CHLORINE & DISINFECTION

CONTINUING EDUCATION
PROFESSIONAL DEVELOPMENT COURSE



Technical Learning College

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Most of our students prefer to do the assignment in Word and e-mail or fax the assignment back to us. We also teach this course in a conventional hands-on class. Call us and schedule a class today.

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Important Information about this Manual

This manual has been prepared to educate students and operators in general safety awareness of dealing with the often-complex and various water disinfectants, including dangerous chemicals, Chlorine and other toxic materials. This CEU course will also cover respirator protection devices, methods, and applications.

This manual will cover general laws, regulations, required procedures and accepted policies relating to the use of disinfectants, DDBPs, Ozone, Ultraviolet Radiation, Respirator Protection Devices, Methods, and Applications. It should be noted, however, that the regulation of respirator protection devices and hazardous materials is an ongoing process and subject to change over time. For this reason, a list of resources is provided to assist in obtaining the most up-to-date information on various subjects.

This manual is a not a guidance document for applicators or operators who are involved with pesticides. It is not designed to meet the requirements of the United States Environmental Protection Agency, Office of Health and Safety Administration (OSHA) or your local State environmental protection agency or health department. This course manual will provide general respirator protection and safety awareness and should not be used as a basis for respirator protection method/device guidance. This document is not a detailed safety manual or a source or remedy for respirator protection or control.

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Chlorine rail tank inside a chlorine storage room. Since 9/11, many water providers have been too scared to utilize the rail car in fear of a terrorist activity.

Technical Learning College's Scope and Function

Technical Learning College (TLC) offers affordable continuing education for today's working professionals who need to maintain licenses or certifications. TLC holds approximately eighty different governmental approvals for granting of continuing education credit.

TLC's delivery method of continuing education can include traditional types of classroom lectures and distance-based courses or independent study. Most TLC's distance based or independent study courses are offered in a print based format and you are welcome to examine this material on your computer with no obligation. Our courses are designed to be flexible and for you do finish the material on your leisure. Students can also receive course materials through the mail. The CEU course or e-manual will contain all your lessons, activities and assignments. Most CEU courses allow students to submit lessons using e-mail or fax, however some courses require students to submit lessons by postal mail. (See the course description for more information.) Students have direct contact with their instructor—primarily by e-mail. TLC's CEU courses may use such technologies as the World Wide Web, e-mail, CD-ROMs, videotapes and hard copies. (See the course description.) Make sure you have access to the necessary equipment before enrolling, i.e., printer, Microsoft Word and/or Adobe Acrobat Reader. Some courses may require proctored exams depending upon your state requirements.

Flexible Learning
At TLC, there are no scheduled online sessions you need contend with, nor are you required to participate in learning teams or groups designed for the "typical" younger campus based student. You will work at your own pace, completing assignments in time frames that work best for you. TLC's method of flexible individualized instruction is designed to provide each student the guidance and support needed for successful course completion.

We will beat any other training competitor's price for the same CEU material or classroom training. Student satisfaction is guaranteed.

Course Structure
TLC's online courses combine the best of online delivery and traditional university textbooks. Online you will find the course syllabus, course content, assignments, and online open book exams. This student friendly course design allows you the most flexibility in choosing when and where you will study.

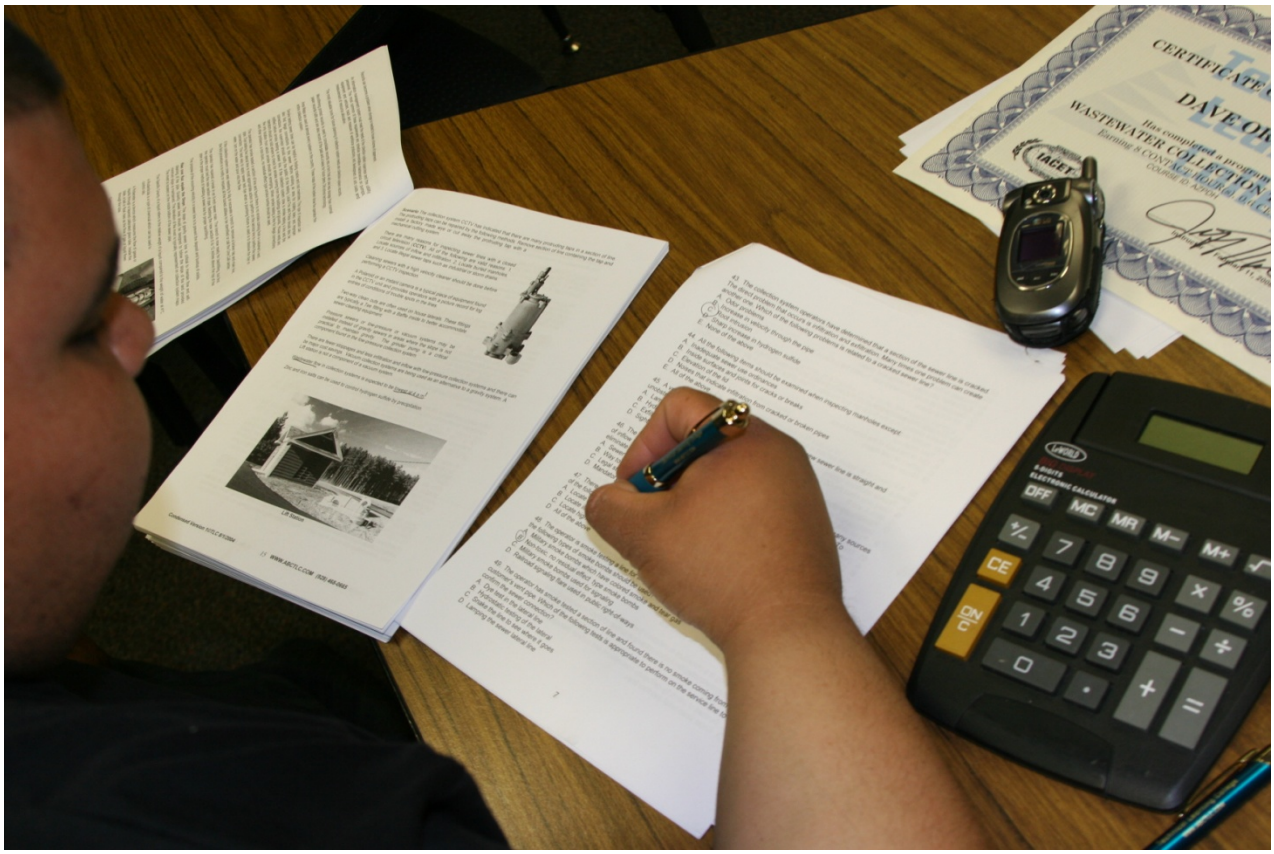
Classroom of One
TLC Online offers you the best of both worlds. You learn on your own terms, on your own time, but you are never on your own. Once enrolled, you will be assigned a personal Student Service Representative who works with you on an individualized basis throughout your program of study. Course specific faculty members are assigned at the beginning of each course providing the academic support you need to successfully complete each course.

Satisfaction Guaranteed
Our Iron-Clad, Risk-Free Guarantee ensures you will be another satisfied TLC student.

We have many years of experience, dealing with thousands of students. We assure you, our customer satisfaction is second to none. This is one reason we have taught more than 20,000 students.

Our administrative staff is trained to provide the best customer service in town. Part of that training is knowing how to solve most problems on the spot with an exchange or refund.

TLC Continuing Education Course Material Development
Technical Learning College (TLC's) continuing education course material development was based upon several factors; extensive academic research, advice from subject matter experts, data analysis, task analysis and training needs assessment process information gathered from other states.



We welcome you to do the electronic version of the assignment and submit the answer key and registration to us either by fax or e-mail. If you need this assignment graded and a certificate of completion within a 48-hour turn around, prepare to pay an additional rush charge of $50.

CEU Course Description

Chlorine & Disinfection CEU Training Course

This course will cover the fundamentals of water disinfection beginning with the different and alternative water disinfectants and ending with the biological analysis of the water, insuring that the water meets federal compliance. Task Analysis and Training Needs Assessments have been conducted to determine or set Needs-To-Know for this course. The goal of this CEU Course is to provide awareness training to help workers recognize the occupational hazards and health effects of different disinfectants, halogens, chlorine exposure and the exposure controls; and to familiarize the participants with the properties and safe handling of chlorine - solid, liquid, gas, and the operation of gas chlorinators and other related equipment. This course covers properties of chlorine, purpose of chlorine, chlorine terminology, dosage calculations, chlorinator equipment, chlorine cylinders, and operation of gas chlorinators, start up and shut down, chlorinator maintenance, troubleshooting common problems, chlorine safety, and chlorine testing procedures.

Water Distribution, Well Drillers, Pump Installers, Water Treatment Operators, Wastewater Operators. The target audience for this course is the person interested in working in a water treatment/wastewater treatment or distribution facility and/or wishing to maintain CEUs for certification license or to learn how to do the job safely and effectively, and/or to meet education needs for promotion. This CEU course will cover the fundamentals of water disinfection beginning with the source of water and ending with the disinfection and distribution making sure that it meets federal compliance.



Final Examination for Credit
Opportunity to pass the final comprehensive examination is limited to three attempts per course enrollment.

Prerequisites None

Course Procedures for Registration and Support
All of Technical Learning College correspondence courses have complete registration and support services offered. Delivery of services will include, e-mail, web site, telephone, fax and mail support. TLC will attempt immediate and prompt service.

Instructions for Written Assignments
The Chlorine and Disinfection CEU training course uses a multiple-choice style answer key. TLC would prefer that the answer key and registration, and survey sheet is faxed or e-mailed to, info@tlch2o.com. If you are unable to do so, please make a copy for yourself and mail me the completed manual. You can find course assistance on the website under the Assignment Page, under the Course Assistance button.

Feedback Mechanism (examination procedures)
Each student will receive a feedback form as part of their study packet. You will be able to find this form in the rear of the course or lesson.

Security and Integrity
All students are required to do their own work. All lesson sheets and final exams are not returned to the student to discourage sharing of answers. Any fraud or deceit and the student will forfeit all fees and the appropriate agency will be notified.

Grading Criteria
TLC will offer the student either pass/fail or a standard letter grading assignment. If TLC is not notified, you will only receive a pass/fail notice. (Certificate)

Recordkeeping and Reporting Practices
TLC will keep all student records for a minimum of seven years. It is your responsibility to give the completion certificate to the appropriate agencies. We will send the required information to Texas, Indiana and Pennsylvania for your certificate renewals.

ADA Compliance
TLC will make reasonable accommodations for persons with documented disabilities. Students should notify TLC and their instructors of any special needs. Course content may vary from this outline to meet the needs of this particular group.

Mission Statement
Our only product is educational service. Our goal is to provide you with the best education service possible. TLC will attempt to make your learning experience an enjoyable educational *opportunity.*

Educational Mission

The educational mission of TLC is:

To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

To provide TLC students opportunities to apply and understand the theory and skills needed for operator certification,

To provide opportunities for TLC students to learn and practice environmental educational skills with members of the community for the purpose of sharing diverse perspectives and experience,

To provide a forum in which students can exchange experiences and ideas related to environmental education,

To provide a forum for the collection and dissemination of current information related to environmental education, and to maintain an environment that nurtures academic and personal growth.

Table of Contents

Introduction	15
Waterborne Pathogens Chapter 1	**19**
Viral Caused Diseases	21
Protozoan Caused Diseases	23
Bacteriological Monitoring	27
Number of Samples	31
Positive Results	32
Heterotrophic Plate Counts	33
Total Coliform	36
Disinfection Rules Chapter 2	**91**
DDBPs	95
Halogens Chapter 3	**99**
Periodic Table	101
pH	103
Fluorine	105
Bromine	106
Iodine	107
Astatine	108
Chlorine Chapter 4	**109**
Chlorine Timeline	113
Chlorine Introduction	119
Exposure Limits	121
Atomic Structure	121
Chlorine Basics	123
Chemistry	127
Chlorinator Components	136
Clor-Alkali Membrane Process	139
Chlorine's Effectiveness	141
Chlorine Gas Section	147
DPD Method	151
Amperometric Titration	152
Sodium Hypochlorite	153
Preparing/Handling/Feeding	157
Exposure	158
Disadvantages	159
Shock Chlorination	161
Calcium Hypochlorite	169
Dose Rates	173
Health Effects	173
Chloramines	177
Chlorine Production	179
System Operation	182
Salt Addition	183
Sodium Hydroxide	184
System Maintenance	185
Equipment Requirements	187

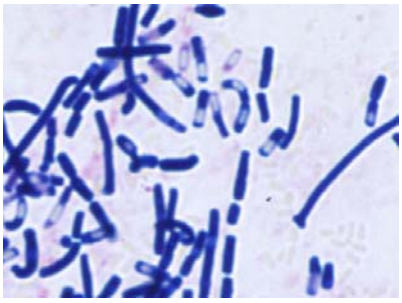
Weigh Scales 188
Health Hazards 191
Advanced Treatment 193
Summary of Toxicology 194
Chlorine Storage 196
Special Requirements 203
Chlorine Dioxide 205
Phosphine 208
Common Questions 209

Alternative Disinfectants Chapter 5 213
Ultraviolet Radiation 213
Photoelectric Cell 215
Advantages and Disadvantages 216
Ozone 217
Advantages 218
Alternatives Summary 218

Respiratory Protection Chapter 6 251
Importance of a Correct Fit 256
OSHA Overview 257
RP Employee Responsibilities 260
Program Statement 260
Program Evaluation 262
Recordkeeping 263
Training Record Example 264
Fit Testing 265
Filter and Canister Replacement 267
Checklist 269
Respiratory Protection Schedule 271
Medical Determination 273
Vapors & Gases 277
Hazard Assessment 282
Respirator Storage 283
SCBA Section 285
SCBA Do's and Don'ts 287
Pressure Demand SCBA 288
Donning 289
Bottle Inspection Form 294
PPE Section 295
Protective Clothing Applications 301
RP Rule 303
Appendix A 305
Appendix B 339
Appendix C 341
Appendix D 346

Disinfection Supplement 361
Sampling Questions 545
Common Water Chemicals 551
Math Conversion Factors 557
Glossary 561

Chlorine Glossary



Anthrax*:* The disease caused by bacillus Anthracis.

AOX: Absorbable organic halogen.

AWWA: American Water Works Association.

°C: degrees Celsius (a/k/a centigrade).

CDC: Centers for Disease Control.

Chlorate ion ($ClO3^-$): A product of the disproportionation of chlorine dioxide, for example by sunlight.

Chlorine dioxide ($ClO2$): A free radical; a powerful, selective oxidant.

Chloride ion (Cl^-): The principal reduction product of chlorine.

Chlorite ion ($ClO2^-$): A product of the partial reduction of chlorine dioxide.

CxT value: The product of the net residual [concentration] of a disinfectant and [time], used as a measure of the amount of disinfection applied to a system.

DOT: (United States) Department of Transportation.

EPA: (United States) Environmental Protection Agency.

°F: degrees Fahrenheit.

FDA: (United States) Food & Drug Administration.

FIFRA: Federal Insecticide Fungicide & Rodenticide Act.

HAA: Haloacetic acid(s), by-products of chlorination of water containing organics which are suspected carcinogens.

ICR: Information Collection Rule.

L: Liter.

Legionella: The microorganism that causes Legionnaire's disease.

MCL: Maximum Contaminant Level.

MCLG: Maximum Contaminant Level Goal.

mg: Milligram.

MRDL: Maximum Residual Disinfectant Level.

MGD: Millions of Gallons per Day

NIH: National Institutes of Health.

OSHA: (United States) Occupational Safety and Health Administration.

Oxidation: The net transfer of electrons from a source to an acceptor.

Pathogen: A disease-causing organism.

ppb: Parts-per-billion.

ppm: parts-per-million; in water, equivalent to mg/L.

SDWA: Safe Drinking Water Act.

Sodium chlorate (NaClO3): The sodium salt of chloric acid; a precursor for chlorine dioxide production, especially for pulp bleaching.

Sodium chlorite (NaClO2): The sodium salt of chlorous acid, a precursor for chlorine dioxide production, especially for drinking water treatment.

Stachybotrys: A particularly virulent type of toxic mold.

TLV: Threshold Limit Value.

TOC: Total Organic Carbon.

TOX: Total organic halogen.

Trihalomethanes: (THM) by-products of chlorination of water containing organics which are suspected carcinogens.

USDA: United States Department of Agriculture.

UV: Ultraviolet light.

WTP: Water treatment plant**.**

Commonly Used Water Disinfectants

Contaminant	MRDL[1] (mg/L)[2]	MRDL[1] (mg/L)[2]	Potential Health Effects from Ingestion of Water	Sources of Contaminant in Drinking Water
Chloramines (as Cl2)	MRDLG=4[1]	MRDL=4.0[1]	Eye/nose irritation; stomach discomfort, anemia	Water additive used to control microbes
Chlorine (as Cl2)	MRDLG=4[1]	MRDL=4.0[1]	Eye/nose irritation; stomach discomfort	Water additive used to control microbes
Chlorine dioxide (as ClO_2)	MRDLG=0.8[1]	MRDL=0.8[1]	Anemia; infants & young children: nervous system effects	Water additive used to control microbes

Disinfection Introduction

Selecting the right disinfection weapon requires understanding the factors governing the particular site and the water or wastewater to be treated. In general, the selection of an appropriate disinfection system should be evaluated against the following six criteria:

1. ***Safety*.** How does the disinfectant work and what types of precautions are needed to transport, store, use, and operate the disinfectant system and associated chemicals? If a system will require significant safety protection—such as use of breathing apparatus and protective clothing—as well as high levels of operator training, it may be advisable to explore other, less intensive systems. In addition, while the disinfectant may be relatively safe to use, consideration also has to be made for the effects of both intentional and unintentional releases to the environment.
2. ***Effectiveness*.** How effective is the disinfectant against the pathogens present in the water or wastewater? Since the intent is to reduce the levels of pathogens to acceptable standards, understanding how effective the proposed disinfectant system is in achieving those target levels, as well as the system's ability to reliably achieve the result, will be important to selecting the right system.
3. ***Cost*.** What are the costs associated with the disinfection system, both in terms of capital outlay and ongoing operations and maintenance? Operating costs can vary in terms of the time it takes to service the disinfectant system regularly, and the costs of supplies and components.
4. ***Complexity of use.*** How does the system operate and does it take specialized training to keep the system within tolerances? Since the outflow from the treatment facility may be subject to various standards and regulations, if the system is too complex it may require additional staff time to ensure that it operates within the desired parameters.
5. ***Environmental/Adverse Effects.*** What are some of the potential downsides to the operation of the system as it relates to the distribution system or watershed in which the treated effluent is discharged? While some systems may provide a net-positive environmental benefit through increased oxygenation of the receiving waters, other systems may need to have additional treatment of the disinfected effluent in order to render it benign when released.
6. ***Flow and Water Characteristics*.** Can the system handle fluctuations within the flow or with changing characteristics of the water or wastewater being processed? If a system has a narrow tolerance for the amount of water or wastewater flow, this could impact the effectiveness of the overall system. In addition, if the system cannot adjust for off-site concerns such as dry or wet weather flow rates of the receiving water body, this may also affect the system's appropriateness for your application.

