and a mean of 2.5 log *Cryptosporidium* removal for both experiments. During the one-hour seeding period of the first experiment, turbidity increased steadily from 0.04 NTU to 0.06 NTU while *Cryptosporidium* removal decreased from 3.3 to 2.3 log.

Emelko et al. (1999) studied design and operational conditions for maximizing *Cryptosporidium* removal by filters during both optimal and challenged operating conditions. A bench-scale experiment was conducted using a dual media filter (anthracite and sand). Water containing a suspension of kaolinite was coagulated in-line at pH 6.9 with 5 mg/L alum and filtered.

After an hour of normal operation the coagulant addition was terminated, resulting in elevated effluent turbidities and decreased removal of *Cryptosporidium*. Turbidity increased from 0.08 NTU to 0.15 NTU while corresponding *Cryptosporidium* removals decreased from 4.2 log to 3.3 log.

5.1.3.3 Sedimentation and Dissolved Air Flotation

One disadvantage of the flocculation/coagulation and sedimentation process is the buoyancy of oocysts and their low settling velocity, which contribute to incomplete removal (Gregory 1994; Swabby-Cahill et al. 1996). Dissolved air filtration (DAF) takes advantage of these properties by floating the oocyst/particle complex to the surface for removal. In DAF, air is dissolved in pressurized water, which is then released into a flotation tank containing the flocculated particles. As the water enters the tank, the dissolved air forms small bubbles, which collide with and attach to floc particles and float to the surface. The smaller the air bubbles, the more bubbles can form, increasing the chances of collision with floc (Gregory and Zabel 1990).

Plummer et al. (1995) investigated the effectiveness of DAF for the removal of *Cryptosporidium parvum* oocysts, comparing removal levels to those achieved by conventional treatment with sedimentation. As shown in Exhibit 5.2, comparison of the prefiltration steps of the conventional treatment process showed only 0 to 0.81 log removal of oocysts by sedimentation and 0.38 to 3.7 log removal by DAF processes, depending on coagulant dose (Plummer et al.,1995).

Edzwald and Kelley (1998) demonstrated a 3 log removal of oocysts using DAF, compared with a 1 log removal using sedimentation in the clarification process before filtration. Braghetta et al. (1997) did not evaluate oocyst removal, but they showed that DAF lengthened microfiltration filter runs and decreased turbidity from mean raw water levels of 2.7 NTU to 0.3 to 1.0 NTU.

Harrington et al. (2001) studied the removal of *Cryptosporidium* and emerging pathogens by filtration, sedimentation, and DAF, using bench-scale jar tests and pilot-scale conventional treatment trains. In the bench-scale experiments, mean log removal of *Cryptosporidium* was 1.2 by sedimentation and 1.7 by DAF, with all experiments run at optimized coagulant doses. *Cryptosporidium* removal was not significantly affected by lower pH or coagulant aid addition, and was similar in all four water sources that were evaluated. However, removal of *Cryptosporidium* was greater at 22°C than at 5°C, and was observed to be higher with alum coagulant than with either polyaluminum hydroxychlorosulfate or ferric chloride. In the pilot scale experiments, water treated with coagulation, flocculation, sedimentation, and granular media filtration had a mean log removal of *Cryptosporidium* of 1.9 in filtered water with turbidity of 0.2 NTU or less. Tenth percentile removal increased as filtered water turbidity dropped

below 0.3 NTU. The authors noted there was no apparent effect of filtration rate on removal efficiency. In comparing *Cryptosporidium* removal by sand, dual media (anthracite/sand), and trimedia (anthracite/sand/garnet) filters, no difference was observed near neutral pH in samples collected from ripened filters. However, at pH 5.7, removal increased significantly in the sand filter, and it outperformed the other filter media configurations. There was no observable effect of a turbidity spike on *Cryptosporidium* removal.

Exhibit 5.2 Data Comparing Sedimentation and Dissolved Air Flotation Removal of *Cryptosporidium*

Raw Water Oocyst Concentration (no./L)	Ferric Chloride Concentration (mg/L)	Removal by Dissolved Air Flotation (log units)	Removal by Sedimentation (log units)
3.5×10^5	2	0.38	0
3.5×10^5	3	2	NR
3.5×10^5	3.5	2.6	0.61
3.5×10^5	4	NR	0.81
3.5×10^5	5	3.7	NR

Source: Plummer et al. 1995.
NR = No marked reduction.

As part of its rule development process, EPA is studying other prefiltration steps systems can use to make filtration more effective (USEPA 2000f). One possible step is installation of pre-settling basins to allow additional settling before or while coagulant is added. Storage has the added advantage of providing time for oocysts and cysts to die off. Systems can also implement additional filtration before and after their usual filtration processes. These include the use of in-bank filtration (passing water through an infiltration gallery or shoreline before withdrawing water), roughing filters (preceding primary filtration), and secondary filtration (including granular activated carbon filters after conventional filtration).

5.1.3.4 Solids Contact Clarifiers

Solids contact clarifiers, also referred to as sludge blanket clarifiers, are processes that incorporate coagulation, flocculation, and sedimentation into one unit process. These processes can provide efficient clarification by recirculating flocculated water, or mixing raw water and coagulants with flocculated water, which enhances chemical precipitation and larger floc formation. Additionally, the flocculated water generally passes directly through the sludge blanket to allow adsorption of the flocs by the sludge blanket. A pilot-scale study of a pulsating solids contact clarifier by New Jersey American Water Company showed over 4-log removal of *Giardia* cysts (Pennsylvania American Water Company 1989). The pulsating design allows the sludge blanket to be uniformly expanded over the entire surface area, which minimizes short circuits.

5.2 Removal of Viruses

This section discusses the factors influencing virus removal in filtered systems. Viruses that are adsorbed to particulate matter may settle out during sedimentation (USEPA 1999a). Sedimentation can remove about 95 percent of poliovirus and coxsackievirus (USEPA 1999b). However, viruses removed as a result of the sedimentation process have not been inactivated (SDWC 1977). They are still capable of causing infection if the settled sludge is improperly disinfected. If untreated sludge is disturbed, viruses can be redistributed into the overlying water (USEPA 1999a).

Removal of viruses in treatment plant filters is highly variable and depends on the filter design and operation, as well as the type of pretreatment provided. Virus particles that are not bound to particulates are usually too small to be retained on sand filters or alternate media used to filter water. Virus retention by such media depends on association of the virus with suspended matter that is large enough to be trapped mechanically. This is because the sand has no affinity for the virus and the virus particles are small enough to pass through the filter pores (Montgomery 1985). When sand filtration follows coagulation and sedimentation, viruses that are sorbed on fine floc particles can be retained efficiently by sand. Under these conditions, virus removal by the filter media ranges from 90 percent to more than 99 percent (SDWC 1977; USEPA 1999a). Payment and Armon (1989) evaluated the percentage removal of indigenous viruses at operational water treatment plants for a combined flocculation and settling step. Results ranged from 0 to 74 percent removal of rotavirus, enterovirus, and bacteriophage during the dry and rainy seasons.

Adsorption is the phenomenon whereby molecules adhere to a surface, such as granular activated carbon (GAC) or resins, with which they come into contact because of forces of attraction at the surface. The degree of adsorption of a virus is pH-dependent, with stronger retention occurring at lower pH (SDWC 1977). Results from bench- and pilot-scale studies performed in 1977 suggest that virus removal onto GAC is inconsistent and hard to control (Montgomery 1985; USEPA 1999a).

More recently, Jancangelo et al. (1995) studied the effectiveness of micro- and ultrafiltration on MS2 bacteriophage viruses. They tested seeded water from three different sources at bench and pilot scales to determine possible mechanisms of action for membrane filtration. At bench scale, with the use of distilled water and worst-case scenario operating conditions, microfiltration provided about 1-log removal of MS2. At pilot scale using raw water, microfiltration consistently achieved 2 or slightly above 2 log of removal for some waters and membranes, but less than 1 log for other sources, suggesting that other mechanisms besides physical straining or adsorption to the membrane are at work. Varying the initial virus concentration had no impact. The authors also tested the impact of adding a clay suspension to the water at bench scale before passing it through a microfilter. Removal was not significantly impacted, suggesting that adsorption (at least to clay suspensions) is not a mechanism. However, precoating the microfilter with clay before filtration, causing the formation of a cake layer, increased log removal from about 1.2 to 3.7, depending on the amount of clay applied. In natural waters at pilot scale, natural cake layer formation had less of an effect, increasing removal by only 0.5 log after 5 hours of operation without backwashing, probably due to the much lower density of the layer. Removal returned to initial levels after backwashing. The authors also found that chemical precipitation, which decreased the rate of flow through the membrane, increased virus removal. The composition of the precipitate was not determined. Ultrafiltration was found to be more effective than microfiltration, exhibiting log removals at or above 6 at both bench-and pilot-scale plants.

Adham et al. (1998) conducted a bench-scale study of virus removal via reverse osmosis. They tested five brands of membranes on water seeded with 10^4 to 10^7 plaque-forming units per milliliter (pfu/mL) of MS2 viruses. Reverse osmosis membranes have nominal cutoffs of 0.001 µm; they block

most particles larger than 0.001 µm in diameter, but variations may occur depending on particle shape and charge. MS2 viruses are 0.025 µm, but some membranes still allowed viruses to pass through. The membranes achieved removes from 1.4 to >7.4 log.

5.3 Conclusion

The studies described above indicate that conventional and direct filtration systems, when operating under appropriate coagulation conditions and achieving a filtered water turbidity level of less than 0.3 NTU, should achieve at least 2 log of *Cryptosporidium* removal. Removal rates vary widely, to over 5 log, depending on water matrix conditions, filtered water turbidity levels, and stage of the filtration cycle.

According to the literature reviewed in this chapter, the highest pathogen removal rates occurred in pilot plants and systems that achieved finished water turbidities of less than 0.1 NTU. Studies of *Cryptosporidium* and *Giardia* removal at times of filter breakthrough and end-of-run show that removal can decline before a significant change in turbidity is detected. Although turbidity is an indicator of filtration performance, it is not a direct indicator of pathogen removal.

DAF may be an effective prefiltration alternative to sedimentation. In two experiments, sedimentation resulted in less than 1 log removal of *Cryptosporidium*, while DAF resulted in up to 3 or more log removal. DAF also has been shown to reduce turbidity.

Virus removal depends heavily on whether adsorption takes place. Viruses adsorb to flocs and either settle or filter out in the treatment process. Other technologies (e.g., slow sand, DE filtration, membranes) can provide equivalent or better pathogen removal than conventional filtration. Slow sand, DE filtration, and bag and cartridge filtration are more suited for smaller systems. Membrane technologies can provide excellent pathogen removal.

6. Observed Finished Water Occurrence

The ICR required finished water sampling by PWSs that detected certain levels of microorganisms in their source water. PWSs were required to conduct finished water monitoring at any treatment plant at which they detected 10 or more *Giardia* cysts, 10 or more *Cryptosporidium* oocysts, or 1 or more total culturable viruses, per liter of any microbial raw water sample (USEPA 1996a). The PWS was required to analyze finished water samples for all organisms, not just those detected in the source water. For each sample of finished water, the PWS reported the concentrations of *Cryptosporidium*, *Giardia*, total culturable viruses, and other indicators (total coliforms, fecal coliforms, and/or *E. coli*). The rule did allow some exceptions to the monitoring requirements. This section presents the finished water microbial occurrence data. Sections 6.1 through 6.4 discuss the *Cryptosporidium*, *Giardia*, virus, and indicator data, respectively.

Along with the systems that reached the trigger levels, other PWSs voluntarily tested their finished water. But because not every system was required to test its finished water, the total number of finished water samples is far less than the number of source water samples.

Exhibit 6.1 presents a summary of sample volumes and volumes analyzed for the two groups of microorganisms of concern: protozoa and viruses. Of the microorganisms tested for in the finished water analysis, protozoa—which include *Cryptosporidium* and *Giardia*—were sampled almost twice as many times as viruses. Coliforms were also sampled more frequently than viruses. Of water sampled each time, only a portion was tested; this was the volume analyzed. The range of the volume analyzed, however, was not significantly different for the two groups. The volume analyzed for viruses was 35 percent lower than the sample volume. For protozoa, the volume analyzed was significantly different from the sample volume; the volume analyzed was 22 percent of the sample volume.

Exhibit 6.1 Summary of Volumes Sampled and Analyzed

Microorganism	Number of Samples (N)	Sample Volume (L)			Volume Analyzed (L)		
		Median	Mean	Range	Median	Mean	Range
Protozoa	1,058	1,015	1,072	100 - 8,509	110	228	1 - 1,855
Viruses	573	1,518	1,550	200 - 2,998	1,000	995	1 - 1,489

6.1 *Cryptosporidium*

Exhibit 6.2 summarizes the results of sampling for various microorganisms. During the 18 months in which ICR data were collected, 1,058 samples of finished water were collected to test for *Cryptosporidium* oocysts. The volumes collected to analyze for *Cryptosporidium* were all close to 1,000 liters (L) (6 percent of samples were 1,200 L or greater). Of the total sample, 11 samples (from 7 plants) had positive detects. These positive detections were 1.04 percent of the total number of samples taken. The average and median concentrations for all 11 positive detections were less than 0.1 oocyst/L. The range of positive concentrations for the *Cryptosporidium* samples was much smaller than that for any of the other microorganisms, except for viruses.

Exhibit 6.2 Summary of Sample Results

Microorganism	Number of Samples	Number of Detects (Percent)		Concentration[1]		Range of Positive Concentrations
				Mean[2]	Median[2]	
Cryptosporidium	1,058	11	(1.04)	0.0057	0.0049	0.001 - 0.011
Giardia	1,058	16	(1.51)	0.033	0.012	0.002 - 0.26
Viruses	573	9	(1.57)	0.0011	0.001	0.001 - 0.002
Total coliform	3,452	24	(0.70)	22.83	1.5	1 - 330
Fecal coliform	2,208	12	(0.54)	2.42	1	1 - 13
E. coli	2,102	5	(0.24)	1	1	1 - 1

[1] Concentrations are in units of number of oocysts/L for *Cryptosporidium*, cysts/L for *Giardia*, most probable number (MPN) of plaque-forming units/L for viruses, and density/100 milliliters (mL) for bacteria.
[2] Mean and median for positive samples.

One plant detected *Cryptosporidium* four times in its finished water; another detected it twice. The other plants with positive detects found *Cryptosporidium* only once. All of the plants with positive detects use conventional filtration and chlorine as primary and secondary disinfectants, except for one that used chloramine as a secondary disinfectant. One plant was found to have oocysts with internal structures still intact. The majority of positive samples, however, had oocysts that contained no internal structures and instead were either amorphous or empty (see Exhibit 6.3).

Exhibit 6.3 Summary of *Cryptosporidium*-Positive Sample Results

Volume Analyzed (L)	Total *Cryptosporidium* Concentration[1]	Oocyst Count			
		Total	Internal Structures	Amorphous	Empty
202	0.01	2	0	1	1
800	0.001	1	0	1	0
180	0.006	1	0	1	0
237	0.004	1	0	0	1
213	0.0094	2	0	0	2
409	0.0049	2	0	1	1
213	0.0047	1	0	0	1
427	0.007	3	0	1	2
454	0.011	5	3	2	0
293	0.0034	1	1	0	0
802	0.001	1	0	0	1

[1] Oocysts per liter.

6.2 *Giardia*

During the 18 months in which ICR data were collected, 1,058 samples of finished water were collected and tested for *Giardia* cysts. Of these, 16 samples tested positive. These positive detects constitute 1.51 percent of the total number of samples taken. The mean and median concentrations for all 16 positive detects are more distinct from one another than for *Cryptosporidium*. The range of the positive concentrations for *Giardia* is much broader than the range of detects for *Cryptosporidium*, even though the same total number of samples were analyzed (see Exhibit 6.2).

The 16 *Giardia*-positive samples were generated by 6 water systems. Five of the plants with positive samples also had positive samples for *Cryptosporidium*. All of the plants with positive samples used conventional filtration and chlorine as primary and secondary disinfectants. The volumes collected to analyze for *Giardia* were all close to 1,000 L, but only a small volume of the 1,000 L of water collected was analyzed. The number of detects per liter of water ranged from 0.002 to 0.26, which is a considerably broader range than for *Cryptosporidium* and for viruses. Counts for *Giardia* were generally higher than for *Cryptosporidium*. This reflects the fact that *Giardia* concentrations are higher than *Cryptosporidium* concentrations in source water (see Chapter 4). Three *Giardia* samples had cysts with one internal structure, and three had cysts with more than one internal structure (see Exhibit 6.4).

Exhibit 6.4 Summary of *Giardia*-Positive Sample Results

Volume Analyzed (L)	Total *Giardia* Concentration[1]	Cyst Count				
		Total	One Internal Structure	Two or More Internal Structures	Amorphous	Empty
202	0.069	14	0	0	0	14
172	0.017	3	0	0	0	3
202	0.01	2	0	0	1	1
237	0.004	1	0	0	0	1
212	0.0094	2	0	0	1	1
102	0.02	2	0	0	2	0
213	0.047	10	1	0	6	3
213	0.0094	2	0	0	2	0
427	0.0023	1	0	0	1	0
205	0.0097	2	0	0	2	0
454	0.026	12	6	3	1	2
293	0.0068	2	0	0	1	1
34	0.26	9	1	2	5	1
120	0.017	2	0	0	2	0
210	0.014	3	0	1	2	0
802	0.002	2	0	0	0	2

[1] Cysts per liter.

6.3 Viruses

During the 18 months in which ICR data were collected, 573 finished water samples were collected to test for viruses. Of these, nine were positive; this constitutes about 1.6 percent of the total number of samples. The average and median concentrations are remarkably similar. Therefore, it can be assumed that very few of the data are outliers. This assumption is confirmed by the range of positive concentrations (0.001-0.002 MPN/L), which is much smaller for virus samples than for any of the other microorganisms (see Exhibit 6.2).

The nine positive sample results were generated by seven plants. The plants with the positive samples used a variety of different water system treatment and disinfection methods (see Exhibit 6.5). The sample volumes collected were approximately 1,500 L, and the volumes analyzed were approximately 1,000 L (see Exhibit 6.1). One of the plants also had positive *Cryptosporidium* and *Giardia* samples.

Exhibit 6.5 Summary of Virus Positive Results

MPN virus/L[1]	Treatment Type	Disinfection	
		Primary	Secondary
0.001	Direct Filtration	Ozone	Chlorine
0.001	Conventional	Chlorine/Chloramine	Chloramine
0.001	Conventional	Chlorine	Chlorine
0.001	Conventional	Chlorine	Chlorine
0.001	Two Stage Softening	Chlorine/Chloramine	Chloramine
0.001	Two Stage Softening	Chlorine/Chloramine	Chloramine
0.002	Two Stage Softening	Ozone	Chloramine
0.001	Two Stage Softening	Chloramine	Chloramine
0.001	Conventional	Chlorine	Chloramine

[1] Most Probable Number of viruses per liter.

6.4 Indicators

The ICR required all PWSs to monitor their source water for total coliforms. Operators could, however, choose between fecal coliform or *E. coli*; some plants monitored for both. Whenever a PWS exceeded a trigger concentration and was required to monitor finished water for protozoa and viruses, it was also required to monitor finished water for total coliform and either fecal coliform or *E. coli*.

6.4.1 Total Coliform

During the 18 months in which ICR data were collected, a total of 3,452 finished water samples were collected to be tested for total coliform. Of that total number, 24 had positive detects. These positive detects account for less than 1 percent of the total number of samples. The mean and median concentrations are quite different (see Exhibit 6.2) because a few outliers (shown in Exhibit 6.6) skew the distribution.

Exhibit 6.6 Summary of Total Coliform Positive Results

Density/100 mL	Treatment Type	Disinfection	
		Primary	Secondary
1	Conventional	None Listed	None Listed
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine/Chloramine	Chloramine
1	Conventional	Chlorine	Chloramine
2	Conventional	Chlorine	Chlorine
3	Conventional	Chlorine	Chlorine
8	Conventional	Chlorine	Chloramine
8	Conventional	Chlorine/Chloramine	Chloramine
11	Conventional	Chlorine	Chlorine
19	Conventional	Chlorine	Chlorine
21	Conventional	Chlorine	Chloramine
90	Conventional	Chlorine/Chloramine	Chloramine
330	Conventional	Chlorine	Chlorine
37	Direct Filtration	Chlorine	Chlorine
1	In-Line Filtration	Chlorine	Chlorine
1	Softening	Chlorine	Chlorine
1	Softening	Chlorine/Chloramine	Chloramine
1	Softening	Ozone	Chlorine
2	Softening	Chlorine	Chlorine
5	Softening	Ozone	Chloramine

6.4.2 Fecal Coliform

During the 18 months in which ICR data were collected, 2,208 finished water samples were collected and tested for fecal coliform. Of that number, 12 samples were positive. These positive detects

account for less than 1 percent of the total number of samples. The results of the positive samples are shown below in Exhibit 6.7.

Exhibit 6.7 Summary of Fecal Coliform Positive Results

Density/100 mL	Treatment Type	Disinfection	
		Primary	Secondary
1	Conventional	None Listed	None Listed
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine/Chloramine	Chloramine
2	Conventional	Chlorine/Chloramine	Chlorine
2	Conventional	Chlorine	Chlorine
4	Conventional	Chlorine	Chlorine
13	Conventional	Chlorine	Chlorine
1	In-Line Filtration	Chlorine	Chlorine

6.4.3 *E. coli*

During the 18 months in which ICR data were collected, 2,102 finished water samples were collected and tested for *E. coli*. Of that number, five samples were positive. These positive detections account for less than 0.5 percent of the total number of samples. The positive results are shown in Exhibit 6.8.

Exhibit 6.8 Summary of *E. coli* Positive Results

Density/100 mL	Treatment Type	Disinfection Type	
		Primary	Secondary
1	In-Line Filtration	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine
1	Conventional	Chlorine	Chlorine

6.5 Finished Water Occurrence—Summary

Of the plants that detected a particular organism in their raw water, less than 2 percent detected the same organism in their finished water. The concentrations of *Cryptosporidium* and *Giardia* in finished water were quite low, with *Cryptosporidium* concentrations never greater than 0.01 oocysts/L and *Giardia* concentrations (with one exception) below 0.07 cysts/L. There was a strong correlation between *Cryptosporidium* and *Giardia*; most plants with positive *Giardia* samples also had positive *Cryptosporidium* samples. Several of these plants also had multiple positive samples, which may indicate frequent process upsets or poorer performance at these plants. Virus detects were also low. Coliform densities in positive samples were generally at or close to 1 per 100 mL for fecal coliform and *E. coli*, while total coliform concentrations for several samples were up to a few hundred times higher. The overall microbial occurrence was low; however, the limitations of the sampling and analysis techniques affected the finished water data as well as source water. As a result, the true finished water occurrence may differ from what the observed data indicate.

7. Modeled Pre-LT2ESWTR Occurrence of *Cryptosporidium* in Finished Water

This chapter estimates the expected occurrence of *Cryptosporidium* in finished water of surface water supplies following implementation of the IESWTR and LT1ESWTR. This expected occurrence (referred to as the Pre-LT2ESWTR occurrence) is used in additional analyses to support the regulatory development of the LT2ESWTR. The *Economic Analysis for the LT2ESWTR* uses this finished water occurrence estimate as a baseline to predict the treatment cost and health benefits of different regulatory scenarios.

It is difficult to quantify *Cryptosporidium* levels in treated drinking water due to the low and variable concentrations typically present after filtration and the relatively high "detection limit" of analytical methods for protozoa. Consequently, EPA estimated finished water occurrence levels based on source water occurrence data and assumptions about the performance of treatment in removing oocysts. Because EPA assesses risk using mean annual exposure, the specific end-point modeled is the expected annual average *Cryptosporidium* concentration in finished water at the plant level.

The modeling of Pre-LT2ESWTR occurrence in finished water of surface water plants was conducted using two-dimensional Monte Carlo simulation procedures structured to address the variability and uncertainty in both annual average source water occurrence and treatment effectiveness among plants.

The algorithm for calculating the finished water *Cryptosporidium* level at an individual water plant is relatively simple, as shown below:

$$C_F = C_S \times 10^{-L} \qquad \text{(Equation 7.1)}$$

where C_F is the finished water *Cryptosporidium* concentration, C_S is the source water *Cryptosporidium* concentration, and L is the log removal achieved by the treatment performed at the plant.

In the Monte Carlo procedure, Equation 7.1 is computed many times. In each iteration of the calculation, different values are selected for C_S and L, reflecting (1) the variability in source water mean occurrence levels and treatment effectiveness expected to occur from one plant to another and (2) the uncertainty in the characterization of both of these factors. The output of this procedure is a set of distributions for C_F. Each of the individual C_F distributions represents one possible depiction of the plant-to-plant variability of finished water *Cryptosporidium* levels. The overall set of those distributions reflects the uncertainty in the "true" plant-to-plant variability. It is important to note that the occurrence modeling performed here, for use in the LT2 rule Economic Assessment, focuses on endemic risk and normal operating conditions at treatment plants and not outbreaks due to extraordinary occurrence levels or breakdowns in the treatment system. It is for this reason that the modeling effort focuses on plant average concentrations in the sources water and not extreme values that may occur rarely. Therefore, it was also considered appropriate to use expected values or means for the treatment effectiveness input value, L, that reflect typical operating conditions at a plant over the long term.

The remainder of this chapter is organized as follows:

- Section 7.1 presents relevant information on the source water *Cryptosporidium* occurrence distributions obtained from each of the four primary data sets (ICR Filtered, ICR Unfiltered, ICRSSM, and ICRSSL).

- Section 7.2 presents information on estimated treatment effectiveness distributions following full implementation of the IESWTR and LT1ESWTR.

- Section 7.3 describes the structure and implementation of the two-dimensional Monte Carlo simulation model.

- Section 7.4 presents the finished water *Cryptosporidium* occurrence distributions resulting from this modeling effort.