With those criteria in mind, there are primarily four basic disinfection systems currently available—chlorination, ozone gas, ultraviolet radiation, and chemical treatment other than chlorine.

A variety of factors come into play in deciding which type of disinfectant system is right for your operation. The decision to install a system could be the result of local concerns and potential to mitigate health risks, as well as improved community relations. In any event, the operator of an onsite water or wastewater treatment plant needs to consider some of the safeguards that need to be in place as well.

"Typical safeguards include operator training and instrumentation monitoring that will perform a shutdown function if something goes above a certain level," says Schilling. "If they detect [for example] an ozone leak, you can do an interconnect and do a plant shutdown. UV has safeguards where you have monitors that tell you what your dosage is, and if you're over or under your dosage it will perform some kind of warning of whatever you want to do."

State and Local Regulations
State and local regulations vary considerably in their requirements to disinfect wastewater, so the decision of what type of system to use can be affected by the chemical and physical composition of the wastewater stream, the environment to which it will be discharged, and the concerns of the local health department. "It's all over the place," says Bach. "The chemical itself is a pesticide and is regulated by the US EPA. The states will specify what sort of *E. coli* or *coliform* counts you're going to have on discharge, and the regulations vary all over the map in the states. You've got a lot of things like that throughout the country and it goes down to ultimately the views of local health departments and reflects the local topography, their local population density, and also their experience of whether or not people have gotten sick."

Alternative Disinfectants More information in the Alternative Section

Unknown Factors Associated with Alternatives
Scientific investigation of risk associated with alternative disinfectants and alternative disinfection by-products is limited. A decision by water facilities to switch from chlorination could be risky because scientists know so little about DBPs from processes other than chlorination.

Drinking Water Disinfectants At a Glance

Disinfectants	Residual Maintenance	State of Information on By-Product Chemistry	Color Removal	Removal of Common Odors
Chlorine	Good	Adequate	Good	Good
Chloramines	Good	Limited	Unacceptable	Poor
Chlorine dioxide	Unacceptable*	Adequate	Good	Good
Ozone	Unacceptable	Limited	Excellent	Excellent
Ultraviolet radiation	Unacceptable	Nil	N/A	N/A

*In Europe, 50% of water distribution systems use chlorine dioxide as the residual disinfectant
Source: Trussell, R., Control Strategy 1; Alternative Oxidants and Disinfectants, 1991

Modern Water Treatment Disinfectants

Many water suppliers add a disinfectant to drinking water to kill germs such as giardia and e coli. Especially after heavy rainstorms, your water system may add more disinfectant to guarantee that these germs are killed.

Chlorine
Some people who use drinking water containing chlorine well in excess of EPA's standard could experience irritating effects to their eyes and nose. Some people who drink water containing chlorine well in excess of the EPA's standard could experience stomach discomfort.

Chloramine
Some people who use drinking water containing chloramines well in excess of EPA's standard could experience irritating effects to their eyes and nose. Some people who drink water containing chloramines well in excess of the EPA's standard could experience stomach discomfort or anemia.

Chlorine Dioxide
Some infants and young children who drink water containing chlorine dioxide in excess of the EPA's standard could experience nervous system effects. Similar effects may occur in fetuses of pregnant women who drink water containing chlorine dioxide in excess of the EPA's standard. Some people may experience anemia.

Disinfectant alternatives will include Ozone, and Ultraviolet light. You will see an increase of these technologies in the near future.

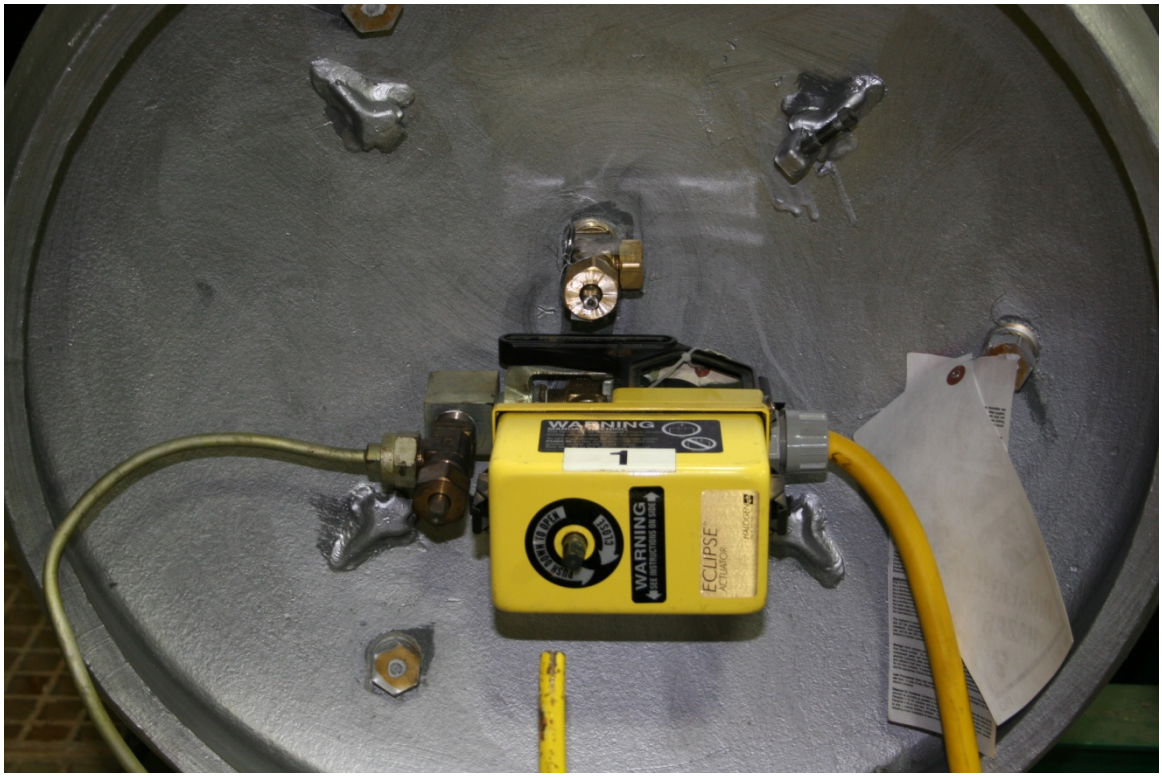
Disinfection Byproducts (DBPS)
Disinfection byproducts form when disinfectants added to drinking water to kill germs react with naturally-occurring organic matter in water.

Total Trihalomethanes
Some people who drink water containing trihalomethanes in excess of the EPA's standard over many years may experience problems with their liver, kidneys, or central nervous systems, and may have an increased risk of getting cancer.

Haloacetic Acids
Some people who drink water containing haloacetic acids in excess of the EPA's standard over many years may have an increased risk of getting cancer.

Bromate
Some people who drink water containing bromate in excess of the EPA's standard over many years may have an increased risk of getting cancer.

Chlorite
Some infants and young children who drink water containing chlorite in excess of the EPA's standard could experience nervous system effects. Similar effects may occur in fetuses of pregnant women who drink water containing chlorite in excess of the EPA's standard. Some people may experience anemia.



1 ton Chlorine containers. Automatic Shutoff device connected to the liquid side only. The top valve is used for the gas. Remember that containers lay on their sides and cylinders stand upright.



Waterborne Pathogens Chapter 1

***The reason we disinfect*.**

Bacteria, viruses and protozoan that cause disease are known as pathogens. Most pathogens are generally associated with diseases that cause intestinal illness and affect people in a relatively short amount of time, generally a few days to two weeks. They can cause illness through exposure to small quantities of contaminated water or food or from direct contact with infected people or animals.



Cryptosporidium

How Diseases are Transmitted.

Pathogens that may cause waterborne outbreaks through drinking water have one thing in common*: *they are spread by the fecal-oral, or feces-to-mouth, route.

Pathogens may get into water and spread when infected humans or animals pass the bacteria, viruses, and protozoa in their stool. For another person to become infected, he or she must take that pathogen in through the mouth. Waterborne pathogens are different from other types of pathogens such as the viruses that cause influenza (the flu) or the bacteria that cause tuberculosis. Influenza virus and tuberculosis bacteria are spread by secretions that are coughed or sneezed into the air by an infected person.

Human or animal wastes in watersheds, failing septic systems, failing sewage treatment plants or cross-connections of water lines with sewage lines provide the potential for contaminating water with pathogens. The water may not appear to be contaminated because feces have been broken up, dispersed, and diluted into microscopic particles. These particles, containing pathogens, may remain in the water and be passed to humans or animals unless adequately treated. Only proper treatment will ensure eliminating the spread of disease. In addition to water, other methods exist for spreading pathogens by the fecal-oral route. The foodborne route is one of the more common methods. A frequent source is a food handler who does not wash his hands after a bowel movement and then handles food with "***unclean***" hands. The individual who eats feces-contaminated food may become infected and ill. It is interesting to note the majority of foodborne diseases occur in the home, not restaurants. Day care centers are another common source for spreading pathogens by the fecal-oral route.

Here, infected children in diapers may get feces on their fingers, then put their fingers in a friend's mouth or handle toys that other children put into their mouths. The general public and some of the medical community usually refer to diarrhea symptoms as "**stomach flu**." Technically, influenza is an upper respiratory illness and rarely has diarrhea associated with it; therefore, stomach flu is a misleading description for foodborne or waterborne illnesses, yet is accepted by the general public. So the next time you get the stomach flu, you may want to think twice about what you've digested within the past few days.

Chain of Transmission

Water is contaminated with feces. This contamination may be of human or animal origin. The feces must contain pathogens (disease-causing bacteria, viruses or protozoa); if the human or animal source is not infected with a pathogen, no disease will result.

The pathogens must survive in the water. This depends on the temperature of the water and the length of time the pathogens are in the water. Some pathogens will survive for only a short time in water, others, such as Giardia or Cryptosporidium, may survive for months.

The pathogens in the water must enter the water system's intake, in numbers sufficient to infect people. The water is either not treated or inadequately treated for the pathogens present. A susceptible person must drink the water that contains the pathogen; illness (disease) will occur. This chain lists the events that must occur for the transmission of disease via drinking water. By breaking the chain at any point, the transmission of disease will be prevented.

Bacterial Diseases

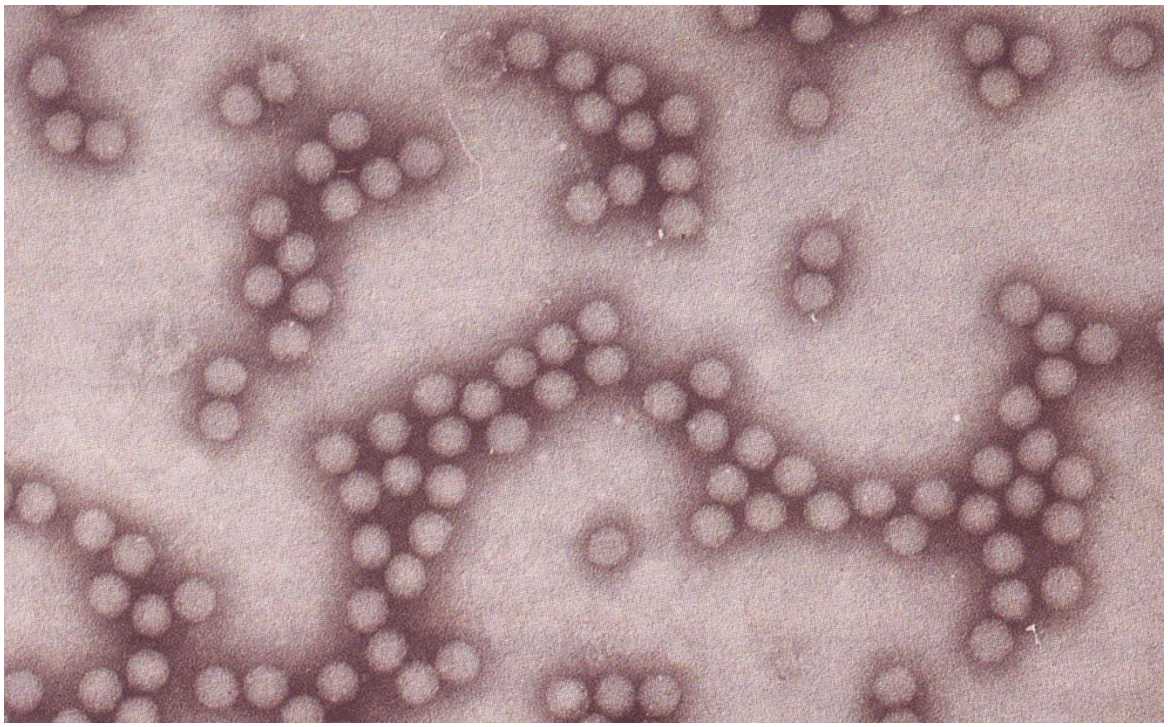
Campylobacteriosis is the most common diarrhea illness caused by bacteria. Other symptoms include abdominal pain, malaise, fever, nausea and vomiting. Symptoms begin three to five days after exposure. The illness is frequently over within two to five days and usually lasts no more than 10 days. Campylobacteriosis outbreaks have most often been associated with food, especially chicken and unpasteurized milk, as well as unchlorinated water.

These organisms are also an important cause of ***"travelers' diarrhea."*** Medical treatment generally is not prescribed for campylobacteriosis because recovery is usually rapid. Cholera, Legionellosis, Salmonellosis, Shigellosis, and Yersiniosis, are other bacterial diseases that can be transmitted through water. All bacteria in water are readily killed or inactivated with chlorine or other disinfectants.



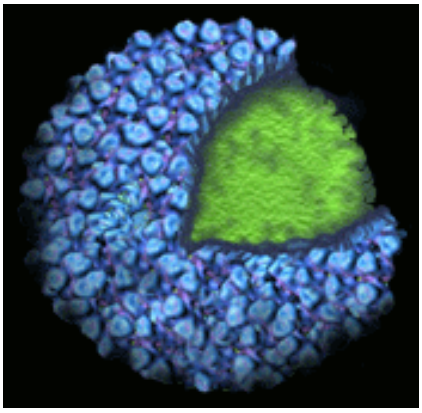
E. coli Bacteria

Viral-Caused Diseases



Hepatitis A is an example of a common viral disease that may be transmitted through water. The onset is usually abrupt, with fever, malaise, loss of appetite, nausea and abdominal discomfort followed within a few days by jaundice. The disease varies in severity from a mild illness lasting one to two weeks to a severely disabling disease lasting several months (rare). The incubation period is 15-50 days and averages 28-30 days. Hepatitis A outbreaks have been related to fecally contaminated water; food contaminated by infected food handlers, including sandwiches and salads that are not cooked or are handled after cooking, and raw or undercooked mollusks harvested from contaminated waters.

Aseptic meningitis, polio, and viral gastroenteritis (**Norwalk agent**) are other viral diseases that can be transmitted through water. Most viruses in drinking water can be inactivated by chlorine or other disinfectants.



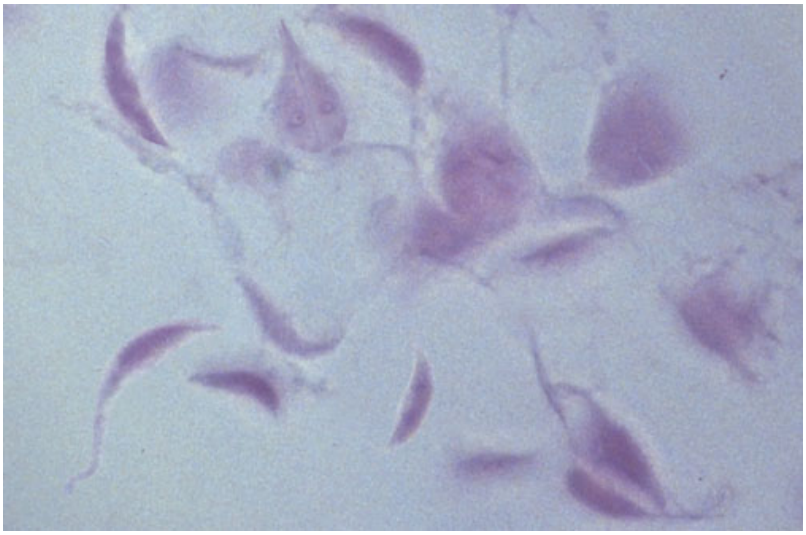
Norwalk Agent

Acronyms

ART: Average residence time
BAT: Best available technology
CWS: Community water system
DBP: Disinfection byproducts
DBPR: Disinfectants and Disinfection Byproducts Rule
DWSRF: Drinking Water State Revolving Fund
EPA: United States Environmental Protection Agency
FACA: Federal Advisory Committee Act
FR: Federal Register
GAC10: Granular activated carbon with ten minute empty bed contact time and 180 day reactivation frequency
GAC20: Granular activated carbon with twenty minute empty bed contact time and 240 day reactivation frequency
GWR: Groundwater Rule
GWUDI: Gound water under the direct influence of surface water
HAA5: Haloacetic acids (five) (sum of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid)
ICR: Information Collection Rule
IDSE: Initial distribution system evaluation
IESWTR: Interim Enhanced Surface Water Treatment Rule
LRAA: Locational running annual average
LT1ESWTR: Long Term 1 Enhanced Surface Water Treatment Rule
LT2ESWTR: Long Term 2 Enhanced Surface Water Treatment Rule
MCL: Maximum contaminant level
MCLG: Maximum contaminant level goal
M-DBP: Microbial and Disinfectants/Disinfection Byproducts
mg/L: Milligrams per liter
MRDL: Maximum residual disinfectant level
MRT: Maximum residence time
NOM: Natural organic matter
NTNCWS: Nontransient noncommunity water system
PWS: Public water system
SBREFA: Small Business Regulatory Enforcement Fairness Act
SDWA: Safe Drinking Water Act, as amended in 1986 and 1996
SWTR: Surface Water Treatment Rule
TCR: Total Coliform Rule
TOC: Total organic carbon
TTHM: Total trihalomethanes
UV: Ultraviolet light
VSS: Very small system

Protozoan Caused Diseases

Protozoan pathogens are larger than bacteria and viruses, but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall called a "**cyst**." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.



Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "**backpacker's disease**" and "**beaver fever**" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days.

Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment, but these are not 100% effective.

Cryptosporidiosis

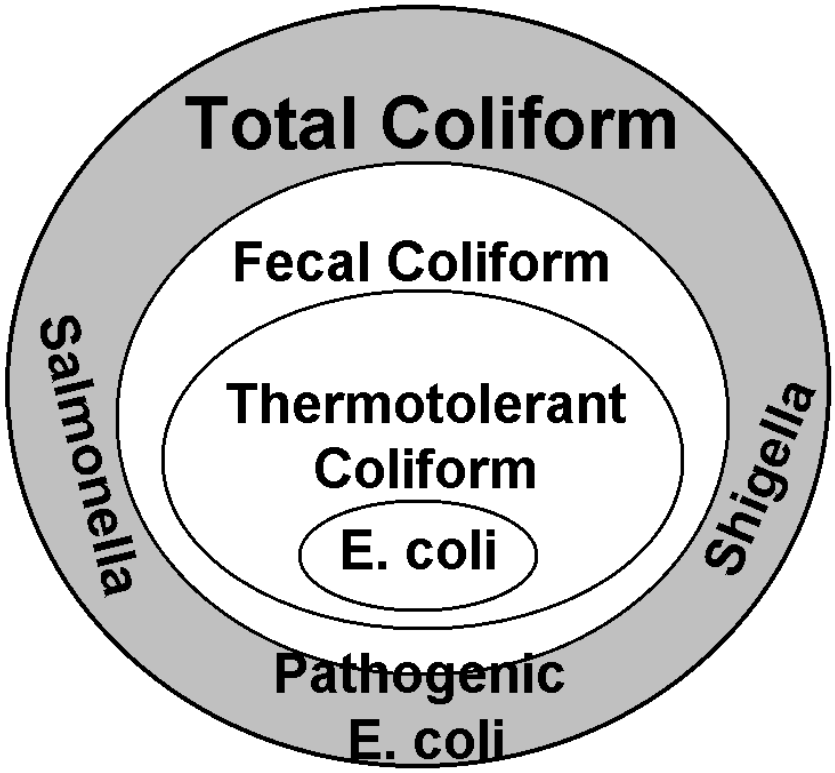


Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days.

Cryptosporidium organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for *Cryptosporidium* infections.

All these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems, such as those with HIV infections or cancer.

By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.



Waterborne Diseases

Name ***Causative organism*** *Source of organism* (Disease)

Viral gastroenteritis ***Rotavirus:*** *lives mostly in young Human feces*, (Diarrhea or vomiting children)

Norwalk-like viruses *Human feces*; also, shellfish; lives in polluted waters (Diarrhea and vomiting)
Salmonellosis ***Salmonella*** (bacterium): *Animal or human feces* (Diarrhea or vomiting)

Escherichia *coli*-- ***E. coli O1*** **57:H7,** ***Other E. coli organisms*** (bacterium): *Human feces* (Symptoms vary with type caused; gastroenteritis)

Typhoid ***Salmonella typhi*** (bacterium): *Human feces, urine* (Inflamed intestine, enlarged spleen, high temperature— sometimes fatal)

Shigellosis ***Shigella*** (bacterium): *Human feces* (Diarrhea)

Cholera ***Vibrio choleras*** (bacterium): *Human feces; also, shellfish; lives in many coastal waters* (Vomiting, severe diarrhea, rapid dehydration, mineral loss —high mortality.)

Hepatitis A **Hepatitis A** virus: *Human feces; shellfish grown*; *lives in polluted waters* (fever Yellowed skin, enlarged liver, vomiting, weight loss, abdominal pain — low mortality, lasts up to four months)

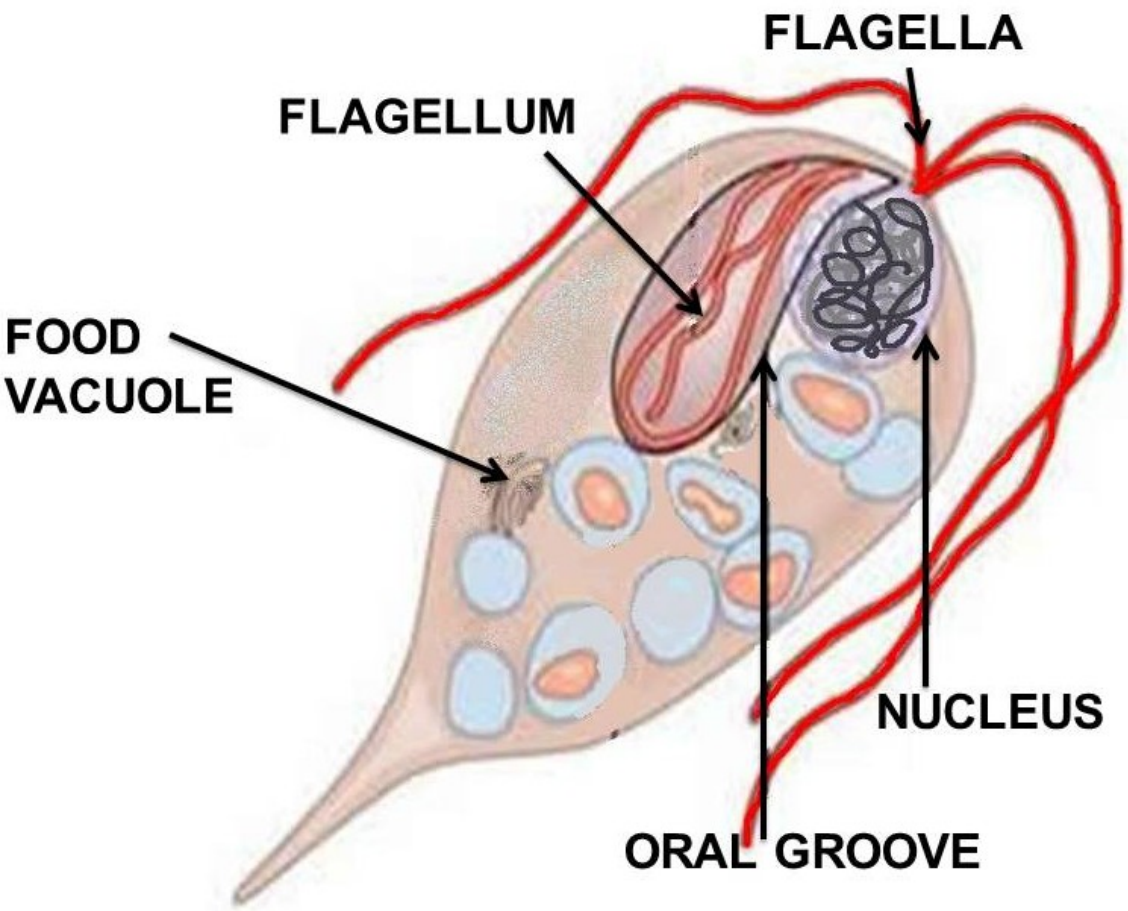
Amebiasis ***Entamoeba histolytica:*** *Human feces* (Mild diarrhea, dysentery, (protozoan) extra intestinal infection)

Giardiasis ***Giardia lamblia*** (protozoan): *Animal or human feces* (Diarrhea, cramps, nausea, and general weakness — lasts one week to months)

Cryptosporidiosis ***Cryptosporidium parvum****: Animal or human feces* (Diarrhea, stomach pain — lasts (protozoan) days to weeks)



E. coli O157



GIARDIA LAMBLIA

Fecal Pollution Questions 45-50

45. Some believe that exposure to fecal pollution through recreational waters or ingestion of contaminated shellfish causes greater health risks if the pollution is of human rather than animal origin.

46. Before the relative risks of human versus animal fecal pollution can be assessed, it is necessary to develop a microbiological method for distinguishing human from animal pollution.

47. Current methods detect fecal pollution but do not reveal the source. The objective of the project entitled “Method to Distinguish Non-Human Fecal Pollution from Human Fecal Pollution” is to develop a gene probe specific for E. coli that inhabit the human intestine for use as an indicator of the presence of human fecal contamination in water.

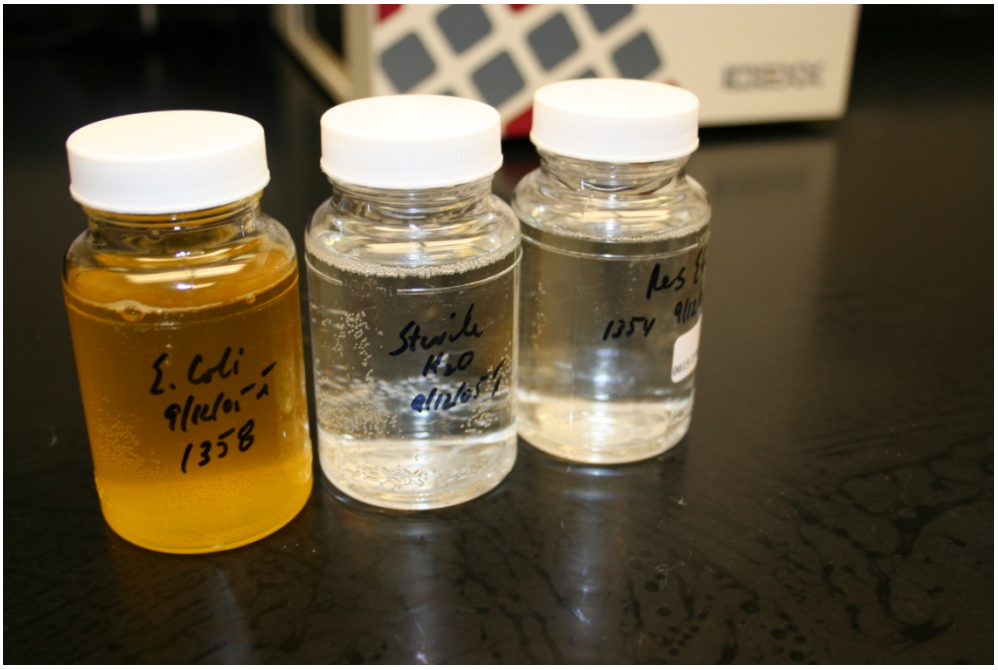
48. The probe will be field tested at several sites in which fecal pollution is exclusively from human sources, exclusively from animal sources and from mixed sources.

Shigella Species

49. Shigella species are among the most common and significant pathogens associated with wastewater and sludge. Because of their low infective doses, these organisms may be hazardous even if present in low numbers in wastewaters that are recycled for potable use or sludges that are applied to agricultural land.

50. Shigellae are very difficult to detect in environmental samples by conventional methods because of their biochemical similarities to E. coli.

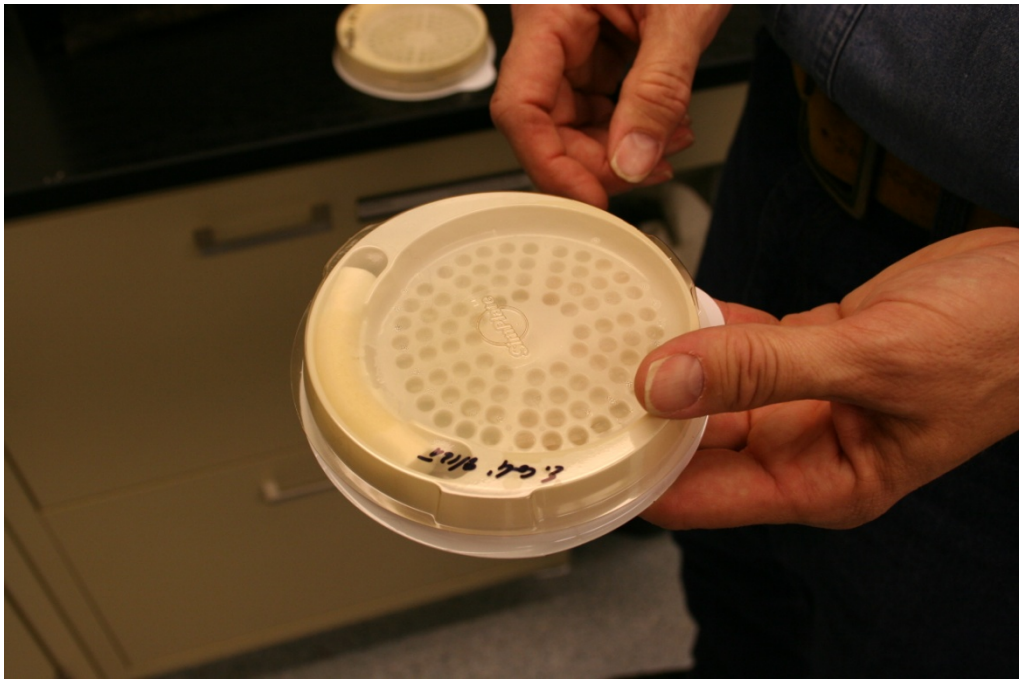
Bacteriological Monitoring Section



Colilert test simultaneously detects and confirms coliforms and E. coli in water samples in 24 hours or less. Simply add the Colilert reagent to the sample, incubate for 24 hours, and read results.

Colilert is easy to read, as positive coliform samples turn yellow, and when E. coli is present, samples fluoresce under UV light.





SimPlate for HPC Multi Dose Method.

IDEXX's HPC method is used for the quantification of heterotrophic plate counts in water. The number of fluorescing wells corresponds to a Most Probable Number (MPN) of total bacteria in the original sample. The MPN values generated by the SimPlate for HPC method correlate with the Pour Plate method using Total Plate Count Agar incubated at 35^{o} for 48 hours as described in *Standard Methods for the Examination of Water and Wastewater, 19th Edition.*



Bacteriological Monitoring

Most waterborne diseases and illnesses have been related to the microbiological quality of drinking water. The routine microbiological analysis of your water is for coliform bacteria. The coliform bacteria group is used as an indicator organism to determine the biological quality of your water. The presence of an indicator or pathogenic bacteria in your drinking water is an important health concern. Indicator bacteria signal possible fecal contamination and therefore, the potential presence of pathogens. They are used to monitor for pathogens because of the difficulties in determining the presence of specific disease-causing microorganisms.

Indicator bacteria are usually harmless, occur in high densities in their natural environment and are easily cultured in relatively simple bacteriological media. Indicators in common use today for routine monitoring of drinking water include total coliforms, fecal coliforms and *Escherichia coli (E. coli).*

Bacteria Sampling

Water samples for bacteria tests must always be collected in a sterile container. Take the sample from an inside faucet with the aerator removed. Sterilize by spraying a 5% household bleach or alcohol solution or flaming the end of the tap with propane torch.

Run the water for five minutes to clear the water lines and bring in fresh water. Do not touch or contaminate the inside of the bottle or cap. Carefully open the sample container and hold the outside of the cap. Fill the container and replace the top. Refrigerate the sample and transport it to the testing laboratory within six hours (in an ice chest). Many labs will not accept bacteria samples on Friday so check the lab's schedule. Mailing bacteria samples is not recommended because laboratory analysis results are not as reliable.

Iron bacteria forms an obvious slime on the inside of pipes and fixtures. A water test is not needed for identification. Check for a reddish-brown slime inside a toilet tank or where water stands for several days.



Standard Sample Coliform Bacteria Bac-T.

Bac-T Sample Bottle, often referred to as a Standard Sample, 100 mls, Notice the white powder inside the bottle. That is Sodium Thiosulfate, a de-chlorination agent. Be careful not to wash-out this chemical while sampling. Notice the custody seal on the bottle.

Coliform Bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.

Laboratory Procedures

The laboratory may perform the total coliform analysis in one of four methods approved by the U.S. EPA and your local environmental or health division:

Methods

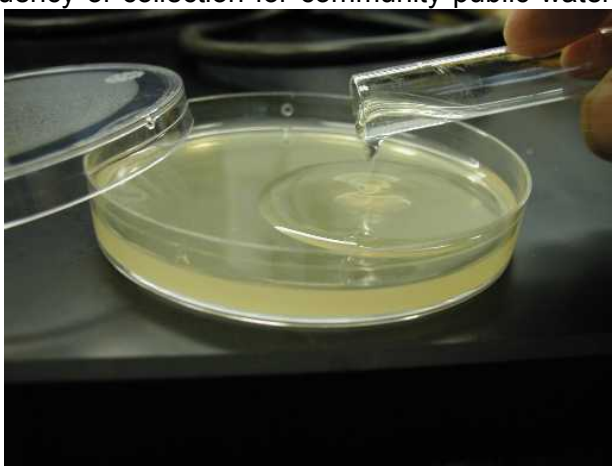
The MMO-MUG test, a product marketed as Colilert is the most common. The sample results will be reported by the laboratories as simply coliforms present or absent. If coliforms are present, the laboratory will analyze the sample further to determine if these are fecal coliforms or E. coli and report their presence or absence.

Types of Water Samples

It is important to properly identify the type of sample you are collecting. Please indicate in the space provided on the laboratory form the type of sample.
The three (3) types of samples are:
1. **Routine:** Samples collected on a routine basis to monitor for contamination. Collection
should be in accordance with an approved sampling plan.
2. **Repeat:** Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect.
3. **Special:** Samples collected for other reasons.
Examples would be a sample collected after repairs to the system and before it is placed back into operation or a sample collected at a wellhead prior to a disinfection injection point.