7.1 Source Water Occurrence

As discussed in Chapter 4, four data sets are used to characterize the occurrence of *Cryptosporidium* in source water for the LT2ESWTR regulatory analysis: the ICR filtered systems data set, the ICR unfiltered systems data set, the ICRSSL data set, and ICRSSM data set. A statistical model was developed and fit to these four data sets to characterize from each a national distribution of source water *Cryptosporidium* concentrations, reflecting both the variability from one plant to another and the uncertainty in the estimates. This statistical model is discussed in Chapter 3 and also in Appendix B.

In the statistical modeling, the four data sets were treated separately, each serving as an alternative picture of the "true" source water occurrence distribution. The data sets were not combined because of differences among the surveys in plant population sampled as well as in sampling and testing methods. The ICR and ICRSS, for example, were conducted at different times, over different numbers of months and numbers of samples per month, and used different laboratory analysis methods. Within the ICRSS, there were two independent surveys of large and medium plants leading to the ICRSSL and ICRSSM data sets, respectively, that characterize these two classes of plants. The ICR surveyed only large plants, but included plants with both filtered and unfiltered source water and is broken into two data sets accordingly. (The ICRSS included too few unfiltered plants to model separately.)

Among the four data sets, ICR unfiltered systems are modeled differently from the others. Because unfiltered systems are required to have watershed protection programs that achieve low turbidity and coliform limits, they generally have high source water quality and are not required to provide physical treatment (they do provide chemical disinfection that does not reliably inactivate *Cryptosporidium*). As a result, their finished water occurrence is the same as their source water occurrence and no further modeling is required. Thus, the source water occurrence modeling described in this section applies to all four data sets, but the finished water modeling described in Sections 7.2 through 7.4 applies only to filtered systems data from the ICR, ICRSSM, and ICRSSL.

Using the simplified, or reduced-form, occurrence model described in Appendix B (section B.6), 1,000 pairs of parameter values—log mean and log standard deviation—were generated by the model based on each data set, each pair defining a national distribution of plant-mean concentrations consistent with that data set. The resulting collections of 1,000 national distribution curves, one collection from each data set, served as input to the cost and benefit models developed for the LT2ESWTR Economic Analysis. Each curve in a set of 1,000 depicts the expected variability in mean source water *Cryptosporidium* concentration among plants, where corrections for test method recovery specific to each data set have been incorporated. The differences from curve to curve, within a collection, represent uncertainty in the estimated plant-mean distribution.

Exhibits 7.1 through 7.5 summarize these four sets of 1,000 distribution curves. A graph with all 1,000 curves would appear as a solid, filled space instead of individual curves and thus provide limited information. Instead, each set is represented by three percentiles—the 50^{th}, representing the median curve, and the 5^{th} and 95^{th} percentiles—comprising a bound that captures all but 10 percent of the values in the 1,000 curves.

None of these percentile curves, however, is an actual curve from among the 1,000 that make up a given set. To generate these bounds, each collection of curves is cross-sectioned vertically at regular intervals. At each evaluated point along the horizontal axis (which shows mean concentration), a single vertical "slice" captures the associated range of vertical axis values (cumulative percentage of plants) across all 1,000 curves. From each slice, the following ordered values are saved: 50th, 500th, and 950th. These saved values represent the 5^{th}, 50^{th}, and 95^{th} percentile at a particular concentration level. To generate the 95-percent bound, for example, a curve is drawn through the saved 95^{th} percentile values, across the full horizontal range of concentration values.

Exhibit 7.1 shows a comparison of ICR, ICRSSL, and ICRSSM median curves across 1,000 modeled source water occurrence distributions. A comparison of the distributions for the four data sets shown in Exhibit 7.1 indicate both some overall differences seen in the mean or median results among the underlying data sets as well as differences in the variability seen from location to location within each of these data sets. These differences among data sets can be considered as the first level of uncertainty that was characterized in the occurrence modeling by virtue of modeling these data sets, especially the three filtered system data sets, separately. A comparison of the curves shows the lower predicted occurrence for the unfiltered systems. It also shows, among the filtered systems data sets, greater variability in the ICR-estimated plant-means. Relative to the ICRSSL and ICRSSM filtered curves, the more gradual slope of the ICR filtered curve results in a higher frequency of both very high and very low plant means.

Exhibits 7.2 through 7.5 capture the second level of uncertainty, specific to each data set, by adding the upper and lower 90-percent bounds to each of the median distribution curves. These bounds reflect uncertainty due to sampling and measurement error (not all public water systems were sampled, small fraction of total source water volume sampled at each participating location, and variable recovery in the laboratory). While differences among data sets and curves reflect uncertainty, each individual curve, by itself, captures the estimated variability in mean source water *Cryptosporidium* concentration from plant-to-plant.

Exhibit 7.1 Comparison of ICR, ICRSSL, and ICRSSM Median Curves Across 1,000 Modeled Source Water Occurrence Distributions

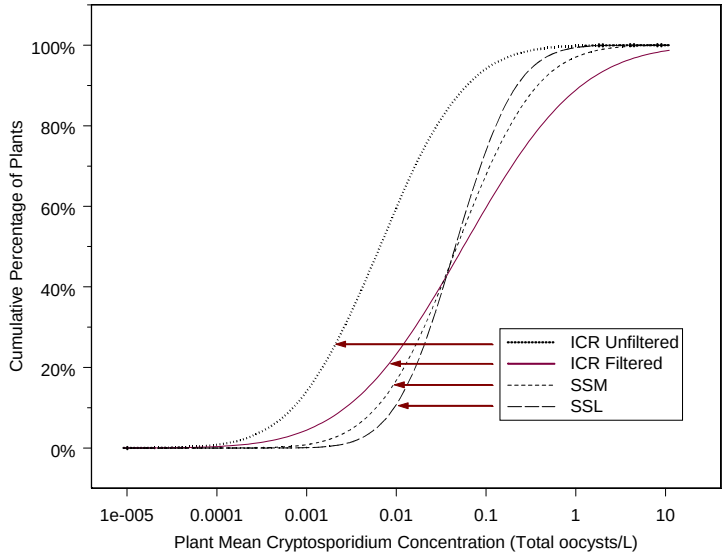


Exhibit 7.2 ICR Filtered Systems
Summary of 1,000 Modeled Source Water Distribution Curves

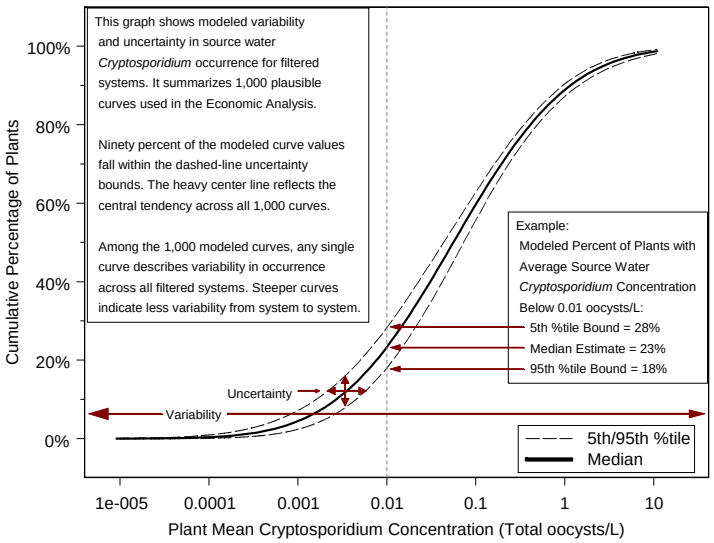


Exhibit 7.3 ICR Unfiltered Systems
Summary of 1,000 Modeled Source Water Distribution Curves

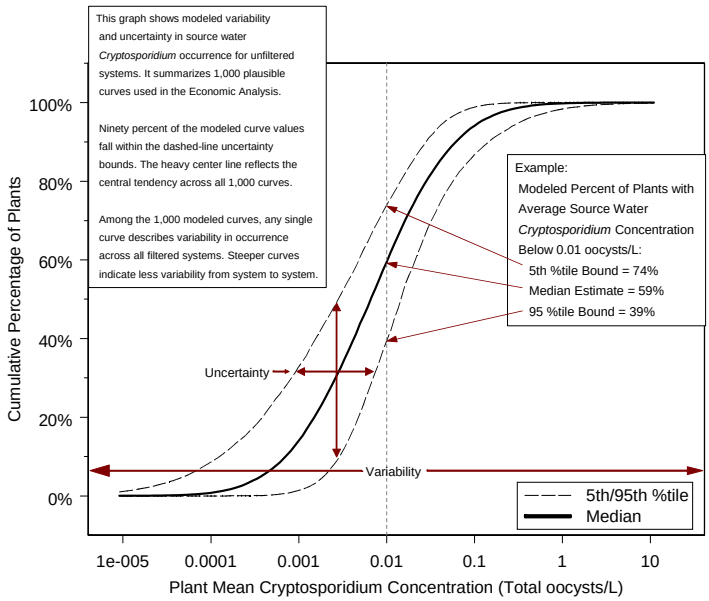


Exhibit 7.4 ICR Supplemental Survey—Large Systems Summary of 1,000 Modeled Source Water Distribution Curves

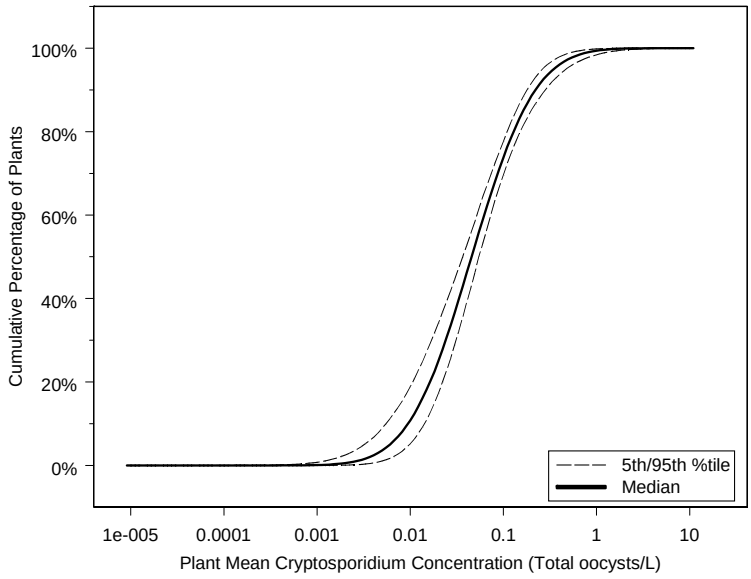


Exhibit 7.5 ICR Supplemental Survey— Medium Systems Summary of 1,000 Modeled Source Water Distribution Curves

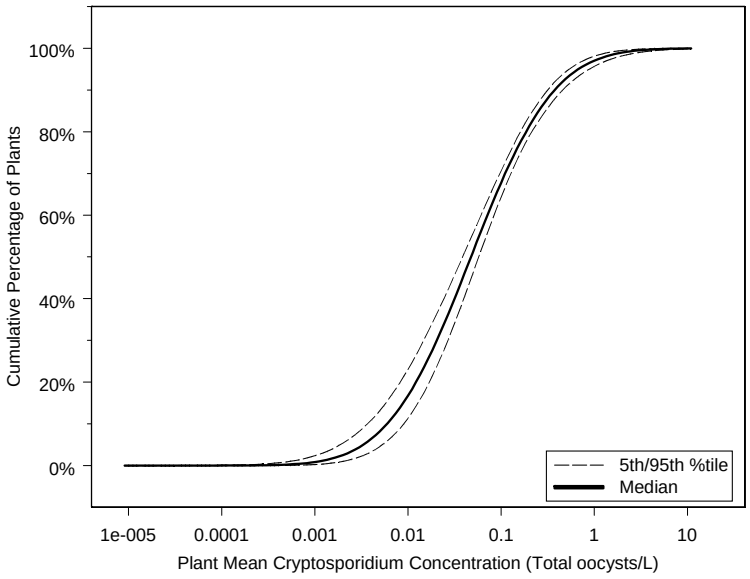


Exhibit 7.6 provides four alternative views of the 1,000 modeled curves from ICR filtered plants data. Starting at the upper left and going clockwise it shows: 1) a copy of Exhibit 7.2, the 5th, and 95th

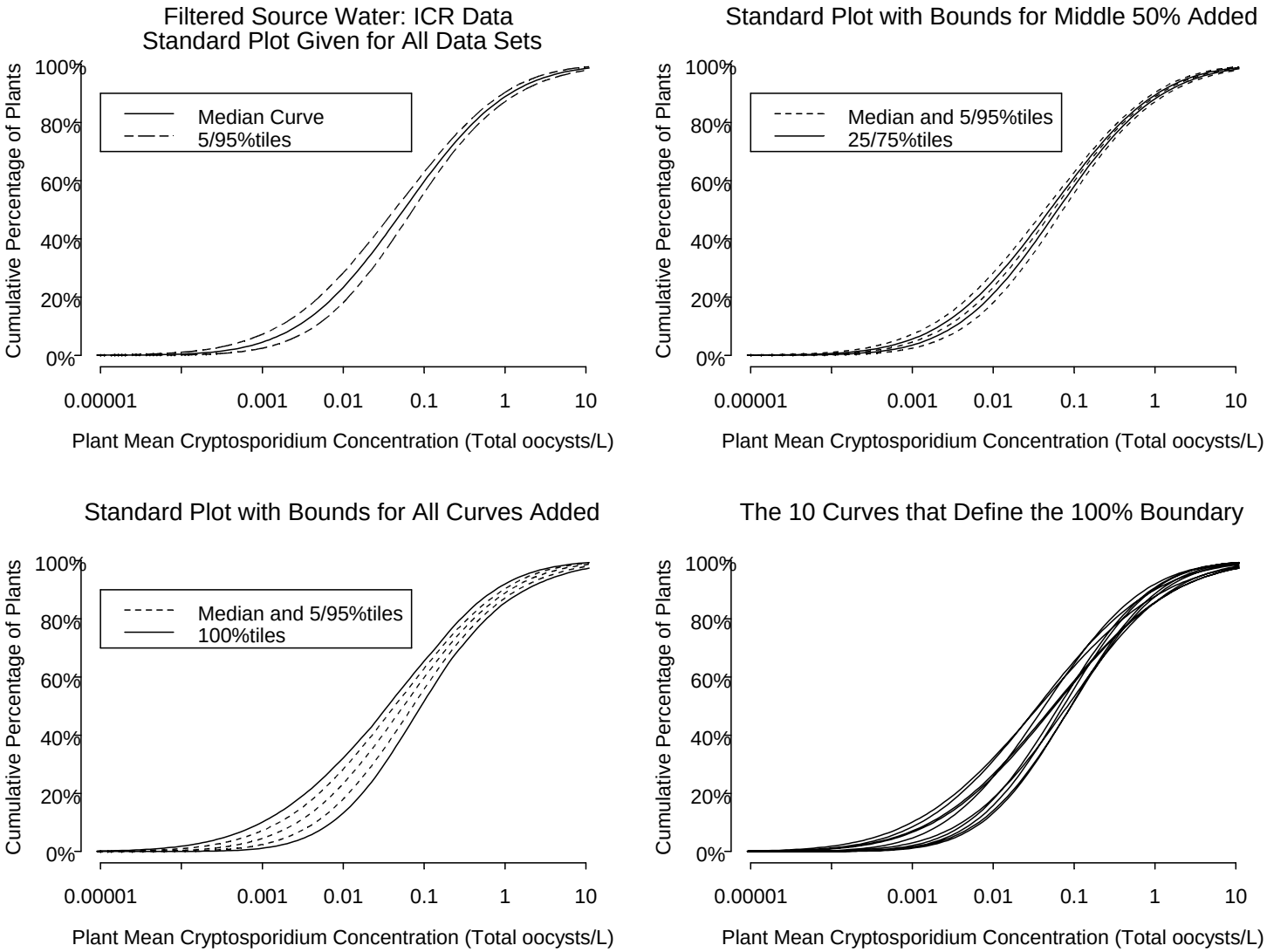
percentiles for the ICR filtered curves; 2) Exhibit 7.2 with 25th and 75th percentile curves added; 3) Exhibit 7.2 with 1st and 100th percentile curves added; and 4) the 10 actual curves that define the 100 percent bounds in the third plot. Although these four plots are all drawn from ICR filtered data, they highlight two characteristics that are common to the sets of 1,000 curves modeled from all four data sets.

First, the actual curves do not cover the bounded 90 percent range uniformly. In each vertical "slice" through the curves, points are distributed in a roughly symmetric, bell-shaped pattern with a higher proportion near the median and fewer out towards the bounds. Comparing the second and third plots in Exhibit 7.2, the relatively narrow range bounded by the 25th and 75th percentiles (top right) contains half of all curve points, while only 10 percent of curve points fall in the wider region between the 90th and 100th percentile bounds (bottom and top region combined).

Second, the 1,000 curves in each set are not arranged in a regular, "parallel" pattern as suggested by the percentile curves. To show this, the fourth graph (bottom right) plots the 10 curves that together define the 100-percent bounds shown in the third graph (bottom left). In other words, each of these 10 curves is either higher or lower, in the vertical direction, than all the other curves in the collection of 1,000 at some point along the horizontal axis, but none of them is higher or lower everywhere. In general, then, percentiles are defined by points from many individual curves that ascend at different rates and cross over one another.

Section 7.3 describes how the collections of modeled distribution curves depicted in the Exhibits 7.2 through 7.5 are used in the Monte Carlo analysis to predict finished water occurrence levels.

Exhibit 7.6 Comparison of Percentiles and Bounds (ICR Filtered Systems)



7.2 Pre-LT2ESWTR Removal of *Cryptosporidium*

EPA estimated average annual removal of *Cryptosporidium* by treatment plants and combined these estimates with the modeled source water occurrence distributions (described in Section 7.1) to derive average finished water *Cryptosporidium* concentrations. The IESWTR and the LT1ESWTR establish filtration requirements designed to provide finished water with a minimum 2 log (99 percent) reduction in *Cryptosporidium* concentrations relative to source water levels. Although systems are required to meet only the 2 log removal target, it is recognized that most systems will achieve greater levels of *Cryptosporidium* removal. For example, some systems have unit processes in addition to conventional treatment that provide higher *Cryptosporidium* removals. Based on studies described below and in Chapter 5, EPA estimates that average yearly *Cryptosporidium* removal levels vary from 2 log to 4 log (99 percent to 99.99 percent) for small surface water systems (serving fewer than 10,000 people), and from 2 log to 5 log (99 percent to 99.999 percent) for medium and large systems (serving at least 10,000 people).

EPA has characterized the distribution of these average removal values among systems as triangular distributions. These triangular distributions are shown in Exhibits 7.7 and 7.8 for large and small systems, respectively. The three points of any single triangular distribution represent the assumed minimum, maximum, and mode (the most likely value) log removal.

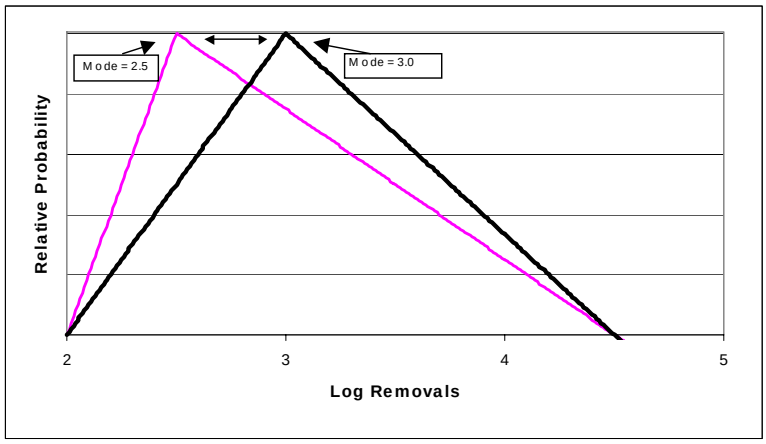
The top and bottom figures in Exhibits 7.7 and 7.8 show two different sets of triangular distributions for each system size category. The "lower-end" distributions represent conventional and direct filtration plants that have no additional treatment processes that could enhance *Cryptosporidium* removal and that minimally meet the effluent turbidity requirements of the IESWTR and LT1ESWTR. To account for those plants that have or will have additional treatment processes prior to the LT2ESWTR or that achieve very low filter effluent turbidity (e.g., combined filter effluent $\leq$0.15 NTU), a second "higher-end" distribution was developed by shifting the lower-end triangle 0.5 log higher.

EPA accounts for uncertainty in the distributions of average removal values by allowing the mode to vary. This creates a set of triangular distributions for each of the four system groups broken out in Exhibits 7.7 and 7.8, with all possible distributions in a set equally likely. For example, the first set of triangular distributions shown in Exhibit 7.7 represents the low-end distributions for medium and large systems. It shows identical end-points for all possible distributions in this set, minimum and maximum values of 2.0 and 4.5. The mode, however, varies uniformly between 2.5 and 3.0, with each possible value defining a different triangular distribution.

The cumulative distributions shown in Exhibits 7.9 and 7.10 bound the range of log removals expected for small and large systems, respectively. Within the range of cumulative distributions shown, all possible curves are equally likely, and the modeling never generates a distribution that falls outside of the depicted bounds.

Exhibit 7.7 Triangular Removal Distributions for Medium and Large Systems

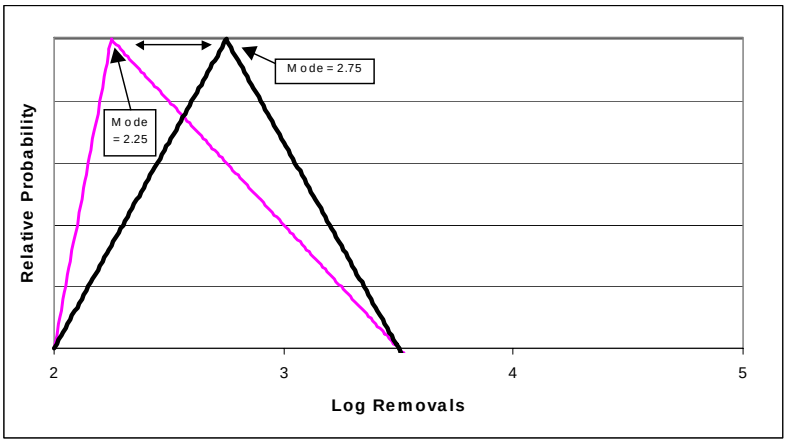
Lower-End Distributions



Higher-End Distributions— Estimate With 0.5 Log Removal Credit

Exhibit 7.8 Triangular Removal Distributions for Small Systems

Lower-End Distribution



Higher-End Distribution—Estimate With 0.5 Log Removal Credit

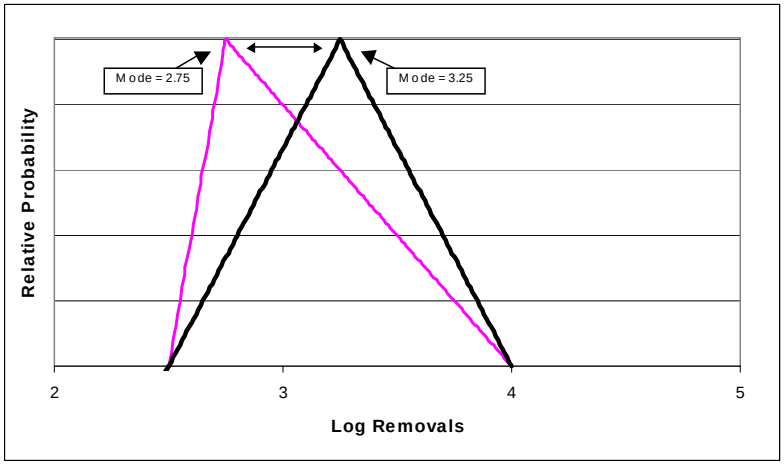


Exhibit 7.9a Cumulative Probability Low-End Distributions for Large and Medium Systems

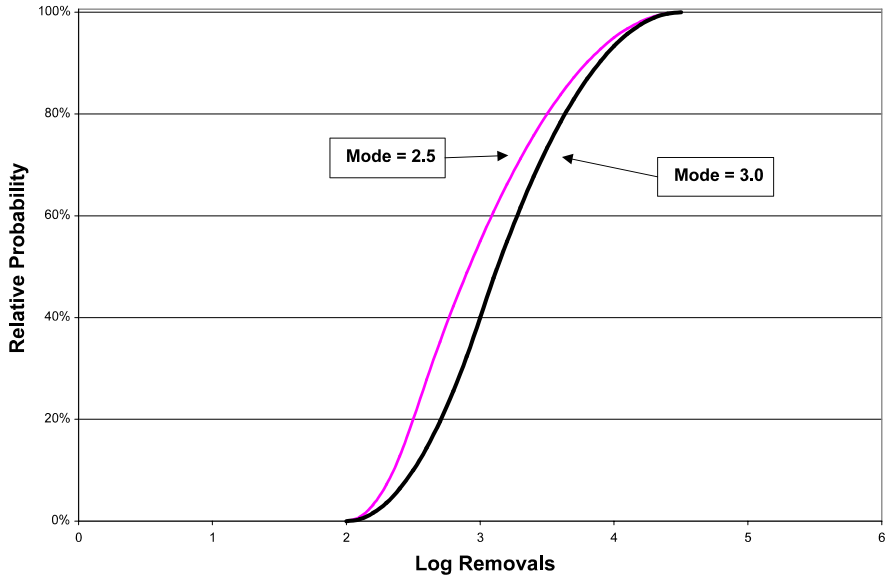


Exhibit 7.9b Cumulative Probability High-End Distributions for Large and Medium Systems