Routine Coliform Sampling

The number of routine samples and frequency of collection for community public water systems is shown in Table 3-1.



Noncommunity and nontransient noncommunity public water systems will sample at the same frequency as a like sized community public water system if:

1. It has more than 1,000 daily population and has ground water as a source, or 2. It serves 25 or more daily population and utilizes surface water as a source or ground water under the direct influence of surface water as its source.

Noncommunity and nontransient, noncommunity water systems with less than 1,000 daily population and groundwater as a source will sample on a quarterly basis.

No. of Samples per System Population

Persons served - Samples per month

up to 1,000	1
1,001-2,500	2
2,501-3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 12,900	10
12,901 to 17,200	15
17,201 to 21,500	20
21,501 to 25,000	25
25,001 to 33,000	30
33,001 to 41,000	40
41,001 to 50,000	50
50,001 to 59,000	60
59,001 to 70,000	70
70,001 to 83,000	80
83,001 to 96,000	90
96,001 to 130,000	100
130,001 to 220,000	120
220,001 to 320,000	150
320,001 to 450,000	180
450,001 to 600,000	210
600,001 to 780,000	240



Repeat Sampling

Repeat sampling replaces the old check sampling with a more comprehensive procedure to try to identify problem areas in the system. Whenever a routine sample is total coliform or fecal coliform present a set of repeat samples must be collected within 24 hours after being notified by the laboratory. The follow-up for repeat sampling is:

1. If only one routine sample per month or quarter is required, four (4) repeat samples must be collected.
2. For systems collecting two (2) or more routine samples per month, three (3) repeat samples must be collected.
3. Repeat samples must be collected from:
   a. The original sampling location of the coliform present sample.
   b. Within five (5) service connections upstream from the original sampling location.
   c. Within five (5) service connections downstream from the original sampling location.
   d. Elsewhere in the distribution system or at the wellhead, if necessary.
4. If the system has only one service connection, the repeat samples must be collected from the same sampling location over a four-day period or on the same day.
5. All repeat samples are included in the MCL compliance calculation.
6. If a system which normally collects fewer than five (5) routine samples per month has a coliform present sample, it must collect five (5) routine samples the following month or quarter regardless of whether an MCL violation occurred or if repeat sampling was coliform absent.

Positive or Coliform Present Results

What do you do when your sample is positive or coliform present?

When you are notified of a positive test result you need to contact either the Drinking Water Program or your local county health department within 24 hours, or by the next business day after the results are reported to you. The Drinking Water Program contracts with many of the local health departments to provide assistance to water systems.

After you have contacted an agency for assistance, you will be instructed as to the proper repeat sampling procedures and possible corrective measures for solving the problem. It is very important to initiate the repeat sampling immediately as the corrective measures will be based on those results.

Some examples of typical corrective measures to coliform problems are:

1. Shock chlorination of a ground water well. The recommended dose of 5% household bleach is 2 cups per 100 gallons of water in the well. This should be done anytime the bell is opened for repair (pump replacement, etc.). If you plan to shock the entire system, calculate the total gallonage of storage and distribution.
2. Conduct routine distribution line flushing. Install blowoffs on all dead end lines.
3. Conduct a cross connection program to identify all connections with non-potable water sources. Eliminate all of these connections or provide approved back flow prevention devices.
4. Upgrade the wellhead area to meet current construction standards as set by your state environmental or health agency.
5. If you continuously chlorinate, review your operation and be sure to maintain a detectable residual (**0.2 mg/l free chlorine**) at all times in the distribution system.
6. Perform routine cleaning of the storage system.

This list provides some basic operation and maintenance procedures that could help eliminate potential bacteriological problems, check with your state drinking water section or health department for further instructions.

Maximum Contaminant Levels (MCLs

State and federal laws establish standards for drinking water quality. Under normal circumstances when these standards are being met, the water is safe to drink with no threat to human health. These standards are known as maximum contaminant levels (**MCL**). When a particular contaminant exceeds its MCL a potential health threat may occur.

The MCLs are based on extensive research on toxicological properties of the contaminants, risk assessments and factors, short term (**acute**) exposure, and long term (**chronic**) exposure. You conduct the monitoring to make sure your water is in compliance with the MCL. There are two types of MCL violations for coliform bacteria. The first is for total coliform; the second is an acute risk to health violation characterized by the confirmed presence of fecal coliform or E.coli.



Quebec Colony Counter→

Heterotrophic Plate Count

Heterotrophic Plate Count (**HPC**) --- formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria and measuring changes during water treatment and distribution in water or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term "***colony-forming units***" (**CFU**).

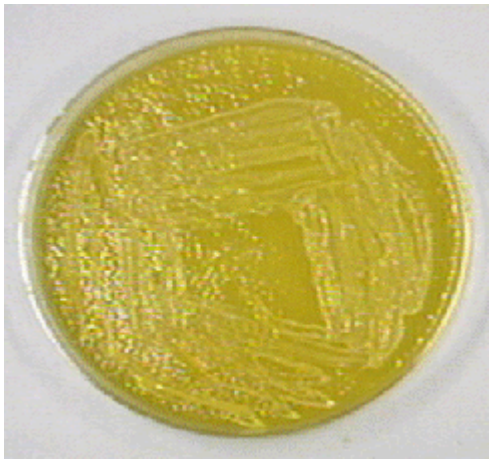
Methods

There are three methods for standard plate count:

1. **Pour Plate Method**
The colonies produced are relatively small and compact, showing less tendency to encroach on each other than those produced by surface growth. On the other hand, submerged colonies often are slower growing and are difficult to transfer.

2. **Spread Plate Method**
All colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Colonies can be transferred quickly, and colony morphology easily can be discerned and compared to published descriptions.

3. **Membrane Filter Method**
This method permits testing large volumes of low-turbidity water and is the method of choice for low-count waters.



Material Needed

i) Apparatus
- Glass rod
- Erlenmeyer flask
- Graduated Cylinder
- Pipet
- Petri dish
- Incubator

ii) Reagent and sample
- Reagent-grade water
- Nutrient agar
- Sample

Procedure*

1.Boil mixture of nutrient agar and nutrient broth for 15 minutes, then cool for about 20 minutes.
2.Pour approximately 15 ml of medium in each Petri dish, let medium solidify.
3.Pipet 0.1 ml of each dilution onto surface of pre-dried plate, starting with the highest dilution.
4.Distribute inoculum over surface of the medium using a sterile, bent glass rod.
5.Incubate plates at 35^{o}C for 48h.
6.Count all colonies on selected plates promptly after incubation, consider only plates having 30 to 300 colonies in determining the plate count..

*Duplicate samples

Computing and Reporting
Compute bacterial count per milliliter by the following equation:
CFU/ml = colonies counted / actual volume of sample in dish.
a)If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies.
b) If plates from all dilutions of any sample have no colony, report the count as less than 1/actual volume of sample in dish estimated **CFU/ml**.
c) Avoid creating fictitious precision and accuracy when computing **CFU** by recording only the first two left-hand digits.

Heterotrophic Plate Count
(Spread Plate Method)
Heterotrophic organisms utilize organic compounds as their carbon source (food or substrate). In contrast, autotrophic organisms use inorganic carbon sources. The Heterotrophic Plate Count provides a technique to quantify the bacteriological activity of a sample. The R2A agar provides a medium that will support a large variety of heterotrophic bacteria. After an incubation period, a bacteriological colony count provides an estimate of the concentration of heterotrophs in the sample of interest.

Laboratory Equipment Needed
100 x 15 Petri Dishes
Turntable
Glass Rods: Bend fire polished glass rod 45 degrees about 40 mm from one end. Sterilize before using.
Pipet: Glass, 1.1 mL. Sterilize before using.
Quebec Colony Counter
Hand Tally Counter

Reagents
1) R2A Agar: Dissolve and dilute 0.5 g of yeast extract, 0.5 g of proteose peptone No. 3, 0.5 g of casamino acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of dipotassium hydrogen phosphate, 0.05 g of magnesium sulfate heptahydrate, 0.3 g of sodium pyruvate, and 15.0 g of agar to 1 L. Adjust pH to 7.2 with dipotassium hydrogen phosphate **before adding agar.** Heat to dissolve agar and sterilize at 121 degrees C for 15 minutes.

2) Ethanol: As needed for flame sterilization.

Preparation of Spread Plates
Immediately after agar sterilization, pour 15 mL of R2A agar into sterile 100 x 15 Petri dishes; let agar solidify. Pre-dry plates inverted so that there is a 2 to 3 g water loss overnight with the lids on. Use pre-dried plates immediately or store up to two weeks in sealed plastic bags at 4 degrees C.

Sample Preparation
Mark each plate with sample type, dilution, date, and any other information before sample application. Prepare at least duplicate plates for each volume of sample or dilution examined. Thoroughly mix all samples by rapidly making about 25 complete up-and-down movements.

Sample Application
Uncover pre-dried agar plate. Minimize time plate remains uncovered. Pipet 0.1 or 0.5 mL sample onto surface of pre-dried agar plate.

Record Volume of Sample Used
Using a sterile, bent glass rod, distribute the sample over surface of the medium by rotating the dish by hand on a turntable. Let the sample be absorbed completely into the medium before incubating. Put cover back on Petri dish and invert for duration of incubation time. Incubate at 28°C for 7 days. Remove Petri dishes from incubator for counting.

Counting and Recording
After incubation period, promptly count all colonies on the plates. To count, uncover plate and place on Quebec colony counter. Use hand tally counter to maintain count. Count all colonies on the plate, regardless of size. Compute bacterial count per milliliter by the following equation:

$$\text{CFU} \Big/ \text{mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish, mL}}$$

To report counts on a plate with no colonies, report the count as less than one (<1) divided by the sample volume put on that plate (remember to account for any dilution of that sample).

If plates of all dilutions for a sample have no colonies, report the count as less than one (<1) divided by the largest sample volume used. Example: if 0.1 mL of a 100:1 and 10000:1 dilution of a sample both turned up with no colonies formed, the reported result would be <1 divided by the largest sample volume 0.001 mL (0.1 mL divided by 100). The final reported result for the sample is <1000 CFU per mL.

Assignment
1. Report the number of colony forming units (**CFU**) found on each plate.

2. Calculate the **CFU** per mL for each plate.

3. The aim of diluting samples is to produce a plate having 30 to 300 colonies, which plates meet these criteria. If no sample produces a plate with a count in this range, use the plate(s) with a count closest to 300. Based on these criteria, use your calculated results to report the **CFU** per mL for each sample. In the conclusion of your lab report, comment on your final results for each sample type as well as the quality of your application of this analysis technique. Feel free to justify your comments using statistical analysis. Also, comment on the general accuracy of this analytical technique and the factors that affect its accuracy and or applicability.

Data Table for Samples

Sample ID	Volume of Sample, mL	Colonies Counted per plate

Total Coliforms

This MCL is based on the presence of total coliforms, and compliance is on a monthly or quarterly basis, depending on your water system type and state rule. For systems which collect *fewer* than 40 samples per month, no more than one sample per month may be positive. In other words, the second positive result (repeat or routine) in a month or quarter results in an MCL violation.

For systems which collect 40 or more samples per month, no more than five (5) percent may be Positive, check with your state drinking water section or health department for further instructions.

Acute Risk to Health (Fecal Coliforms and E. Coli)
An acute risk to human health violation occurs if either one of the following happen:
1. A routine analysis shows total coliform present and is followed by a repeat analysis which indicates fecal coliform or E. coli present.
2. A routine analysis shows total and fecal coliform or E. coli present and is followed by a repeat analysis which indicates total coliform present. An acute health risk violation requires the water system to provide public notice via radio and television stations in the area. This type of contamination can pose an immediate threat to human health and notice must be given as soon as possible, but no later than 72 hours after notification from your laboratory of the test results.

Certain language may be mandatory for both these violations and is included in your state drinking water rule.

Public Notice
A public notice is required to be issued by a water system whenever it fails to comply with an applicable MCL or treatment technique, or fails to comply with the requirements of any scheduled variance or permit. This will inform users when there is a problem with the system and give them information.



A public notice is also required whenever a water system fails to comply with its monitoring and/or reporting requirements or testing procedure. Each public notice must contain certain information, be issued properly and in a timely manner, and contain certain mandatory language.

The timing and place of posting of the public notice depends on whether an acute risk is present to users. Check with your state drinking water section or health department for further instructions.

The following are acute violations
1. Violation of the MCL for nitrate.
2. Any violation of the MCL for total coliforms, when fecal coliforms or E. coli are present in the distribution system.
3. Any outbreak of waterborne disease, as defined by the rules.

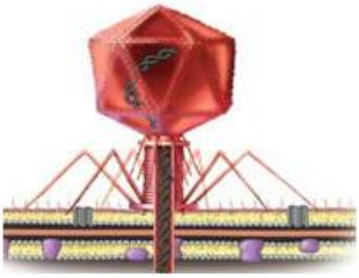
Microorganism Appendix

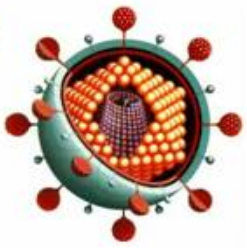


COCCI
(SPHERICAL)



SPIRILLI
(SPIRAL)

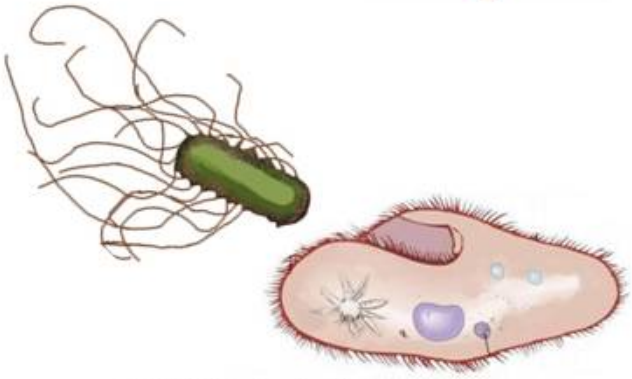




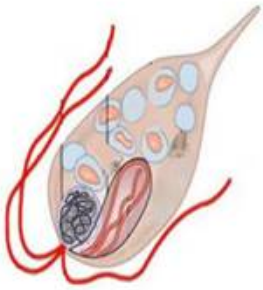
VIRUSES



BACILLI
(ROD-SHAPED)



BACTERIA

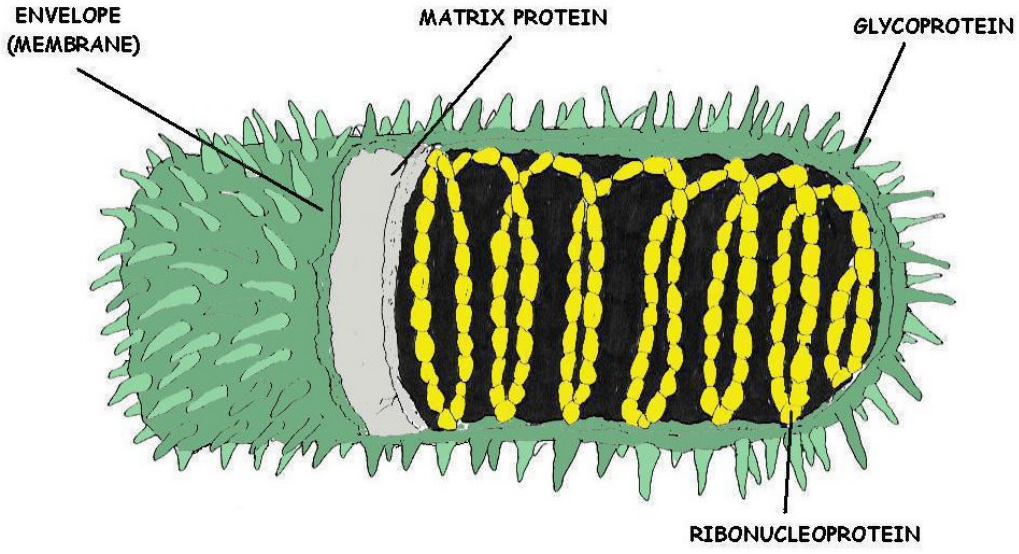


PROTOZOA

BACTERIA TYPES

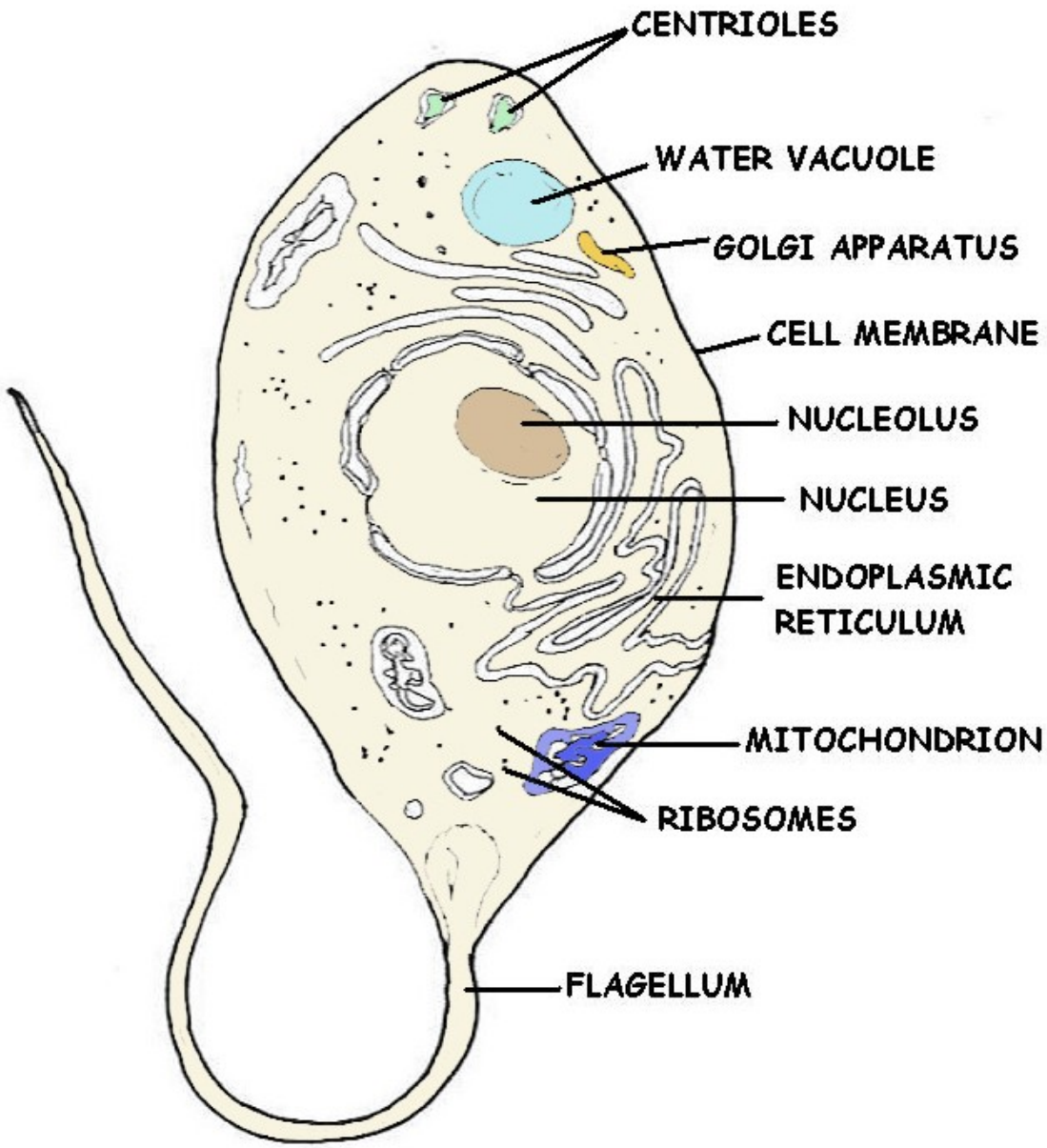


This section will give a close-up and short explanation of the major microorganisms found in water and in wastewater.