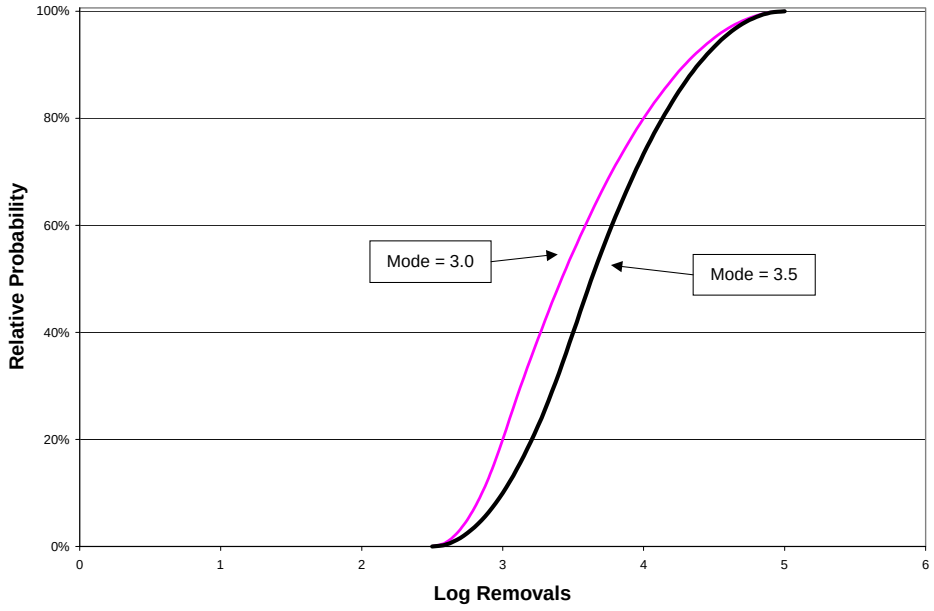


Exhibit 7.10a Cumulative Probability Low-End Distributions for Small Systems

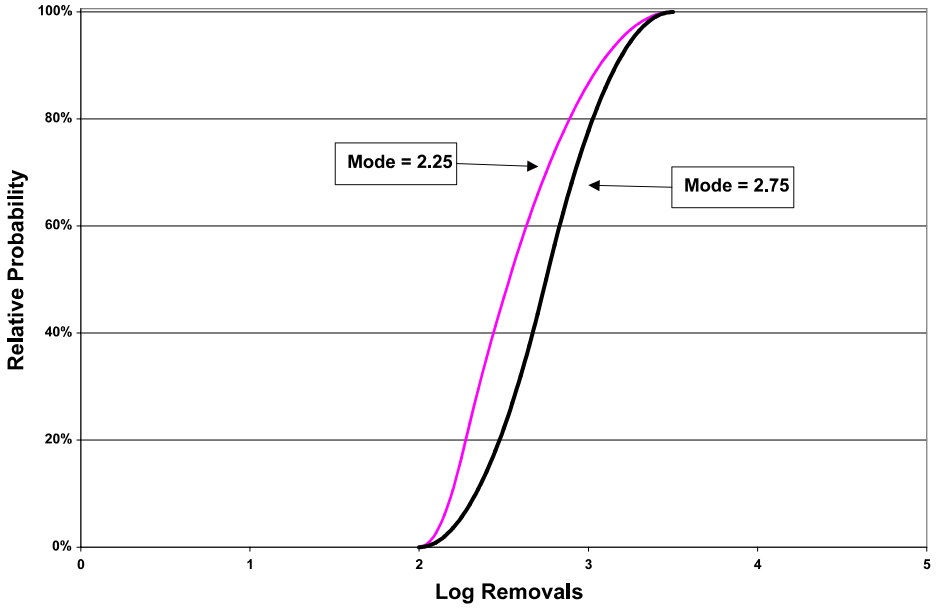
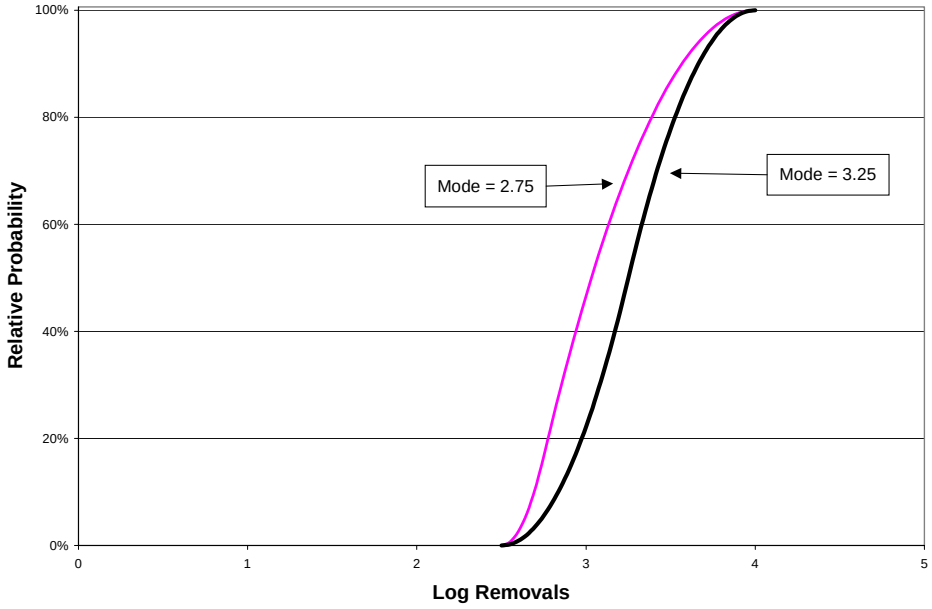


Exhibit 7.10b Cumulative Probability High-End Distributions for Small Systems



These triangular log-removal distributions were estimated from analysis of various filtration studies described in Chapter 5. These studies covered a wide range of source waters and treatment conditions. There are many challenges in estimating the *Cryptosporidium* removal achieved by full-scale water treatment plants. Most full-scale studies show few detections of *Cryptosporidium* in finished water due to the low source water occurrence of *Cryptosporidium* and insensitivity of the available analytical methods. Pilot studies can overcome the source water concentration limitations by seeding the influent with very high concentrations; however, the relationship between full-scale plant and pilot plant performance is questionable. Another approach for addressing the challenge of estimating *Cryptosporidium* removal is to assess the removal of other particles, such as aerobic spores and total particle counts, as indicators of *Cryptosporidium* removal. Pilot studies have established strong correlations between *Cryptosporidium* removal and the removal of spores and particles (McTigue et al. 1998, Dugan et al. 2001), and it is often possible to quantify removal of these indicators in full-scale plants.

The key studies that support the minimum, modes, and maximum log removal values of the triangular distributions are provided below. The values are based on indicator measurements (spores or particles) at full-scale plants or for *Cryptosporidium* at pilot-scale plants, with the exception of one study by Nieminski and Ongerth, (1995) which seeded *Cryptosporidium* in a full-scale plant that was not in operation at the time of the study.

7.2.1 Large and Medium System Triangular Distributions

The large and medium system lower-end triangle represents conventional or direct filtration plants that do not have additional unit processes to enhance *Cryptosporidium* removal and that minimally meet IESWTR effluent turbidity requirements. Several studies support the log removal estimations presented in Exhibit 7.7 with 2.5 and 3.0 log modes (being the most likely), 2.0 log minimum, and 4.5 log maximum.

7.2.1.1 Modes

McTigue et al. (1998) conducted an on-site survey of 100 treatment plants and reported a median particle removal of 2.8 log for particles greater than 0.2 μm, with removals ranging of 0.04 to 5.5 log. Part of their investigation also included a pilot study that showed a significant correlation between particle and protozoa removal when operating at similar temperature and coagulation conditions. Nieminski and Bellamy (2000) sampled raw and finished water at 24 utilities and found that the median removal of aerobic spores was 2.8 log. They also tested for *Cryptosporidium* and *Giardia*, but these protozoa were rarely detected in finished water due to their relatively low occurrence and the insensitivity of analytical methods. Nieminski and Ongerth (1995) reported average *Cryptosporidium* removal of 3 log for conventional and direct filtration pilot studies at relatively low influent and effluent turbidities of 4 NTU and 0.1 - 0.2 NTU, respectively. They also conducted full-scale studies that yielded lower average *Cryptosporidium* removals of 2.25 log for conventional and 2.8 log for direct filtration. The reported data may be lower than the actual removals because the authors reported *Cryptosporidium* log removals only when *Cryptosporidium* were detected in the effluent (six of eight trials).

These studies support the choice of 2.5 and 3.0 log as modes of the triangular distribution for those plants that achieve relatively lower effluent turbidity levels and lack additional unit processes. Data from both the McTigue et al. (1998) and Nieminski and Bellamy (2000) studies covered a large number

of utilities, which indicates their median log removals are more likely to be representative of national averages than studies that sample one or two different utilities or source waters.

7.2.1.2 Maximum

Dugan et al. (2001) conducted several seeded runs at varying effluent turbidities. Under optimal coagulation conditions, total *Cryptosporidium* removals (after sedimentation and filtration) was greater than 5.0 log for runs with effluent turbidity ranging from 0.02 to 0.15 NTU. McTigue (1998) conducted pilot studies and reported average *Cryptosporidium* removals of 4 log during stable operation. Patania et al. (1995) also reported average and median values of approximately 4 log removal and a range of 1.4 to 6.2 log removal for *Cryptosporidium* from pilot studies at four different locations. These studies show that greater than 5.0 log removal of *Cryptosporidium* can be achieved by filtration during optimal conditions. The maximum log removal of 4.5 log represents an upper estimate of the mean log removal full scale plants could achieve over the course of a year.

7.2.1.3 Minimum

The minimum 2 log removal is based on studies cited above and IESWTR and LT1ESWTR requirements. The IESWTR and LT1ESWTR require an effluent turbidity of 0.3 NTU for 95 percent of the time; plants meeting this limit are assumed for purposes of regulatory compliance to achieve at least a 2 log removal of *Cryptosporidium*. The minimum 2.0 log removal in the triangular distribution represents the plants that operate frequently under stressed or sub-optimal conditions or with overall poorer performance. Investigations by Huck et al. (2000) showed that *Cryptosporidium* removals diminished when the filters were operating under sub-optimal conditions. Coagulant dose was decreased in two direct filtration pilot-scale plants to achieve a target turbidity of 0.2 to 0.3 NTU. Median *Cryptosporidium* removals decreased from 5.6 to 3.2 log and from 3.2 to 1 log. Emelko et al. (2000) also studied *Cryptosporidium* removal during vulnerable filtration periods with a direct filtration system. During an early breakthrough period, effluent turbidity increased from very low (0.04-0.08 NTU) to approximately 0.2 NTU while the *Cryptosporidium* removal decreased from an average of 5.5 log to 2.1 log.

7.2.1.4 High-End Distribution

The high-end triangular distribution represents treatment plants with unit processes in addition to conventional filtration and/or plants that achieve very low effluent turbidity. For example, EPA estimates a pre-sedimentation basin can provide an additional 0.5 log *Cryptosporidium* removal. EPA also has assumed that under the LT2ESWTR, plants that achieved combined filter effluent turbidity $\leq$0.15 NTU in 95 percent of samples would receive an additional 0.5 log credit towards *Cryptosporidium* treatment requirements. Considering that many systems have or will have these processes in place prior to the LT2ESWTR or will achieve low effluent turbidity levels through programs like the Partnership for Safe Water, a second triangular distribution (high-end removal) was developed by increasing the log removal modes and endpoints by 0.5 log.

7.2.2 Small System Triangular Distributions

For small systems, the predicted maximum and most likely *Cryptosporidium* removal levels are lower than the large and medium system values. Smaller plants have fewer filters than larger plants; therefore, fluctuations in the operation of a single filter have larger impacts on plant performance. For example, backwashing and shutting down a filter for maintenance cause hydraulic fluctuations that can substantially affect operational stability. In addition, if one filter performs poorly, the effect is greater in a plant with fewer filters. Plant control is generally less automated at smaller plants, which makes water quality control more difficult. The minimum estimated log removal is the same for small and larger systems since systems are assumed to be in compliance with the IESWTR and LT1ESWTR and achieve at least 2.0 log *Cryptosporidium* removal. Note, source water quality for small systems is assumed to be the same as medium and large systems.

7.3 Description of Monte Carlo Model Used to Predict Finished Water Occurrence

As noted above, the predictions of finished water occurrence levels of *Cryptosporidium* were derived through a two-dimensional Monte Carlo simulation model. This section describes the structure and implementation of that model.

Two-dimensional Monte Carlo models are used in simulations where it is important to differentiate between model inputs that describe uncertainty in a variable and those that describe variability. As shown in Equation 7.1, presented at the beginning of this chapter, the basic algorithm for calculating finished water occurrence at the individual system (or plant) level is a fairly simple function of two variables: source water occurrence (C_S) and log removal (L). There is, however, plant-to-plant variability in both of these inputs, and uncertainty in the "true" characterization of that plant-to-plant variability. As noted previously, the Monte Carlo model considers normal operating conditions at each plant, and therefore uses a constant treatment efficacy for the modeled time period of one year (i.e., a given treatment efficacy was selected at random from the distribution of L values that vary from plant to plant and then applied to all measurements in a year for that plant).

As noted earlier, one level of uncertainty considered in this analysis is embodied in the three separate sets of filtered system data on source water occurrence. The use of these three data sets as alternative views of the true occurrence distributions falls outside of the Monte Carlo simulation analysis. These three source water occurrence data sets are used to produce three alternative views of finished water occurrence for filtered systems.

Within each data set, uncertainty is incorporated in the Monte Carlo simulations through the selection of alternative distributions as depicted in Exhibits 7.2 through 7.4. In similar fashion, the uncertainty in the effectiveness of treatment is captured through the selection of alternative log removal distributions described by the "space" depicted in the cumulative distributions shown in Exhibits 7.9 and 7.10.

For each combination of data set and plant size group, the Monte Carlo simulation was carried out in two loops. The first was an uncertainty loop with 100 iterations, each with two steps—select a distribution of plant-mean concentrations at random and select a single distribution of log removals. For example, for the combination of the ICR filtered data set and the small plant category, the plant-mean distribution was selected from among the 1,000 curves summarized in Exhibit 7.2, and the log-removal distribution was obtained from the range of curves shown in Exhibit 7.10.

With these two distributions (plant-mean concentrations and log removals) defined in the uncertainty loop, 250 values were then chosen at random from each distribution in a variability loop, the second loop in the Monte Carlo simulation. From these values, 250 finished water values were computed as shown in Equation 7.1. In total, 25,000 finished water concentrations were simulated in 100 uncertainty loops, with each collection of 250 values representing one possible national distribution of mean finished water *Cryptosporidium* concentrations and the differences among sets of 250 representing uncertainty about the true national distribution.

From each distribution of 250 estimated finished water plant-mean concentrations, summary statistics including the mean, median, and percentiles were saved. The result was 100 estimates of each distribution statistic, one from each uncertainty loop. To summarize this collection of estimates, the 5^{th}, 50^{th}, and 95^{th} value across the uncertainty dimension (100 estimates per percentile) was taken at every 5^{th} percentile across the plant-to-plant variability dimension. Connecting all these 50^{th} percentiles, for example, results in the median curves displayed in Exhibit 7.11. This method for summarizing the data is similar to the method described in Exhibit 7.1 for summarizing collections of source water occurrence curves. The finished water occurrence curves are presented for large and small systems and all four data sets (three filtered and one unfiltered) in the next section (Exhibits 7.12 through 7.19).

7.4 Estimates of Pre-LT2 Finished Water Occurrence

Exhibits 7.11 and 7.12 compare the estimated finished water distribution curves across all three filtered system and the one unfiltered system data sets, for small and large systems, respectively. The distribution curves are the 50^{th} percentile or median curves. Exhibits 7.13 through 7.19 present all the combinations of data set and system size category one at a time, adding the 5^{th} and 95^{th} percentile curves to show 90-percent confidence bounds. Comparison of these finished water curves to the analogous source water curves in Exhibits 7.2 through 7.5 reveals the impact of the 2.5 to 3.5 log removal for *Cryptosporidium* modeled in Section 7.2.

Exhibit 7.20 gives a table of summary statistics for the estimated distributions for plant-mean finished water *Cryptosporidium* concentration in Exhibits 7.13 through 7.19. Like the exhibits, the table is broken out by both data set and system size category. The rows of the table reflect the variability within each distribution curve for each data set. The first three rows for the ICR filtered data set, for example, show the estimated mean, median, and 90^{th} percentile finished water concentrations for each of the three distribution curves for large and small systems. Columns reflect uncertainty in the distributions. The first column in Exhibit 7.20 shows the summary statistics for the median occurrence distribution for large systems for each of the four data sets. The second column shows the same statistics for the 5^{th} percentile distribution (the lower end of the 90-percent confidence interval) and the third column shows statistics for the 95^{th} percentile distribution (the upper end of the 90-percent confidence interval). For example, looking at the first row of the table, the mean concentration of the median distribution for large plants, based on ICR data, is 1.54×10^{-4} oocysts/L, and a 90-percent confidence interval for this concentration is 5.38×10^{-5} to 3.83×10^{-4}.

Exhibit 7.11 All Data Sets, Small Systems
Comparison of Median Finished Water Distribution Curves

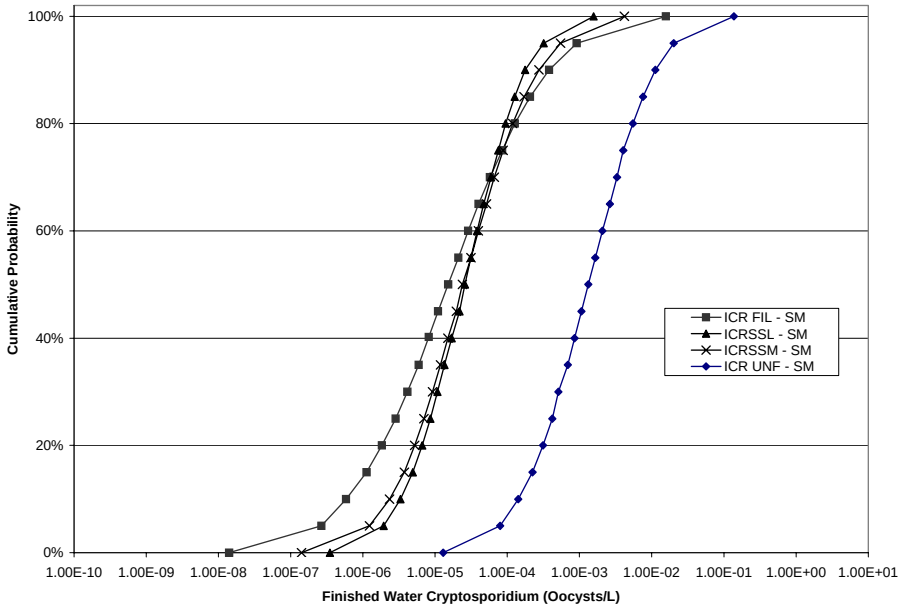


Exhibit 7.12 All Data Sets, Large Systems
Comparison of Median Finished Water Distribution Curves

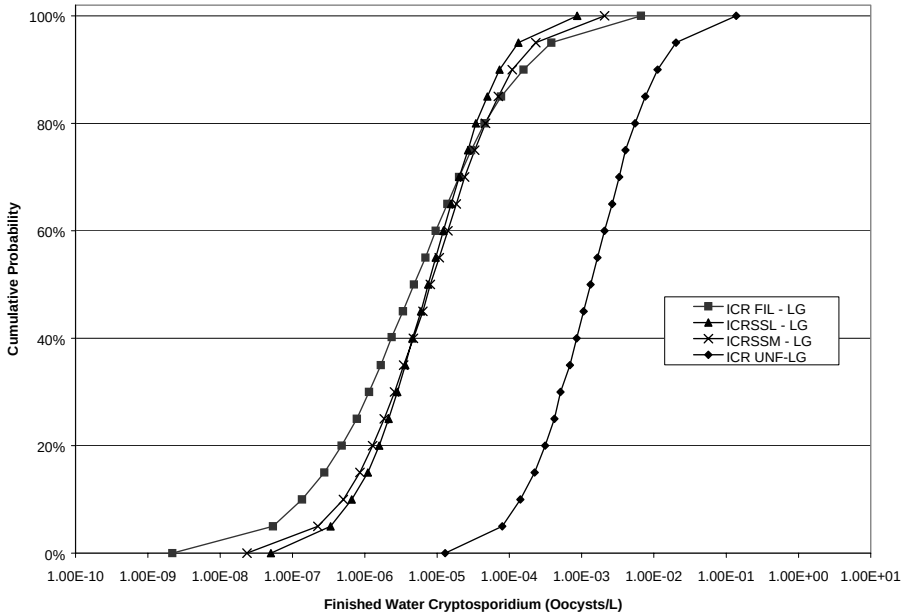


Exhibit 7.13 ICR Filtered Data, Small Systems Median Curve and 90-Percent Confidence Bounds

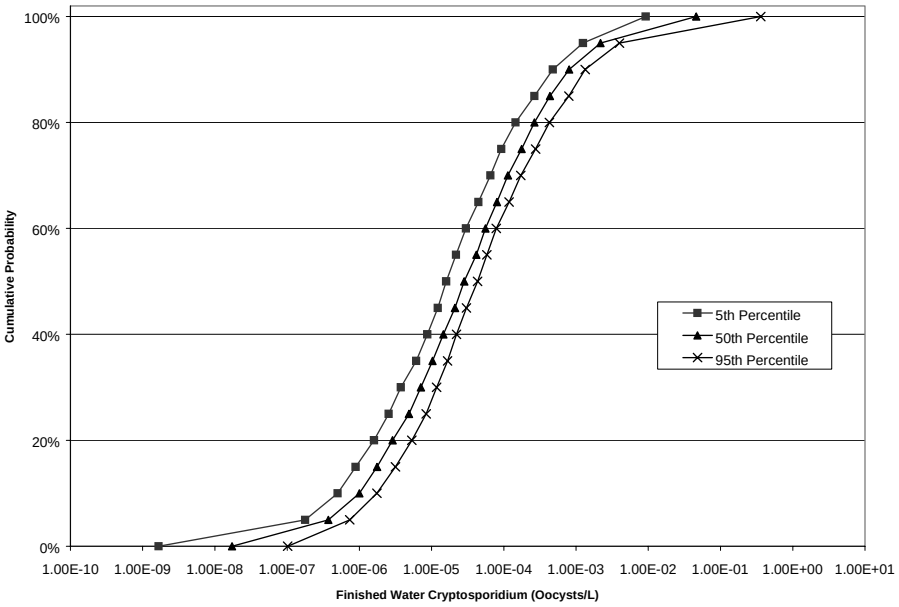


Exhibit 7.14 ICR Filtered Data, Large Systems Median Curve and 90-Percent Confidence Bounds

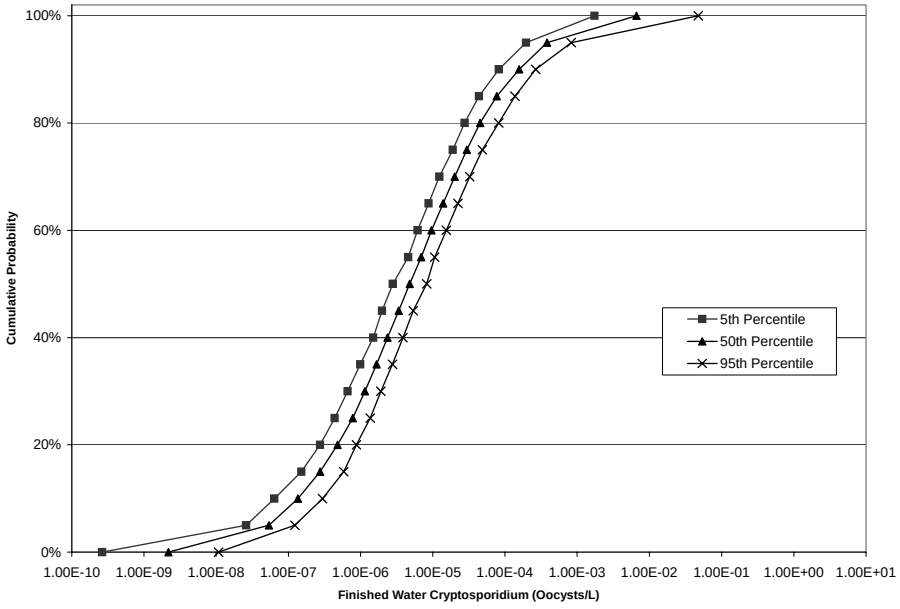


Exhibit 7.15 ICRSSL Data, Small Systems
Median Curve and 90-Percent Confidence Bounds

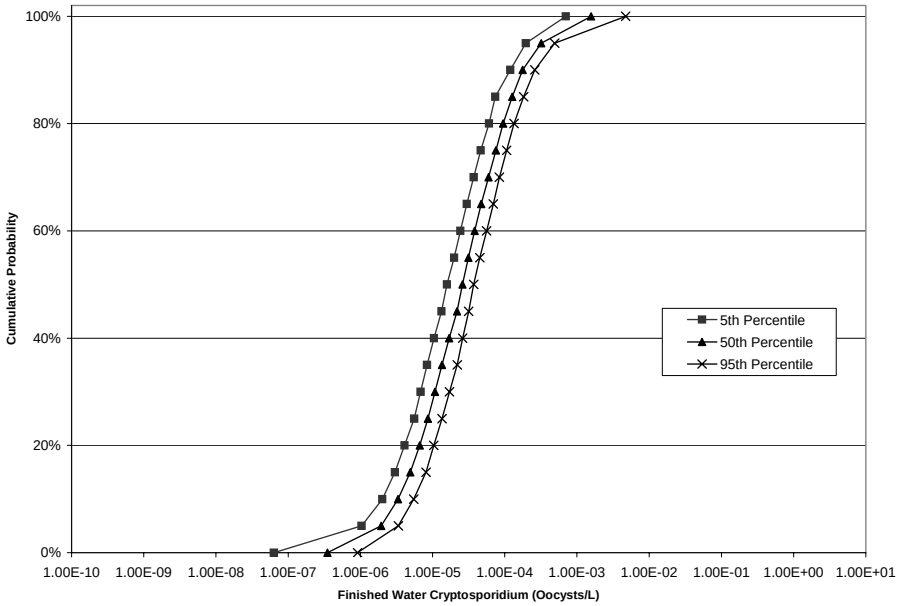


Exhibit 7.16 ICRSSL Data, Large Systems
Median Curve and 90-Percent Confidence Bounds