VIRUS

Protozoa



PROTOZOAN CELL

Protozoa are around 10–50 micrometer, but can grow up to 1 mm and can easily be seen under a microscope. Protozoa exist throughout aqueous environments and soil. Protozoa occupy a range of trophic levels. As predators, they prey upon unicellular or filamentous algae, bacteria, and microfungi.

Protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. Protozoa also play a vital role in controlling bacteria populations and biomass. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important. Protozoa such as the malaria parasites (Plasmodium spp.), trypanosomes and leishmania are also important as parasites and symbionts of multicellular animals.

Most protozoa exist in 5 stages of life which are in the form of trophozoites and cysts. As cysts, protozoa can survive harsh conditions, such as exposure to extreme temperatures and harmful chemicals, or long periods without access to nutrients, water, or oxygen for a period of time. Being a cyst enables parasitic species to survive outside of the host, and allows their transmission from one host to another. When protozoa are in the form of trophozoites (Greek, tropho=to nourish), they actively feed and grow. The process by which the protozoa takes its cyst form is called encystation, while the process of transforming back into trophozoite is called excystation.

Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, and some both (e.g. Coccidia). An individual protozoan is hermaphroditic.

Classification
Protozoa were commonly grouped in the kingdom of Protista together with the plant-like algae and fungus-like water molds and slime molds. In the 21st-century systematics, protozoans, along with ciliates, mastigophorans, and apicomplexans, are arranged as animal-like protists. However, protozoans are neither Animalia nor Metazoa (with the possible exception of the enigmatic, moldy Myxozoa).

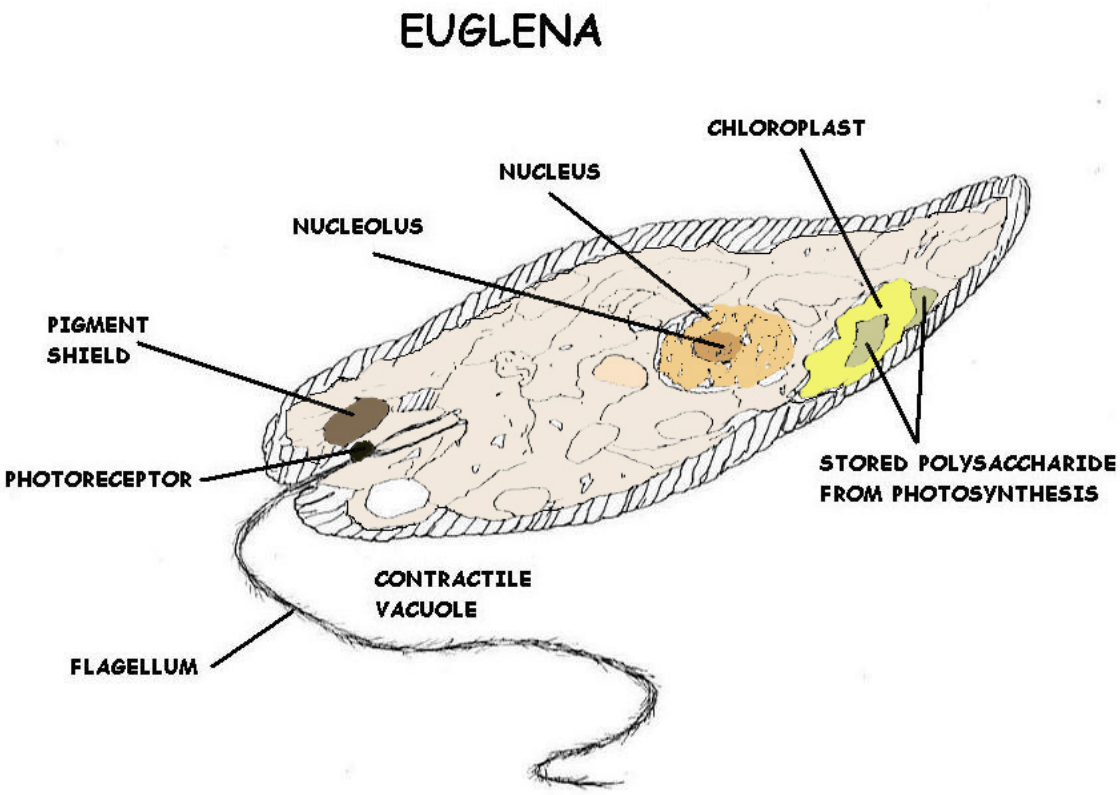
Sub-groups
Protozoa have traditionally been divided on the basis of their means of locomotion, although this is no longer believed to represent genuine relationships:
* Flagellates (e.g. Giardia lambia)
* Amoeboids (e.g. Entamoeba histolytica)
* Sporozoans (e.g. Plasmodium knowlesi)
* Apicomplexa
* Myxozoa
* Microsporidia
* Ciliates (e.g. Balantidium coli)

There are many ways that infectious diseases can spread. Pathogens usually have specific routes by which they are transmitted, and these routes may depend on the type of cells and tissue that a particular agent targets. For example, because cold viruses infect the respiratory tract, they are dispersed into the air via coughing and sneezing.

Once in the air, the viruses can infect another person who is unlucky enough to inhale air containing the virus particles.

Agents vary greatly in their stability in the environment. Some viruses may survive for only a few minutes outside of a host, while some spore-forming bacteria are extremely durable and may survive in a dormant state for a decade or more.

Protozoa Section



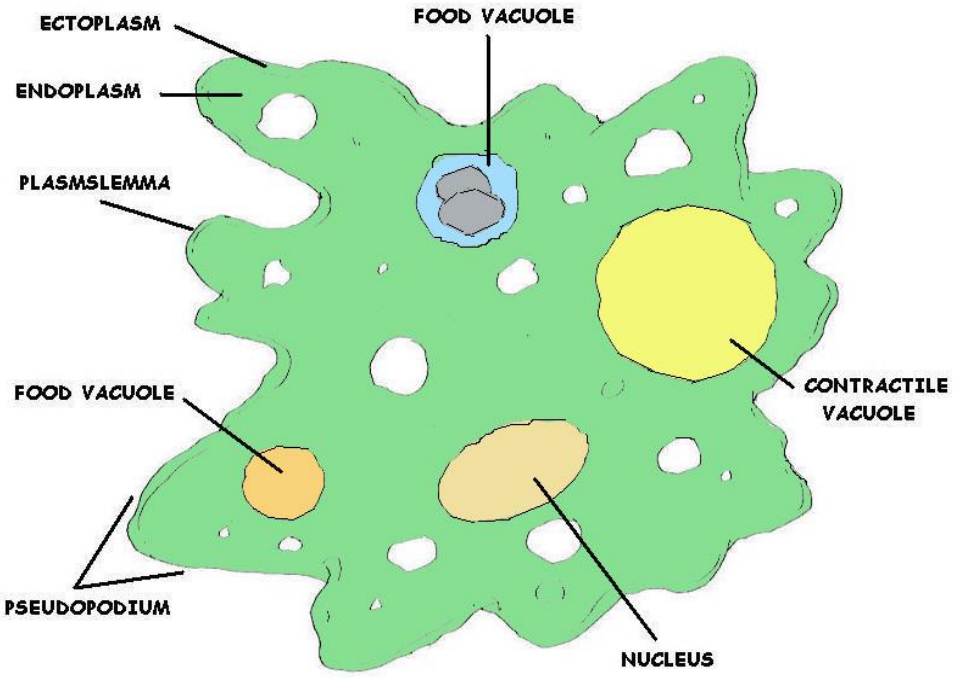
The diverse assemblage of organisms that carry out all of their life functions within the confines of a single, complex eukaryotic cell are called protozoa.

Paramecium, Euglena, and Amoeba are well-known examples of these major groups of organisms. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. The unicellular photosynthetic protozoa are sometimes also called algae and are addressed elsewhere. This report considers the status of our knowledge of heterotrophic protozoa (protozoa that cannot produce their own food).

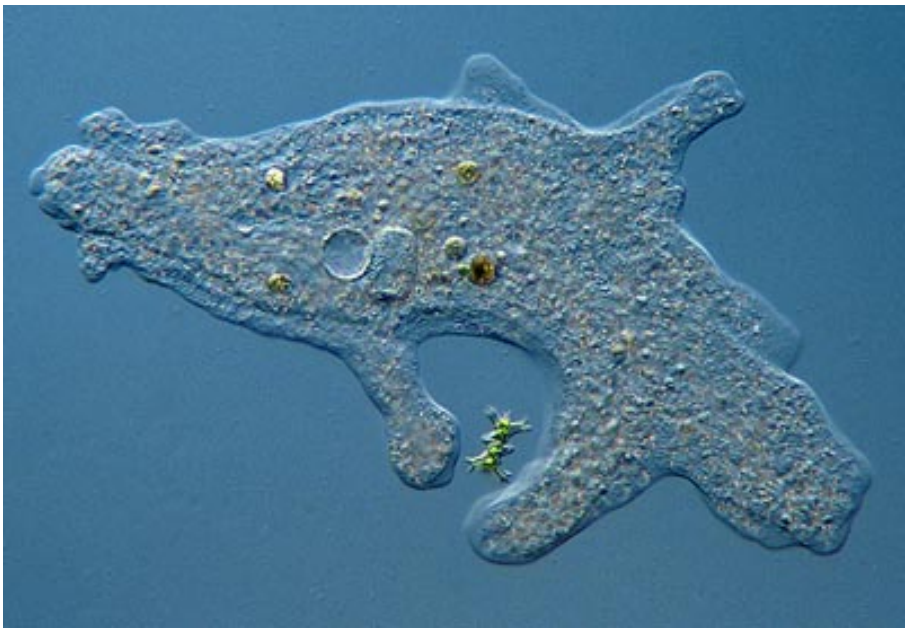
Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.



AMOEBA



Amoeba proteus*, pseudopods slowly engulf the small desmid *Staurastrum.

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey, they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information
Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa
Parasites
Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease
The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following pages.

Symbionts
Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa
While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great.

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).

Contractile Vacuoles
Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure. Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short. These are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes, and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles
Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

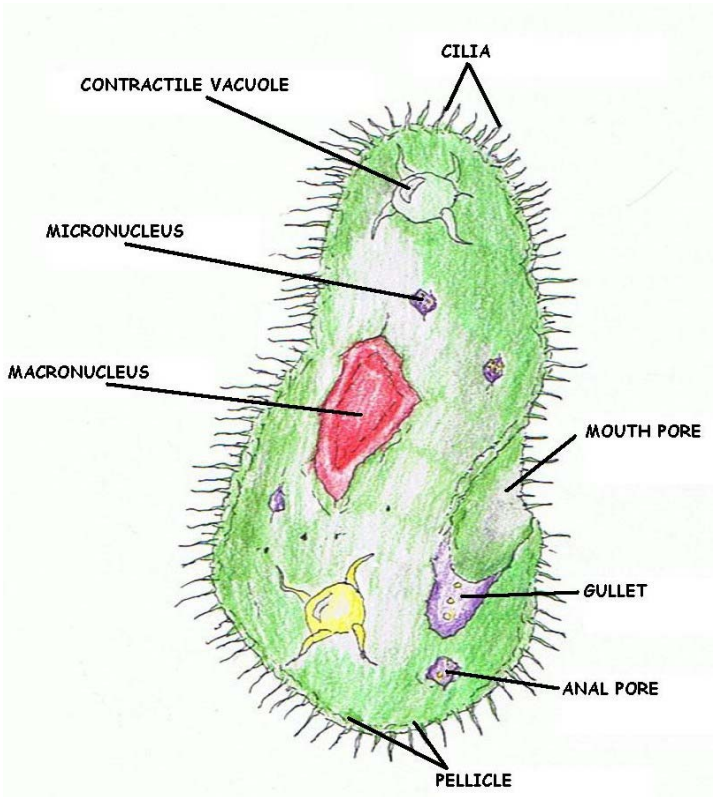
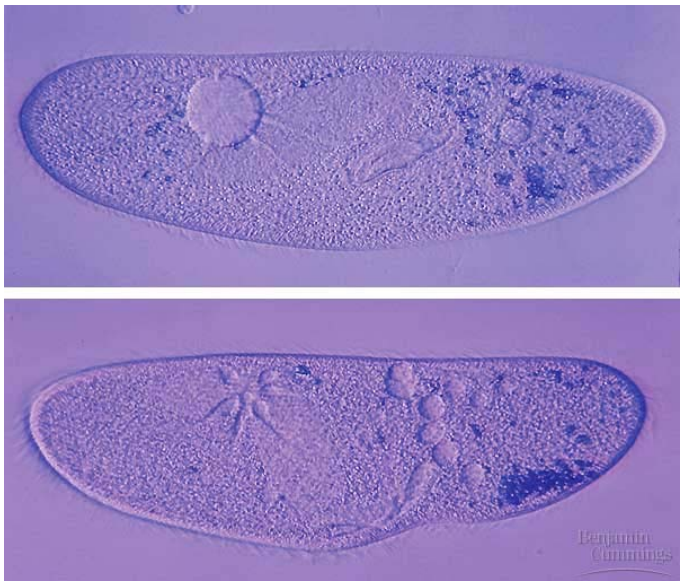


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.



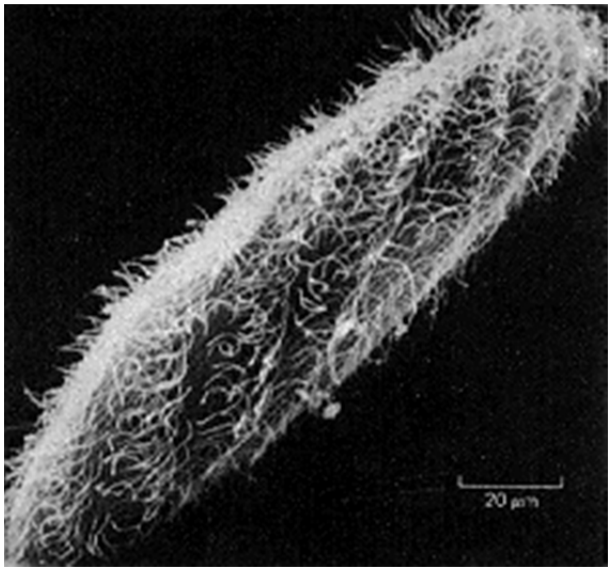
Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration.

If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).





Here is a wastewater Inspector is utilizing the auto sampler's manual to help adjust the time and adjust for the correct flow for a composite sample. You can see that she is pouring off the pickle jar. This inspector is also the course author.