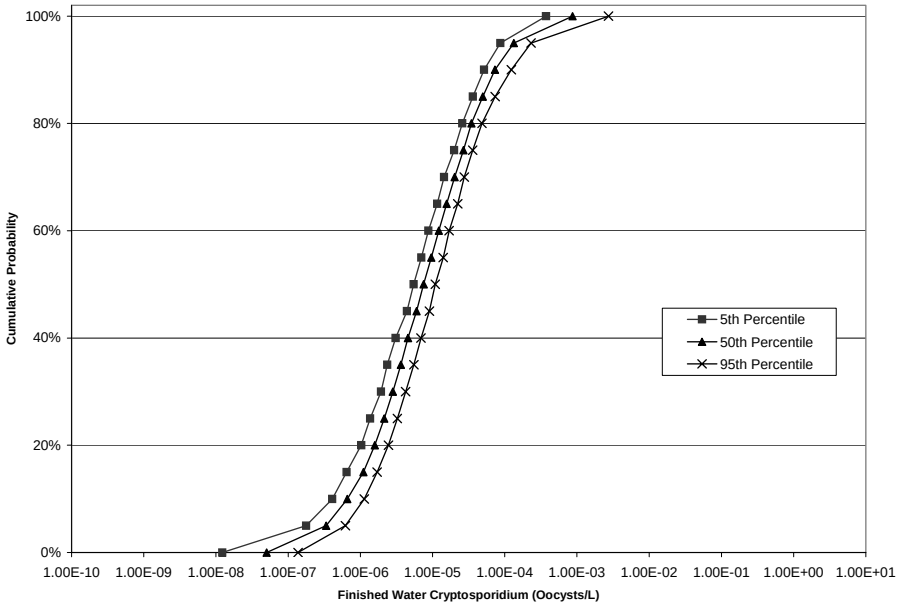


Exhibit 7.17 ICRSSM Data, Small Systems
Median Curve and 90-Percent Confidence Bounds

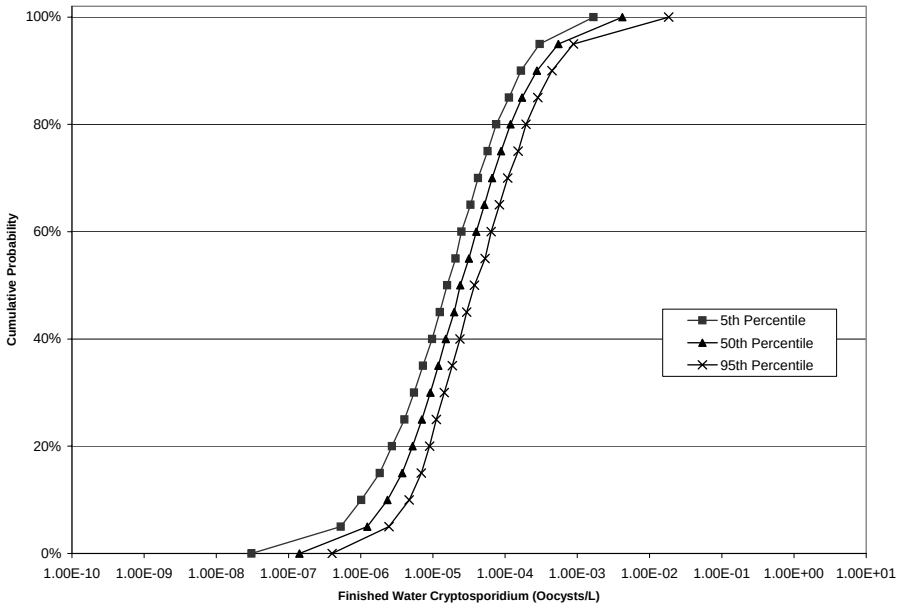


Exhibit 7.18 ICRSSM Data, Large Systems
Median Curve and 90-Percent Confidence Bounds

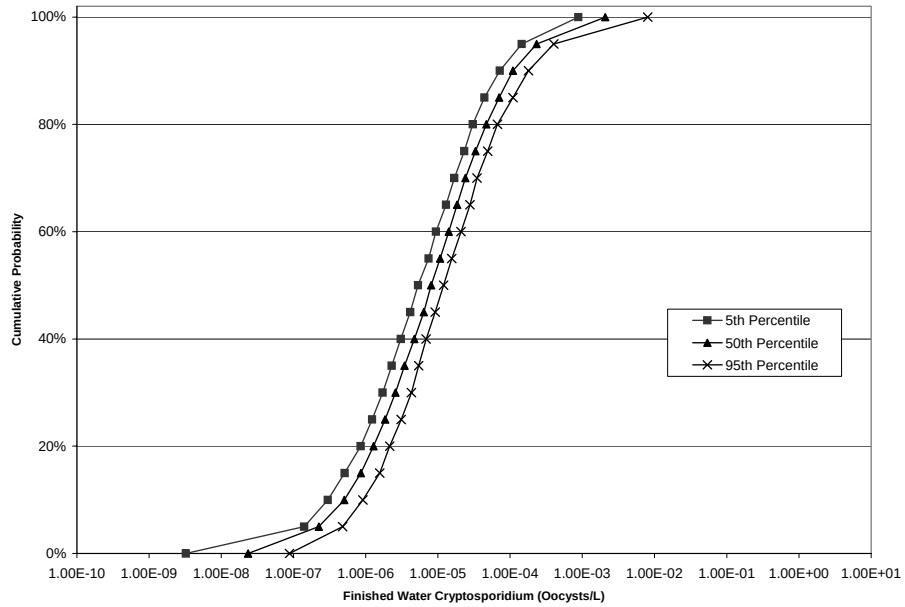


Exhibit 7.19 ICR Unfiltered Data, Large Systems Median Curve and 90-Percent Confidence Bounds

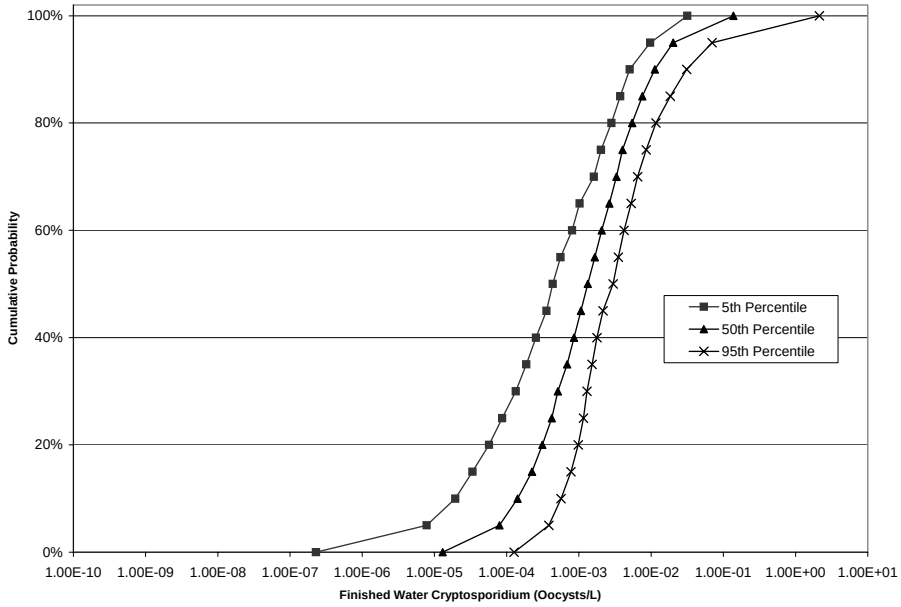


Exhibit 7.20. Summary of Finished Water Occurrence Distributions by Data Source and System Size

		Large Systems			Small Systems		
Data Source	Statistic	Median Distribution	5th Percentile Distribution	95th Percentile Distribution	Median Distribution	5th Percentile Distribution	95th Percentile Distribution
ICR	Mean	1.54×10^{-4}	5.38×10^{-5}	3.83×10^{-4}	3.62×10^{-4}	1.33×10^{-4}	7.70×10^{-4}
	Median	5.05×10^{-6}	2.79×10^{-6}	8.22×10^{-6}	1.63×10^{-5}	9.75×10^{-6}	2.44×10^{-5}
	90th %ile	1.63×10^{-4}	8.22×10^{-5}	2.66×10^{-4}	3.85×10^{-4}	2.10×10^{-4}	6.24×10^{-4}
ICRSSL	Mean	3.50×10^{-5}	2.34×10^{-5}	5.28×10^{-5}	8.33×10^{-5}	4.86×10^{-5}	1.30×10^{-4}
	Median	7.75×10^{-6}	5.48×10^{-6}	1.09×10^{-5}	2.60×10^{-5}	1.58×10^{-5}	3.73×10^{-5}
	90th %ile	7.74×10^{-5}	5.17×10^{-5}	1.23×10^{-4}	1.82×10^{-4}	1.19×10^{-4}	2.60×10^{-4}
ICRSSM	Mean	6.24×10^{-5}	3.66×10^{-5}	9.64×10^{-5}	1.46×10^{-4}	7.87×10^{-5}	2.61×10^{-4}
	Median	8.28×10^{-6}	5.32×10^{-6}	1.20×10^{-5}	2.56×10^{-5}	1.57×10^{-5}	3.75×10^{-5}
	90th %ile	1.17×10^{-4}	7.20×10^{-5}	1.80×10^{-4}	2.85×10^{-4}	1.66×10^{-4}	4.46×10^{-4}
Unfiltered	Mean	1.95×10^{-2}	2.71×10^{-3}	2.00×10^{-2}	1.95×10^{-2}	2.71×10^{-3}	2.00×10^{-2}
	Median	1.47×10^{-3}	4.36×10^{-4}	2.97×10^{-3}	1.47×10^{-3}	4.36×10^{-4}	2.97×10^{-3}
	90th %ile	1.39×10^{-2}	5.05×10^{-3}	3.11×10^{-2}	1.39×10^{-2}	5.05×10^{-3}	3.11×10^{-2}

Note: Data provided in Exhibit 7.20 are in oocysts per L.

7.5 Comparison of EPA Estimates with Aboytes et al. (2000)

A study by Aboytes et al. (2000) provides an alternative perspective on *Cryptosporidium* occurrence in finished drinking water and the efficacy of treatment. This study involved collecting 100-L finished water samples on a monthly basis from 80 surface water utilities. Samples were analyzed for infectious *Cryptosporidium parvum* with a cell culture-PCR (CC-PCR) method. The objective of the study was to "assess the adequacy of treatment to protect against infectious *Cryptosporidium* in drinking water." All utilities in the study were enrolled in the Partnership for Safe Water, a voluntary cooperative program that seeks to optimize treatment plant performance. Most samples had turbidity below 0.1 NTU and all were below 0.3 NTU, the standard set by the IESWTR. Among 1,674 samples of 100 L each, 24 were positive for infectious *C. parvum* (LeChevallier 2001). The authors reported an average CC-PCR recovery efficiency of 32.3 percent. Hence, if it is assumed that one infectious oocyst accounted for each positive sample, and the oocyst count is adjusted for average recovery, these results produce a mean concentration of infectious oocysts of 4.4×10^{-4} oocysts/L, or 0.044 oocysts/100 L.

To compare results from Aboytes et al. with EPA finished water *Cryptosporidium* estimates based on results from the ICR and ICRSS, it is necessary to consider the fraction of oocysts that are infectious. Because oocysts lose viability in the environment, it is expected that infectious oocysts are only a fraction of the total number of oocysts in a water sample. While the CC-PCR method registers only infectious oocysts, the ICR Method and EPA Methods 1622/23 count total oocysts without regard to whether they are viable and infectious. To estimate the fraction of oocysts that may be infectious, EPA evaluated a study by LeChevallier et al. (2003) that analyzed several hundred source water samples from six utilities using both the CC-PCR method and Method 1623. Oocysts were detected in 60 of 593 samples (10.1 percent) by Method 1623, and infectious oocysts were detected in 22 of 560 samples (3.9 percent) by the CC-PCR procedure. Recovery efficiencies for the two methods were similar. According to the authors, these results suggest that approximately 37 percent of the oocysts detected by Method

1623 were viable and infectious. Based on these results, as well as consideration of the structure of counted oocysts, EPA assumes that the fraction of oocysts that is infectious may average from 15 to 25 percent for the ICR and 30 to 50 percent for the ICRSS (as described in Section 5.2.4 of the Economic Analysis for the LT2ESWTR).

If the estimates of mean large plant finished water oocyst concentrations from the median distributions from Exhibit 7.20 are multiplied by 40 percent to adjust for the fraction of oocysts that are infectious, the mean finished water concentrations of infectious oocysts are as follows:

ICR Mean = 6.1×10^{-5}; ICRSSM Mean = 2.5×10^{-5}; ICRSSL = 1.4×10^{-5} (oocysts/L)

Thus, the mean finished water infectious oocyst level reported in Aboytes et al. of 4.4×10^{-4} oocysts/L is a factor of 7 greater than the EPA mean estimate based on the ICR and a factor of 30 greater than the ICRSSL estimate. While the reason for this significant discrepancy is unknown, it cannot be fully attributed to potential error in factors such as the fraction of oocysts that are infectious. Rather, it may indicate that the ICR and ICRSS underestimate source water *Cryptosporidium* occurrence and/or that EPA has overestimated treatment efficacy, as discussed below.

Unfortunately, source water data are not available for plants in the Aboytes et al. study, so it is not possible to directly determine their average treatment efficiency. However, removal efficiency may be estimated based on other survey data. The ICRSS was conducted during a time frame similar to Aboytes et al., and the filtration and separation steps used in the CC-PCR method of Aboytes et al. are similar to those in Method 1622/23 used in the ICRSS. Consequently, the ICRSS source water data may be somewhat comparable to the Aboytes et al. finished water data. The mean source water *Cryptosporidium* concentration of plants in the ICRSS, adjusted for average recovery of 43 percent, was 0.14 oocysts/L. If this value is multiplied by 40 percent as an estimate of the fraction of oocysts that are infectious, the result is a mean source water concentration of 0.056 infectious oocysts/L. If this were the mean source water concentration in the Aboytes et al. study, then the plants in that survey achieved an average removal of 1.7 log to produce the mean finished water concentration of 0.0011 oocysts/L that was measured. For the plants in the Aboytes et al. survey to have achieved a mean oocyst removal of 2.5 log, which was the lowest mean removal assumed in EPA estimates, the mean source water oocyst concentration would have to have been over twice that measured during the ICRSS. Either hypothesis indicates that EPA may have underestimated the risk from *Cryptosporidium* in drinking water by underestimating finished water oocyst levels.

7.6 Summary

Through modeling efforts that encompass the uncertainty and variability of *Cryptosporidium* concentrations in source waters, treatment efficiencies, sampling, and analytical methods, the occurrence of *Cryptosporidium* in finished water was estimated for Pre-LT2ESWTR conditions. While estimated finished water occurrence levels are very low, the presence of any *Cryptosporidium* in drinking water poses the risk of consumption, which can lead to adverse health effects. In the *Economic Analysis for the LT2ESWTR*, EPA uses these finished water occurrence distributions to estimate risk prior to the LT2ESWTR, and subsequently, to estimate potential health benefits realized from the LT2ESWTR.

8. Population Profile for Exposure Assessment

Accurately assessing the risk associated with the LT2ESWTR regulatory options requires estimating and characterizing the population served by PWSs affected by the rule. This chapter describes the data sources, assumptions, and methodology used to estimate the population at risk from exposure to microbial contaminants.

8.1 Population Profile

The LT2ESWTR applies to all PWSs that use surface water or ground water under the direct influence of surface water (GWUDI) and, by extension, the populations they serve. Characterization of population served is available from two major sources: the Safe Drinking Water Information System (SDWIS), as summarized in the Drinking Water Baseline Handbook (USEPA 2001b), and the 1996 ICR. Detailed system information, including system counts and population served, as reported by states to EPA, is maintained within SDWIS. The SDWIS inventory is "frozen" near the end of the calendar year and distributed to state drinking water programs for verification of the number and types of systems. These data are incorporated into the Baseline Handbook for use in conducting cost-benefit assessments.

The second source of population data is the 1996 ICR. Treatment plant data, including population served, were collected from nearly 300 large surface and ground water systems. The ICR provided EPA with extensive information on chemical byproducts, microbial contaminants, and treatment capabilities to control these contaminants and was used to develop *Cryptosporidium* occurrence profiles for LT2ESWTR risk analyses. Population served by the large water systems as reported under the ICR does not correlate with SDWIS data, mainly due to the way in which wholesale populations were assigned. Under the ICR, systems reported total population served, including retail and wholesale portions. Under SDWIS, systems report only their retail population served—systems that purchase water from other systems and distribute it to their retail populations are considered stand-alone systems in SDWIS. To maintain consistency in estimating population exposed across all system size categories, the SDWIS population inventory was selected for all LT2ESWTR risk analyses.

Exhibit 8.1 presents the total population served by public water from SDWIS (4^{th} quarter 2000 data) for community water systems (CWSs), nontransient noncommunity water systems (NTNCWSs), and transient noncommunity water systems (TNCWSs). Because a person need ingest only one *Cryptosporidium* oocyst to become infected, LT2ESWTR risk analyses must consider all types of PWSs, including those that provide drinking water only part of the time or to transient populations. The total population served presented in Exhibit 8.1 (279.8 million) is 99.4 percent of the total population in 2000, as reported by the United States Census Bureau. The inclusion of TNCWSs in population estimates may result in some double-counting; for instance, people who drink the water at a rest area TNCWS may be served by a CWS at home. These estimates represent the broad universe of populations and systems to be considered in the risk assessment.

Exhibit 8.1 Estimates of Population Served By System Size (2000)

Size Category	Population Served by PWSs	Population Served by Surface Water and GWUDI Systems	Percent of Total Population Served by PWSs	Percent of Surface Water and GWUDI Population Served in Each Size Category
Large PWSs (serving over 100,000)	115,597,748	97,821,628	35.0%	55.3%
Medium PWSs (serving 10,001–100,000)	96,660,320	60,639,692	21.7%	34.0%
Small PWSs (serving 10,000 or fewer)	67,536,316	18,980,512	6.7%	10.7%
Total Population	**279,794,384**	**177,441,832**	**63.4%**	**100%**

Source: USEPA 2000g

In additional to system type, source water and system size are important distinctions in evaluating the risks of microbial contamination. Source water for drinking water treatment systems can be ground water, surface water, ground water under the direct influence of surface water (GWUDI), or a combination of the three. GWUDI is any ground water source with significant occurrence of microorganisms or significant and relatively rapid shifts in water characteristics that closely correlate with surface water conditions. It is important to note that system classification in SDWIS is not by predominant water source. A system with any input of surface water, even if it is a very small portion of the total flow, is categorized as a surface water system. Systems using surface water or GWUDI are susceptible to microbial contamination and are targets of the LT2ESWTR. They provide water to more than 63 percent of the total population served by PWSs.

8.2 Characterization of Population, Including Sensitive Subpopulations

Several population subgroups are of particular importance with respect to the potential health risks posed by microbial contaminants, especially *Cryptosporidium* and *Giardia*. In general, people with compromised immune systems, infants, and the elderly (if already weakened by other conditions) are at higher risk for the negative health effects caused by *Cryptosporidium* and *Giardia*. This section discusses the size of these sensitive subpopulations and possible future population trends within these subgroups.

Individuals with compromised immune systems, most notably three subgroups comprised of people undergoing treatment for cancer, recipients of organ transplants, and those with acquired immunodeficiency syndrome (AIDS), have a greater risk than immunocompetent individuals of developing severe, life-threatening illness if they become infected (Gerba et al. 1996). Estimates of the number of persons in each subgroup, compiled from Centers for Disease Control and Prevention (CDC) data and other surveys, are presented in Exhibit 8.2. The portion of immunocompromised persons served by surface and GWUDI systems is estimated to be 0.6 percent of all people served by these systems. The numbers in Exhibit 8.2 were calculated by multiplying the number of immunocompromised people in each category by the percentage of the total U.S. population served by surface water and GWUDI systems and by the percentages of the surface water and GWUDI population served by large, medium, and small systems. These estimates assume random distribution of the sensitive subpopulation among ground

water, surface water, and GWUDI systems, among systems of different sizes, and between individuals served and not served by PWSs. They also do not account for those who use alternative water sources because of their illness.

Exhibit 8.2 Estimates of Immunocompromised Population Served By Surface Water and GWUDI Systems By System Size

Size Category	Population Served by Surface Water and GWUDI	Number of People Living with AIDS[1]	Number of New Cancer Cases[2]	Number of Organ Recipients Living[3]	Total Immuno-Compromised
Large PWSs (serving over 100,000)	97,821,628	112,227	440,754	49,848	602,829
Medium PWSs (serving 10,001–100,000)	60,639,692	69,570	273,224	30,367	373,160
Small PWSs (serving 10,000 or fewer)	18,980,512	21,776	85,520	9,167	116,463
Total	**177,441,832**	**203,572**	**799,498**	**89,382**	**1,092,452**
Percentage of Population Served by Surface Water and GWUDI Systems That Is Immunocompromised		**0.11%**	**0.45%**	**0.05%**	**0.62%**
Percentage of Total U.S. Population		**0.07%**	**0.28%**	**0.03%**	**0.39%**

Source: 1. CDC 2000c
2. U.S. Census Bureau 2001a
3. United Network of Organ Sharing 2000

Notes: Not all new cancer cases are immunocompromised, since not all undergo chemotherapy. However, for purposes of simplicity, all new cases were assumed to be immunocompromised. In addition, cancer patients diagnosed in previous years may be immunocompromised but are not included here. Cancer cases do not include certain skin cancers and most in situ carcinomas.

Organ recipients is the number of recipients living who received a donation from Oct. 1, 1987 to Dec. 31, 1999.

Infectious diseases can have a greater impact on the elderly because immune function declines with age, antibiotic treatment is less effective, and malnutrition is more common (Meyers 1989). However, not all elderly are at increased risk for *Cryptosporidium*; only those with underlying health problems are. Because it is difficult to determine the percentage of elderly that are at increased risk, the entire elderly population is shown in Exhibit 8.3, as a very conservative estimate of those that could be at risk. As the relatively large generation born between 1946 and 1964 (the "Baby Boomers") ages, the age-based population groups at risk for infections also will increase. Exhibit 8.3 provides estimates of the current and future population age 65 and older served by surface and GWUDI systems, derived from U.S. Census Bureau (2001b) projections. Population projections assume the proportion of the total population served by surface water and GWUDI systems is constant through time, along with the percentages of that population served by small, medium, and large systems. These estimates also assume random distribution of this population subgroup among ground water, surface water, and GWUDI systems and among systems of different sizes.

As shown in Exhibit 8.3, the population age 65 and older affected by the LT2ESWTR is expected to more than double from 2000 to 2050. Based strictly on this size increase in a sensitive subpopulation,

waterborne disease cases in the elderly could double in the next 50 years. Also, the percentage of those 65 and older served by surface and GWUDI systems is expected to increase from 7.8 to 10.4 percent of the total U.S. population by 2020, indicating an increase in the relative proportion of the elderly population to the general population.

Exhibit 8.3 Estimates of U.S. Population 65 and Older Served By Surface Water and GWUDI Systems by System Size

Size Category	2000	2010	2020	2050
Large PWSs (>100,000)	12,156,774	13,797,619	18,676,693	28,487,748
Medium PWSs (10,001–100,000)	7,545,584	8,564,039	11,586,844	17,682,051
Small PWSs (10,000 or fewer)	2,360,753	2,679,392	3,625,124	5,532,104
Total	**22,063,110**	**25,041,050**	**33,879,661**	**51,701,902**
Percent of Total Projected U.S. Population	**7.8%**	**8.3%**	**10.4%**	**12.8%**

Source: Derived from U.S. Census Bureau 2001b and Exhibit 8.1

Young children are a vulnerable population, subject to more severe health effects caused by *Giardia*, *Cryptosporidium*, and other waterborne pathogens. In particular, infants who have not yet developed immune responses are vulnerable to exposure and are at higher risk of severe dehydration caused by diarrhea. Although direct exposure to *Cryptosporidium* in tap water is minimal for infants (tap water used for formula is generally boiled) and low for children under 5 (USEPA 2001b), children are more vulnerable to severe health effects when they are exposed. Exhibit 8.4 estimates the population of children under 5 years of age by system size for those served by surface and GWUDI systems and the projected increases in this subpopulation. Population projections assume the proportion of the total population served by surface water and GWUDI systems is constant through time, as are the percentages of that population served by small, medium, and large systems. These estimates also assume random distribution of this population subgroup among ground water, surface water, and GWUDI systems and among systems of different sizes.

Exhibit 8.4 Estimates of U.S. Population under Age 5 Served by Surface Water and GWUDI Systems by System Size

Size Category	2000	2010	2020	2050
Large PWSs (>100,000)	6,662,045	6,982,710	7,626,124	9,350,349
Medium PWSs (10,001–100,000)	4,135,063	4,334,096	4,733,456	5,803,665
Small PWSs (10,000 or fewer)	1,293,718	1,355,989	1,480,935	1,815,766
Total	**12,090,826**	**12,672,795**	**13,840,516**	**16,969,780**
Percent of Total Projected U.S. Population	**4.3%**	**4.2%**	**4.3%**	**4.2%**

Source: Derived from U.S. Census Bureau 2001b and Exhibit 8.1

8.3 Population Profile for Exposure Assessment—Summary

Risk assessments of various LT2ESWTR regulatory scenarios must take into account the characteristics of the population affected. The total population served by public water systems is estimated to be 279.8 million, based on SDWIS data. More than 63 percent of this population (177.4 million) is served by surface water or GWUDI systems and is affected by the LT2ESWTR. Sensitive subpopulations, namely the immunocompromised, children under the age of 5, and the elderly, make up approximately 35.2 million of the 177.4 million individuals, or 20 percent of the population served by surface water and GWUDI systems. Not everyone in these groups is at increased risk, since not all elderly have the same vulnerability and since children may have limited exposure to drinking water. However, the number of immunocompromised individuals is expected to increase over the next several decades, making accurate risk assessment for these sensitive subpopulations all the more critical.

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