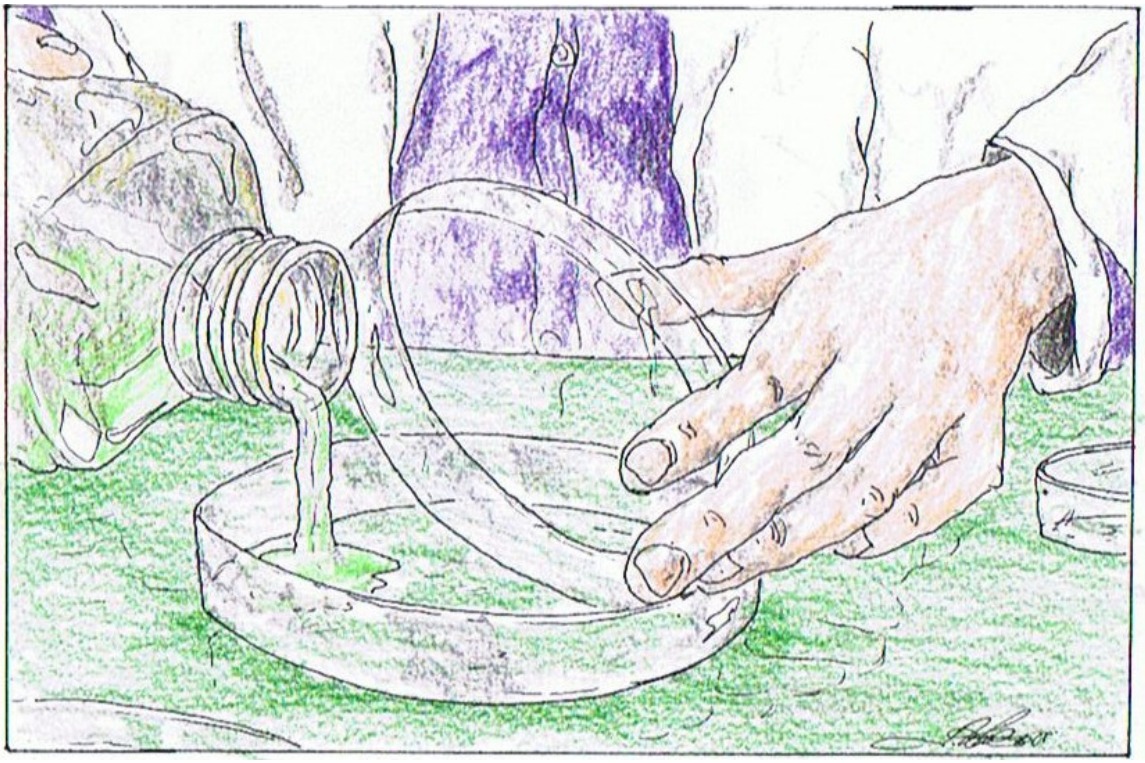
Protozoan Diseases

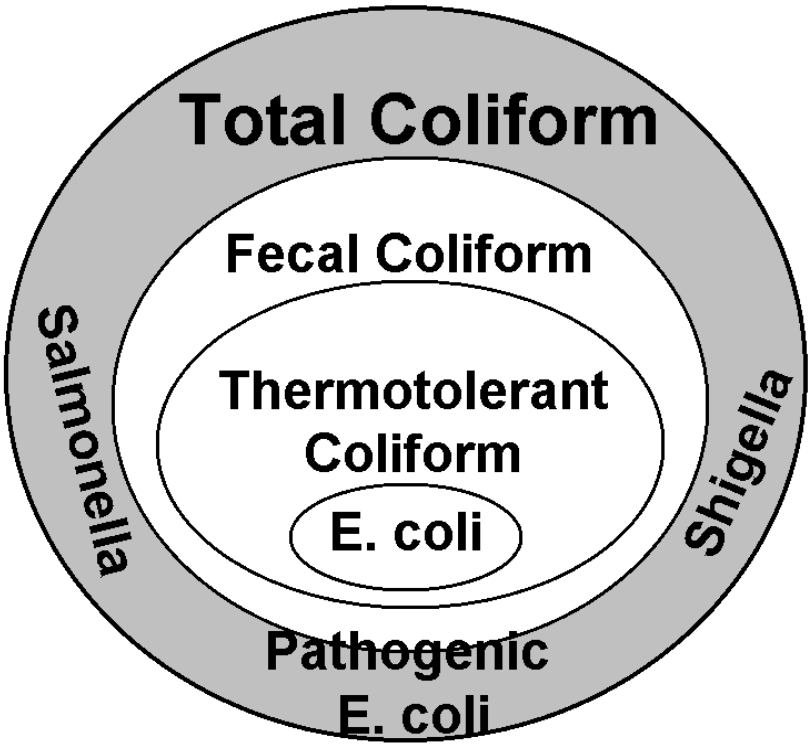
Protozoan pathogens are larger than bacteria and viruses, but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall called a "***cyst***." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days. Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

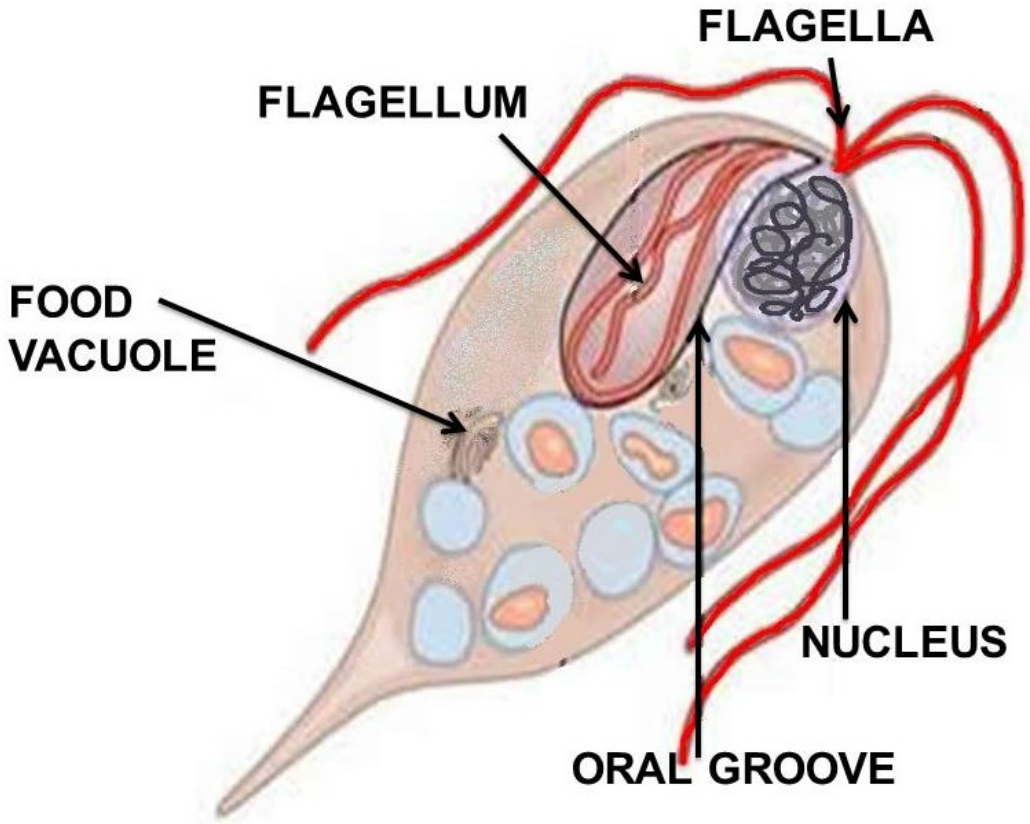
Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.




Total Coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Shigella
Pathogenic E. coli

Giardia Lamblia



GIARDIA LAMBLIA

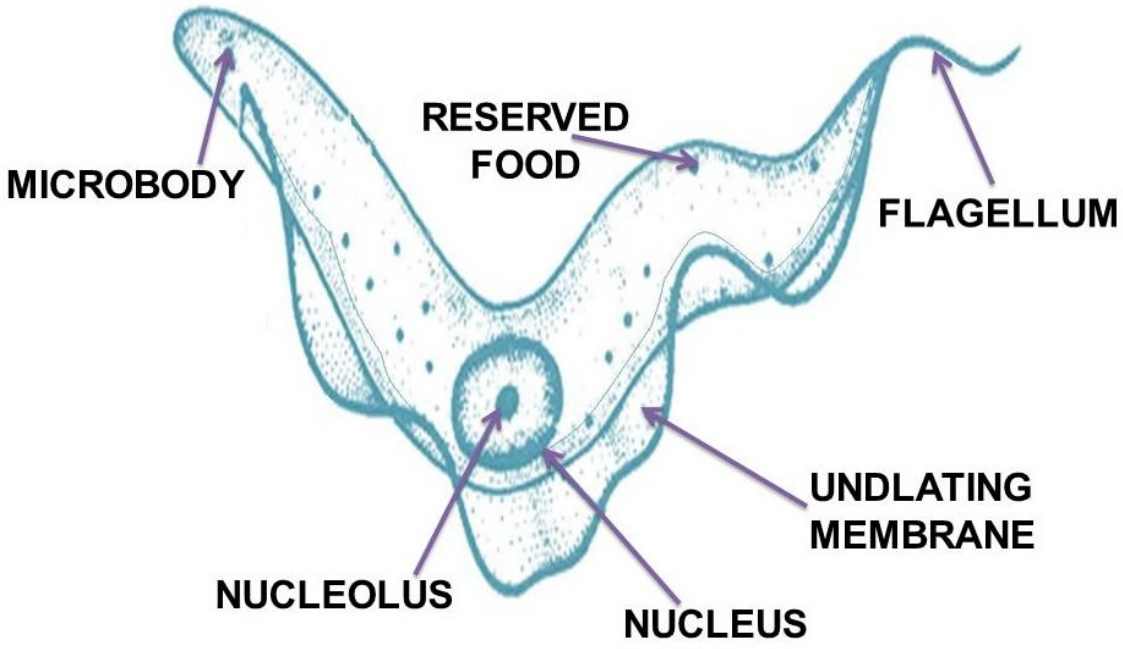
Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

Giardia infection can occur through ingestion of dormant cysts in contaminated water, or by the fecal-oral route (through poor hygiene practices). The Giardia cyst can survive for weeks to months in cold water and therefore can be present in contaminated wells and water systems, and even clean-looking mountain streams, as well as city reservoirs, as the Giardia cysts are resistant to conventional water treatment methods, such as chlorination and ozonolysis. Zoonotic transmission is also possible, and therefore Giardia infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes, especially the artificial lakes formed by beaver dams (hence the popular name for giardiasis, "Beaver Fever"). As well as water-borne sources, fecal-oral transmission can also occur, for example in day care centers, where children may have poorer hygiene practices.

Those who work with children are also at risk of being infected, as are family members of infected individuals. Not all Giardia infections are symptomatic, so some people can unknowingly serve as carriers of the parasite.

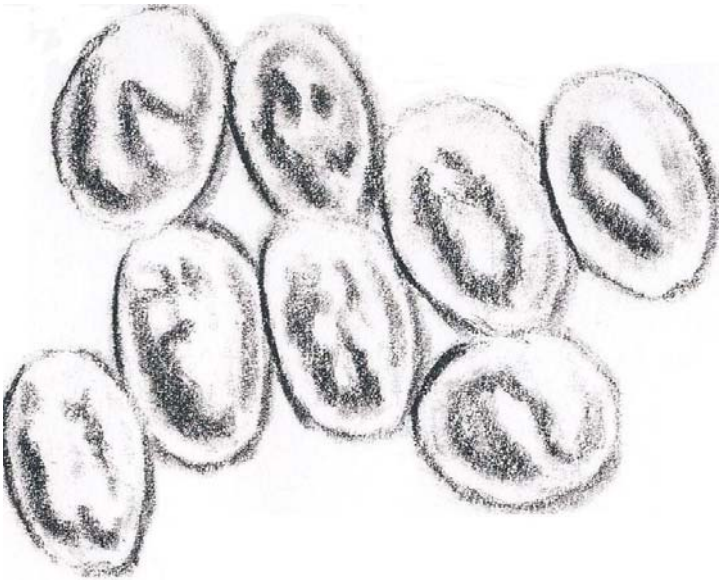
The life cycle begins with a non-infective cyst being excreted with feces of an infected individual. Once out in the environment, the cyst becomes infective. A distinguishing characteristic of the cyst is 4 nuclei and a retracted cytoplasm. Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the feces. While the trophozoites may be found in the feces, only the cysts are capable of surviving outside of the host.

Distinguishing features of the trophozoites are large karyosomes and lack of peripheral chromatin, giving the two nuclei a halo appearance. Cysts are distinguished by a retracted cytoplasm. This protozoa lacks mitochondria, although the discovery of the presence of mitochodrial remnant organelles in one recent study "indicate that Giardia is not primitively amitochondrial and that it has retained a functional organelle derived from the original mitochondrial endosymbiont"



PROTOZOAN PARASITE

Cryptosporidium

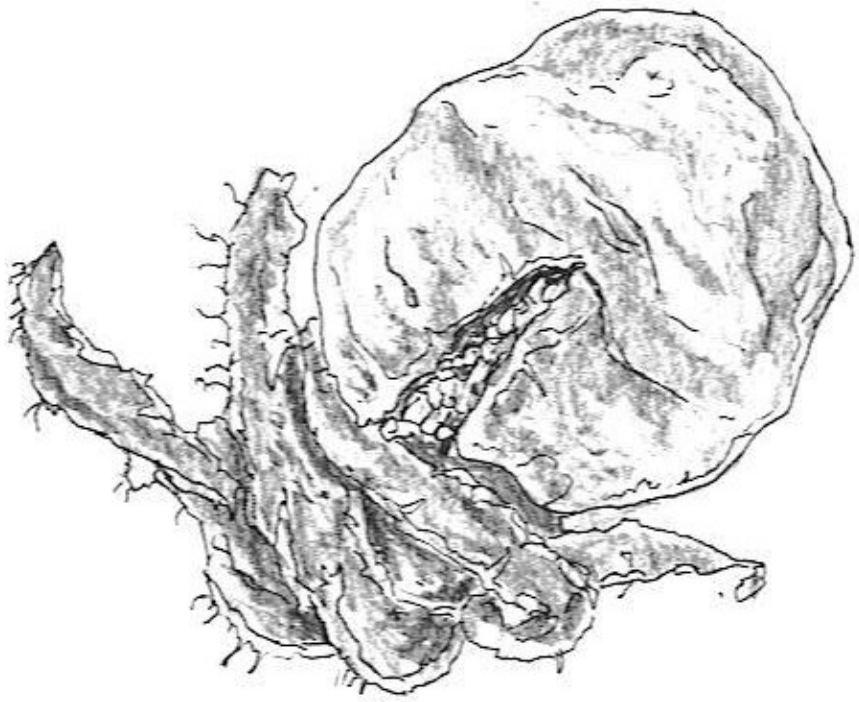


Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

A number of species of Cryptosporidium infect mammals. In humans, the main causes of disease are C. parvum and C. hominis (previously C. parvum genotype 1). C. canis, C. felis, C. meleagridis, and C. muris can also cause disease in humans. In recent years, cryptosporidiosis has plagued many commercial Leopard gecko breeders. Several species of the Cryptosporidium family (C. serpentes and others) are involved, and outside of geckos it has been found in monitor lizards, iguanas, tortoises as well as several snake species.

Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals. The parasite is transmitted by environmentally hardy cysts (oocysts) that, once ingested, excyst in the small intestine and result in an infection of intestinal epithelial tissue.

The genome of Cryptosporidium parvum was sequenced in 2004 and was found to be unusual amongst Eukaryotes in that the mitochondria seem not to contain DNA. A closely-related species, C. hominis, also has its genome sequence available. CryptoDB.org is a NIH-funded database that provides access to the Cryptosporidium genomics data sets.



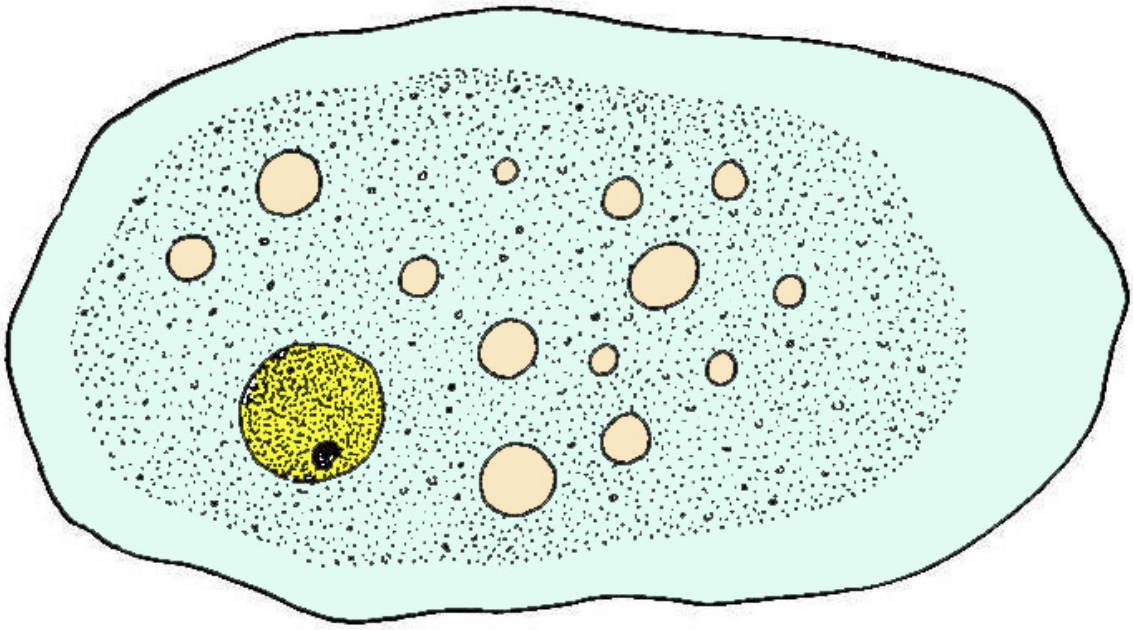
CRYPTO-PARVUM

When *C. parvum* was first identified as a human pathogen, diagnosis was made by a biopsy of intestinal tissue (Keusch, *et al*., 1995). However, this method of testing can give false negatives due the "patchy" nature of the intestinal parasitic infection (Flanigan and Soave, 1993). Staining methods were then developed to detect and identify the oocysts directly from stool samples. The modified acid-fast stain is traditionally used to most reliably and specifically detect the presence of cryptosporidial oocysts.

There have been six major outbreaks of cryptosporidiosis in the United States as a result of contamination of drinking water (Juranek, 1995). One major outbreak in Milwaukee in 1993 affected over 400,000 persons. Outbreaks such as these usually result from drinking water taken from surface water sources such as lakes and rivers (Juranek, 1995). Swimming pools and water park wave pools have also been associated with outbreaks of cryptosporidiosis. Also, untreated groundwater or well water public drinking water supplies can be sources of contamination.

The highly environmentally resistant cyst of *C. parvum* allows the pathogen to survive various drinking water filtrations and chemical treatments such as chlorination. Although municipal drinking water utilities may meet federal standards for safety and quality of drinking water, complete protection from cryptosporidial infection is not guaranteed. In fact, *all* waterborne outbreaks of cryptosporidiosis have occurred in communities where the local utilities met all state and federal drinking water standards (Juranek, 1995).

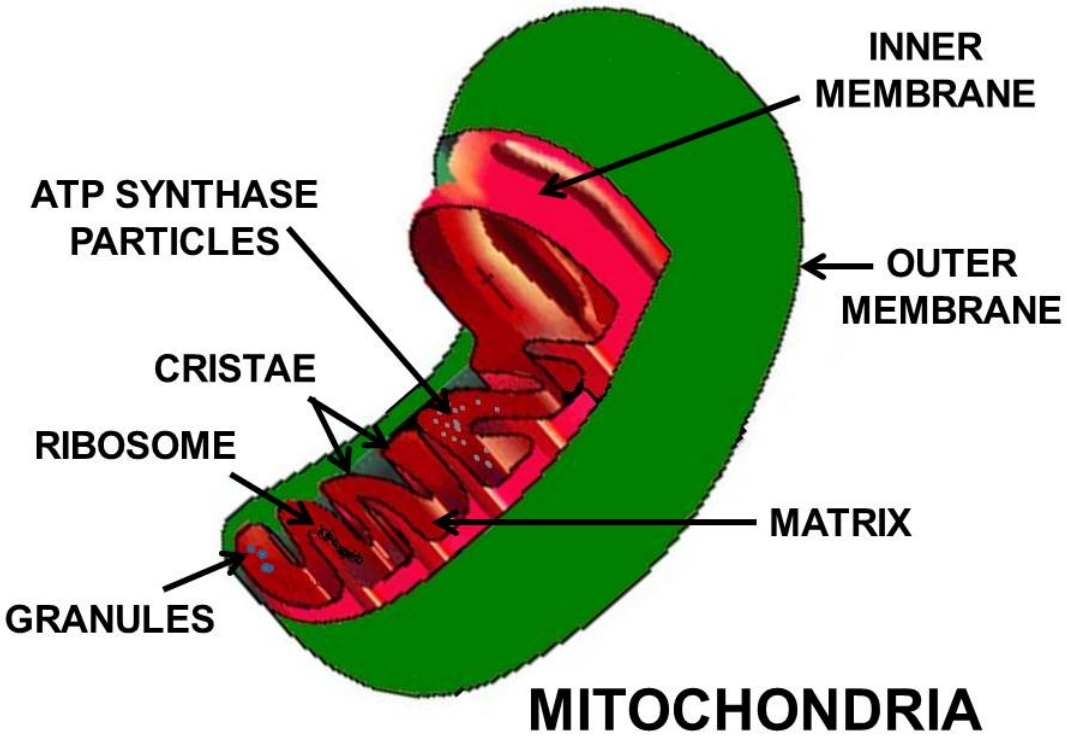
Entamoeba histolytica



Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

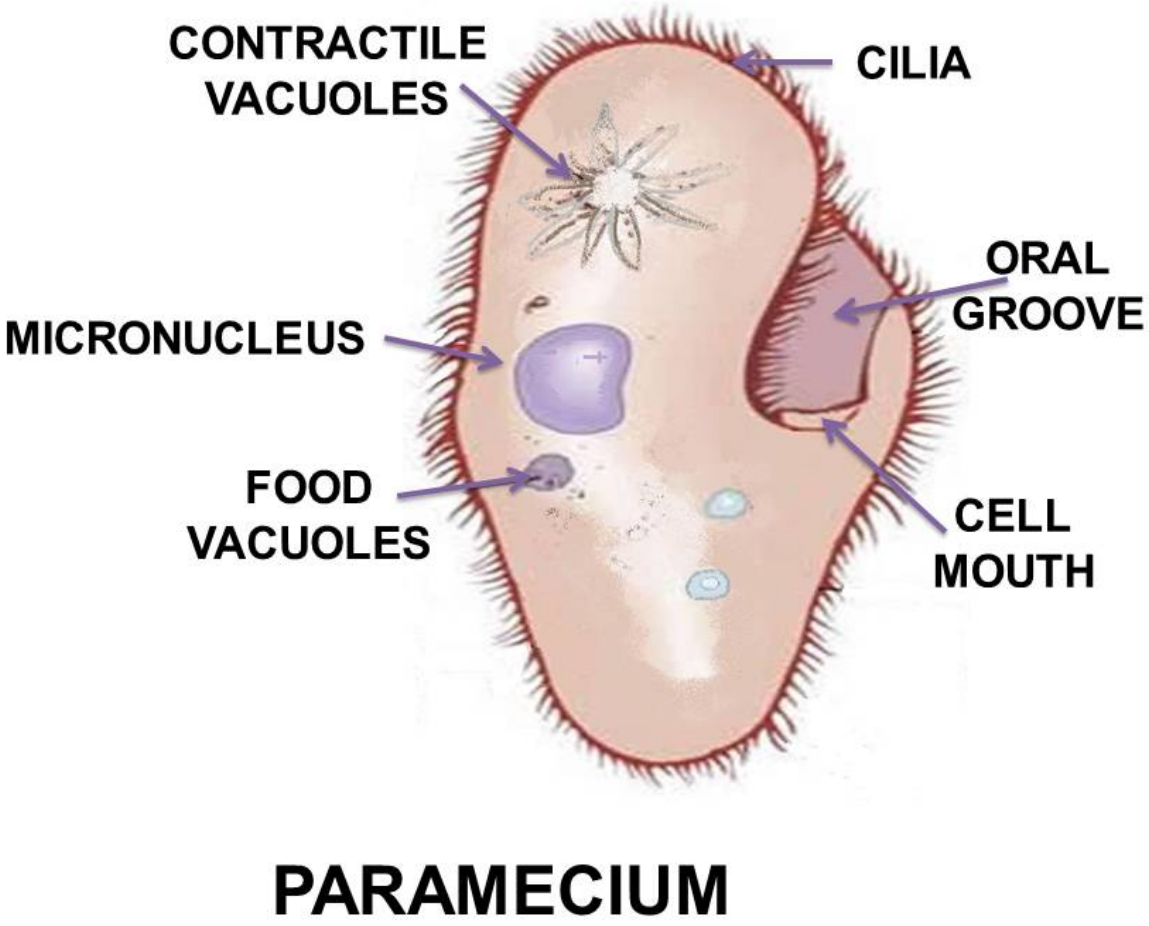
On average, about one in 10 people who are infected with *E. histolytica* becomes sick from the infection. The symptoms often are quite mild and can include loose stools, stomach pain, and stomach cramping. Amebic dysentery is a severe form of amebiasis associated with stomach pain, bloody stools, and fever. Rarely, *E. histolytica* invades the liver and forms an abscess. Even less commonly, it spreads to other parts of the body, such as the lungs or brain.

Scientific classification
Domain: Eukaryota
Phylum: Amoebozoa
Class: Archamoebae
Genus: Entamoeba
Species: E. histolytica



Mitochondria

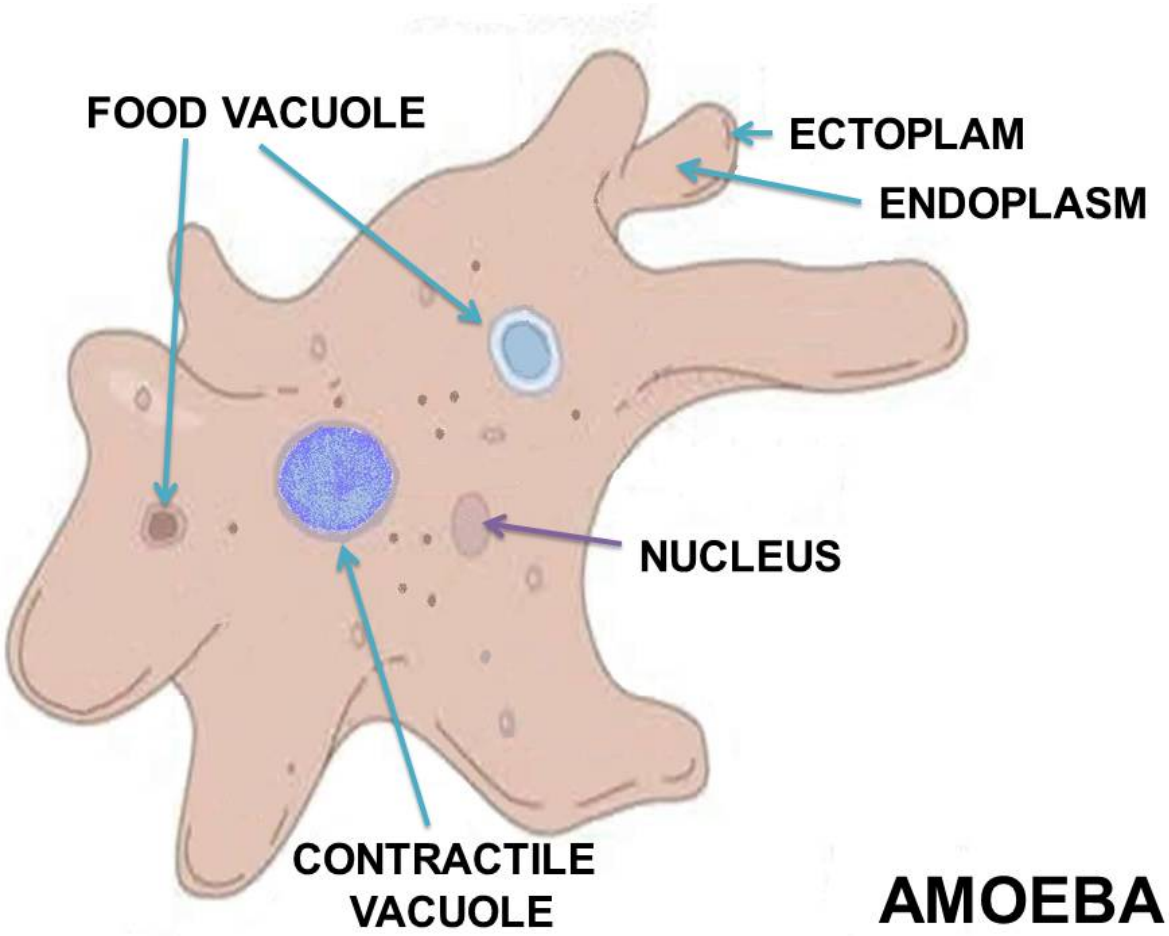
The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. As they are prokaryotes, bacteria do not tend to have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures. They consequently lack a nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells, such as the Golgi apparatus and endoplasmic reticulum.



PARAMECIUM

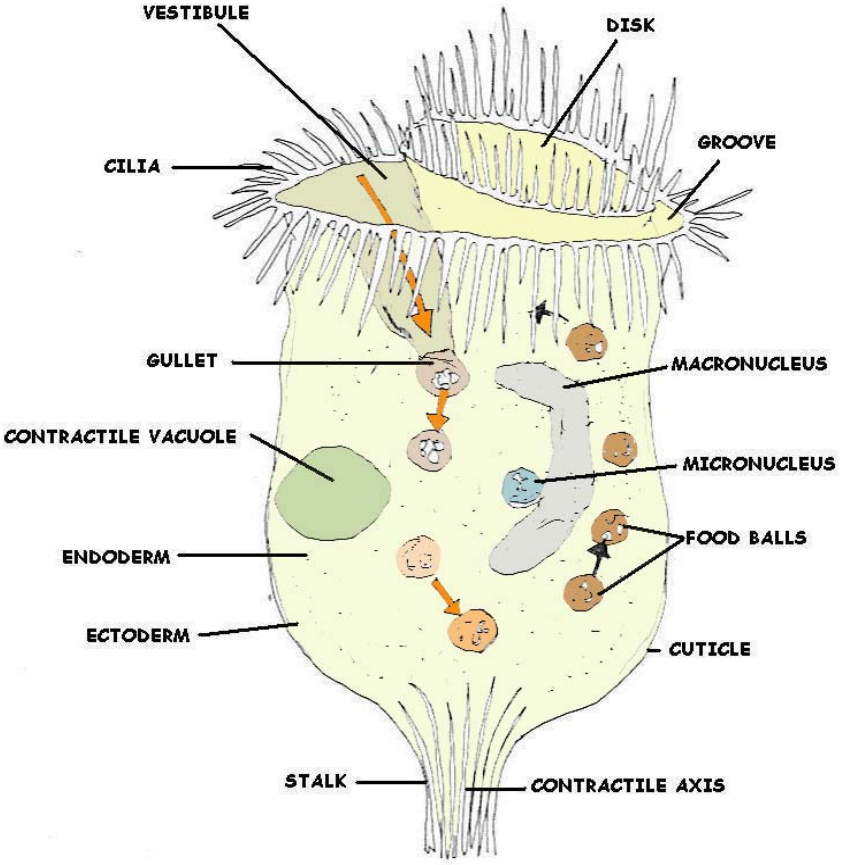
Paramecia

Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).



Amoeba

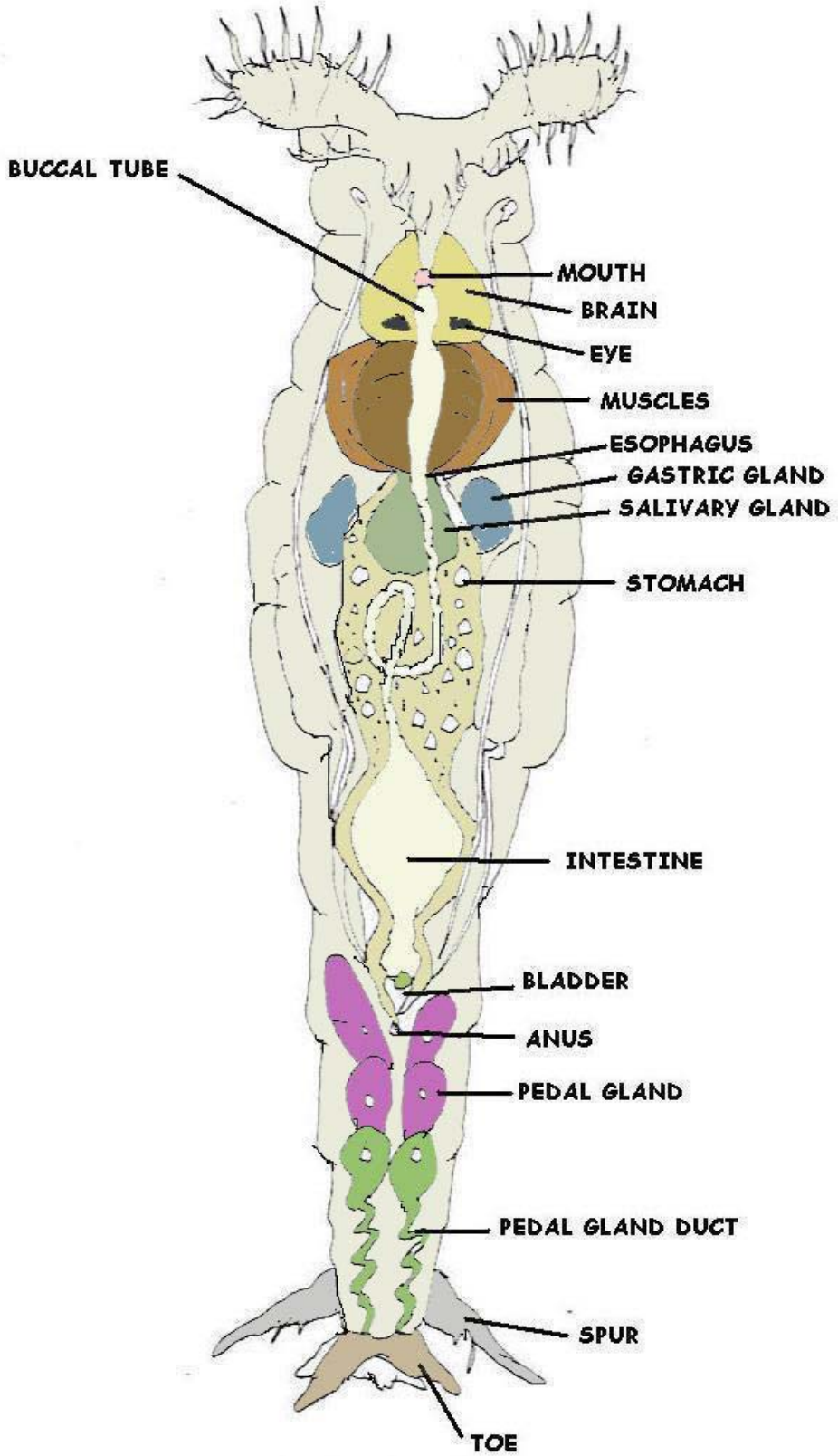
Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.



Vorticella

Vorticella is a genus of protozoa, with over 100 known species. They are stalked inverted bell-shaped ciliates, placed among the peritrichs. Each cell has a separate stalk anchored onto the substrate, which contains a contracile fibril called a myoneme. When stimulated this shortens, causing the stalk to coil like a spring. Reproduction is by budding, where the cell undergoes longitudinal fission and only one daughter keeps the stalk. Vorticella mainly lives in freshwater ponds and streams - generally anywhere protists are plentiful. Other genera such as Carchesium resemble Vorticella but are branched or colonial.

Domain: Eukaryota
Phylum: Ciliophora
Class: Oligohymenophorea
Subclass: Peritrichia
Order: Sessilida
Family: Vorticellidae
Genus: Vorticella


BUCCAL TUBE
MOUTH
BRAIN
EYE
MUSCLES
ESOPHAGUS
GASTRIC GLAND
SALIVARY GLAND
STOMACH
INTESTINE
BLADDER
ANUS
PEDAL GLAND
PEDAL GLAND DUCT
SPUR
TOE

ROTIFER

Rotifer

The rotifers make up a phylum of microscopic and near-microscopic pseudocoelomate animals. They were first described by John Harris in 1696 (Hudson and Gosse, 1886). Leeuwenhoek is mistakenly given credit for being the first to describe rotifers but Harris had produced sketches in 1703. Most rotifers are around 0.1-0.5 mm long, and are common in freshwater throughout the world with a few saltwater species. Rotifers may be free swimming and truly planktonic, others move by inch worming along the substrate, whilst some are sessile, living inside tubes or gelatinous holdfasts. About 25 species are colonial (e.g. Sinantherina semibullata), either sessile or planktonic.

Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

Like many other microscopic animals, adult rotifers frequently exhibit eutely - they have a fixed number of cells within a species, usually on the order of one thousand. Males in the class Monogononta may be either present or absent depending on the species and environmental conditions. In the absence of males, reproduction is by parthenogenesis and results in clonal offspring that are genetically identical to the parent. Individuals of some species form two distinct types of parthenogenetic eggs; one type develops into a normal parthenogenetic female, while the other occurs in response to a changed environment and develops into a degenerate male that lacks a digestive system, but does have a complete male reproductive system that is used to inseminate females thereby producing fertilized 'resting eggs'. Resting eggs develop into zygotes that are able to survive extreme environmental conditions such as may occur during winter or when the pond dries up. These eggs resume development and produce a new female generation when conditions improve again. The life span of monogonont females varies from a couple of days to about three weeks.

Bdelloid rotifers are unable to produce resting eggs, but many can survive prolonged periods of adverse conditions after desiccation. This facility is termed anhydrobiosis, and organisms with these capabilities are termed anhydrobionts. Under drought conditions, bdelloid rotifers contract into an inert form and lose almost all body water; when rehydrated, however, they resume activity within a few hours. Bdelloids can survive the dry state for prolonged periods, with the longest well-documented dormancy being nine years. While in other anhydrobionts, such as the brine shrimp, this desiccation tolerance is thought to be linked to the production of trehalose, a non-reducing disaccharide (sugar), bdelloids apparently lack the ability to synthesize trehalose. Bdelloid rotifer genomes contain two or more divergent copies of each gene. Four copies of hsp82 are, for example, found. Each is different and found on a different chromosome, excluding the possibility of homozygous sexual reproduction.

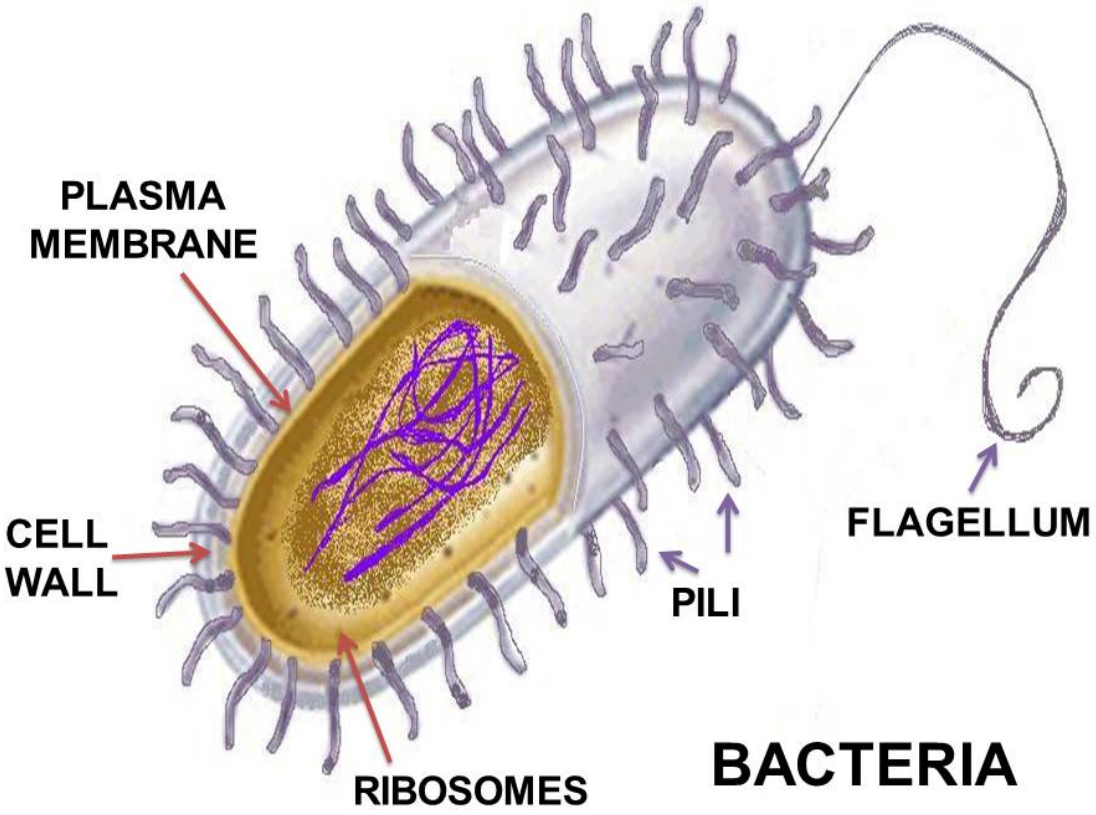
Waterborne Diseases

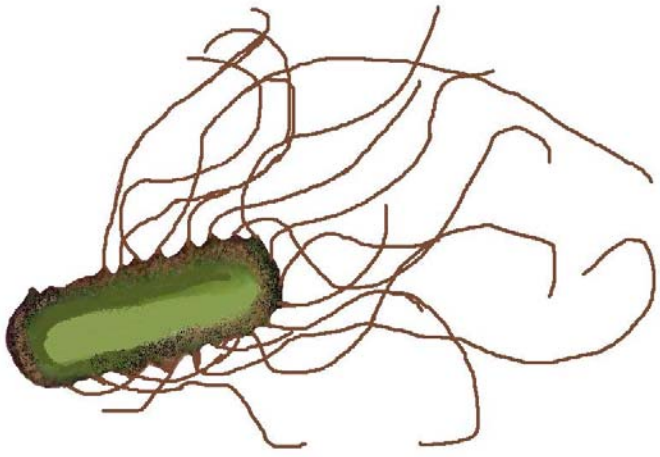
Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) [*1]	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:

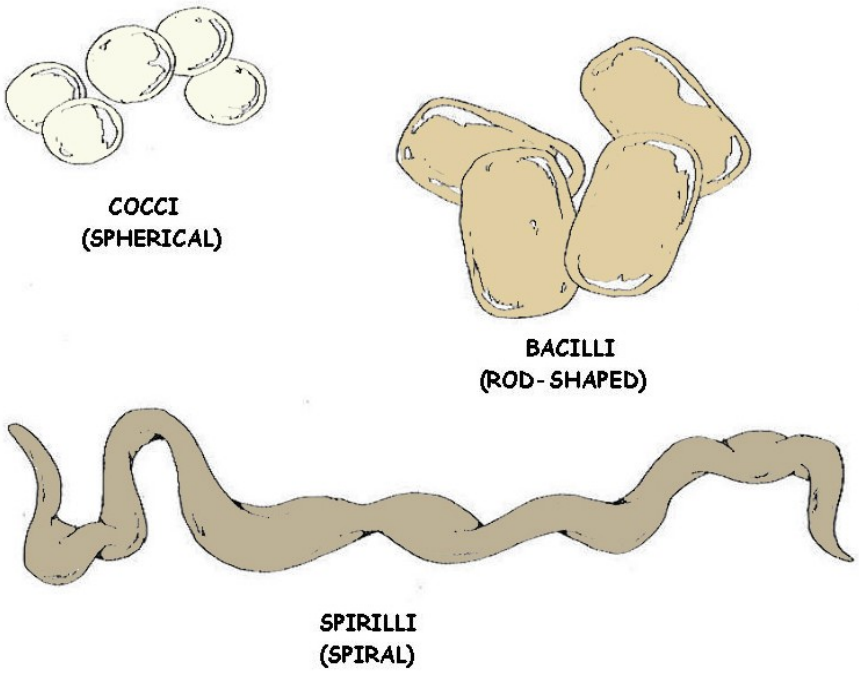
*1 http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm

Bacteria Section





Peritrichous Bacteria



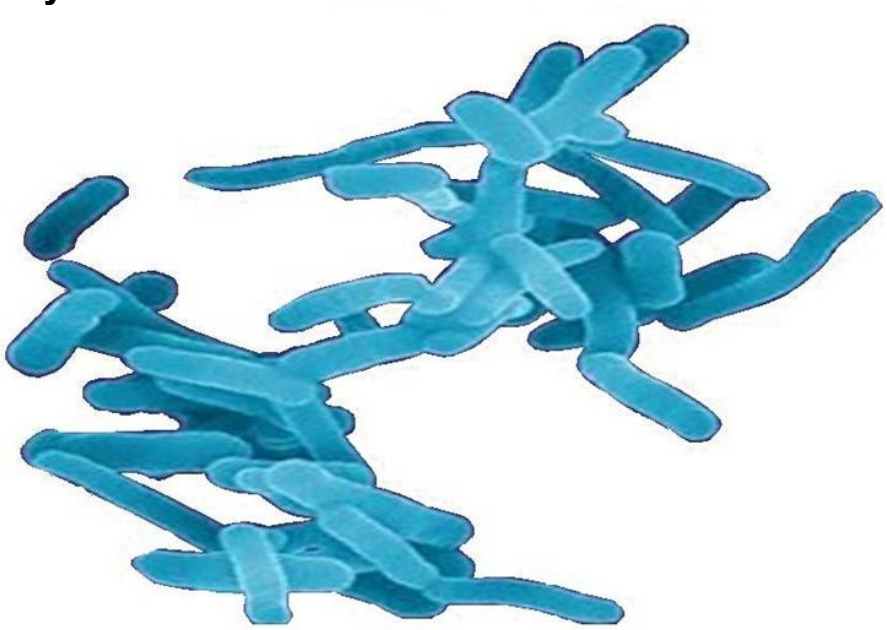
BACTERIA TYPES

Microbiologists broadly classify bacteria according to their shape: spherical, rod-shaped, and spiral-shaped. Pleomorphic bacteria can assume a variety of shapes. Bacteria may be further classified according to whether they require oxygen (aerobic or anaerobic) and how they react to a test with Gram's stain. Bacteria in which alcohol washes away Gram's stain are called gram-negative, while bacteria in which alcohol causes the bacteria's walls to absorb the stain are called gram-positive.



COLORLESS FILAMENTOUS
SULFUR BACTERIA

Shigella dysenteriae



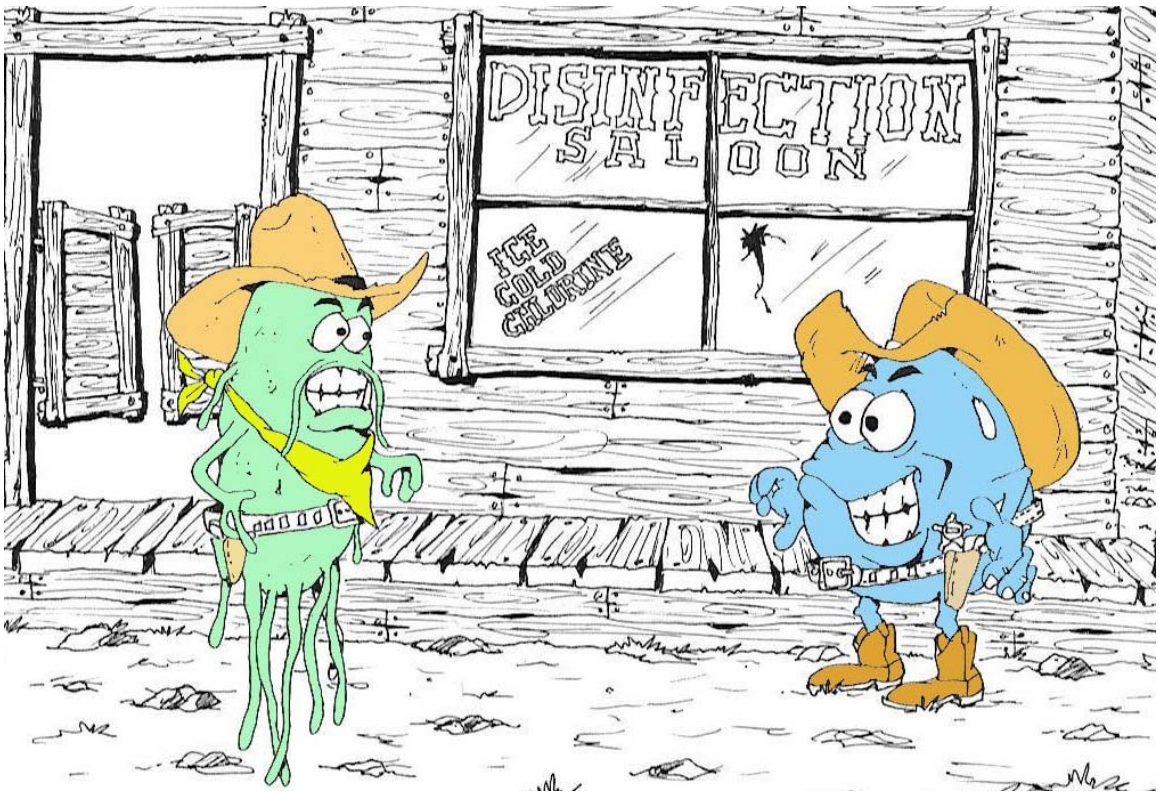
SHIGELLA DYSENTERIAE

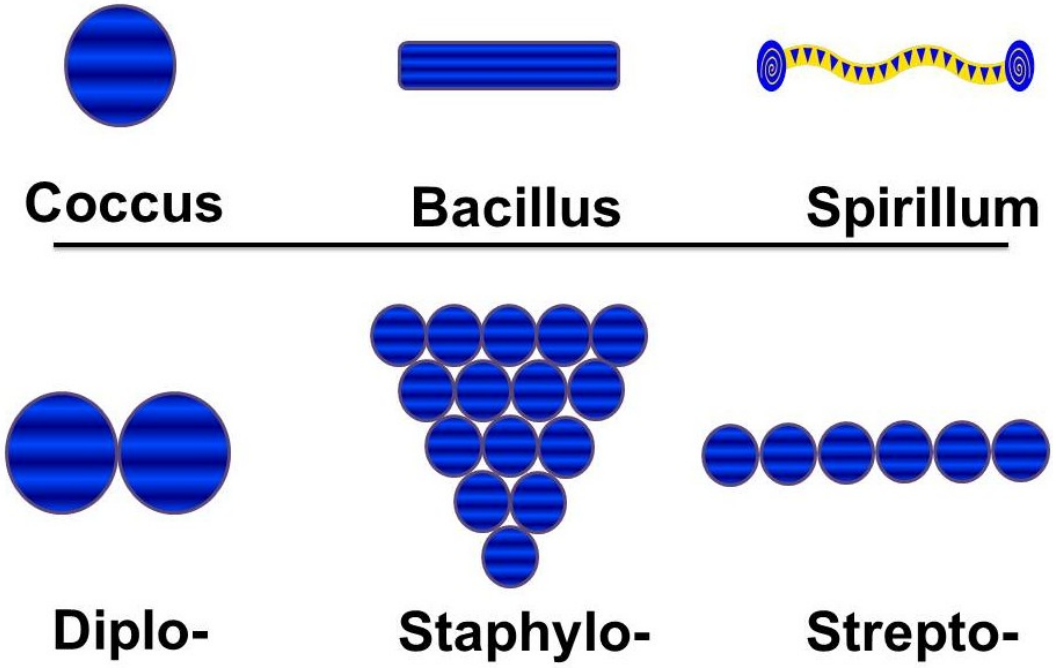
Shigella dysenteriae is a species of the rod-shaped bacterial genus Shigella. Shigella can cause shigellosis (bacillary dysentery). Shigellae are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.

S. dysenteriae, spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents. Shigella infection is typically via ingestion (fecal–oral contamination); depending on age and condition of the host as few as ten bacterial cells can be enough to cause an infection. Shigella causes dysentery that result in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. Some strains produce enterotoxin and Shiga toxin, similar to the verotoxin of E. coli O157:H7. Both Shiga toxin and verotoxin are associated with causing hemolytic uremic syndrome.

Shigella invades the host through epithelial cells of the large intestine. Using a Type III secretion system acting as a biological syringe, the bacterium injects IpaD protein into cell, triggering bacterial invasion and the subsequent lysis of vacuolar membranes using IpaB and IpaC proteins. It utilizes a mechanism for its motility by which its IcsA protein triggers actin polymerization in the host cell (via N-WASP recruitment of Arp2/3 complexes) in a "rocket" propulsion fashion for cell-to-cell spread.

The most common symptoms are diarrhea, fever, nausea, vomiting, stomach cramps, and straining to have a bowel movement. The stool may contain blood, mucus, or pus (e.g. dysentery). In rare cases, young children may have seizures. Symptoms can take as long as a week to show up, but most often begin two to four days after ingestion. Symptoms usually last for several days, but can last for weeks. Shigella is implicated as one of the pathogenic causes of reactive arthritis worldwide.

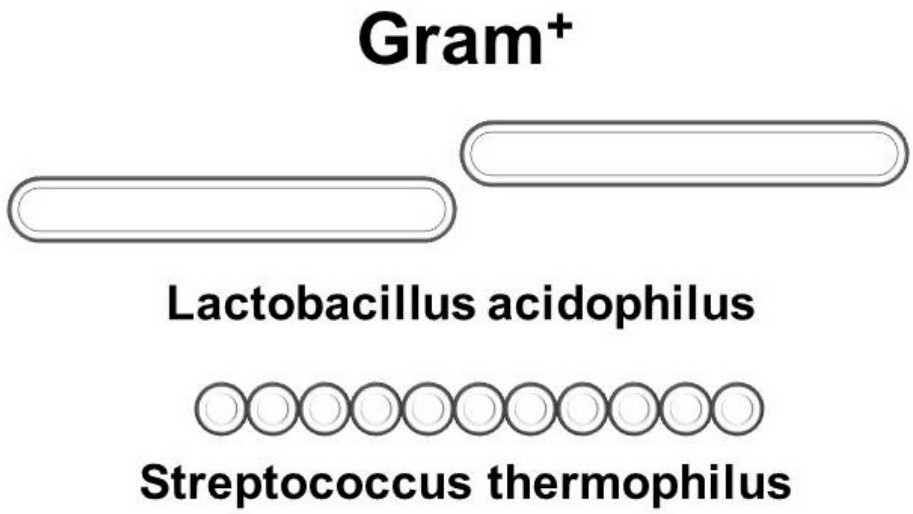


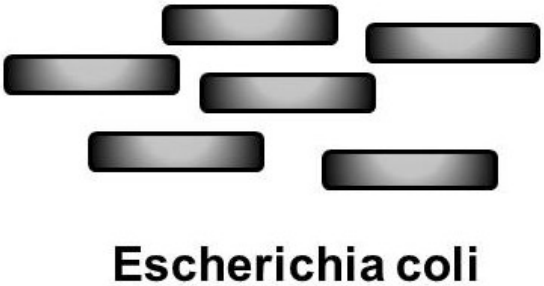


Bacteria Types

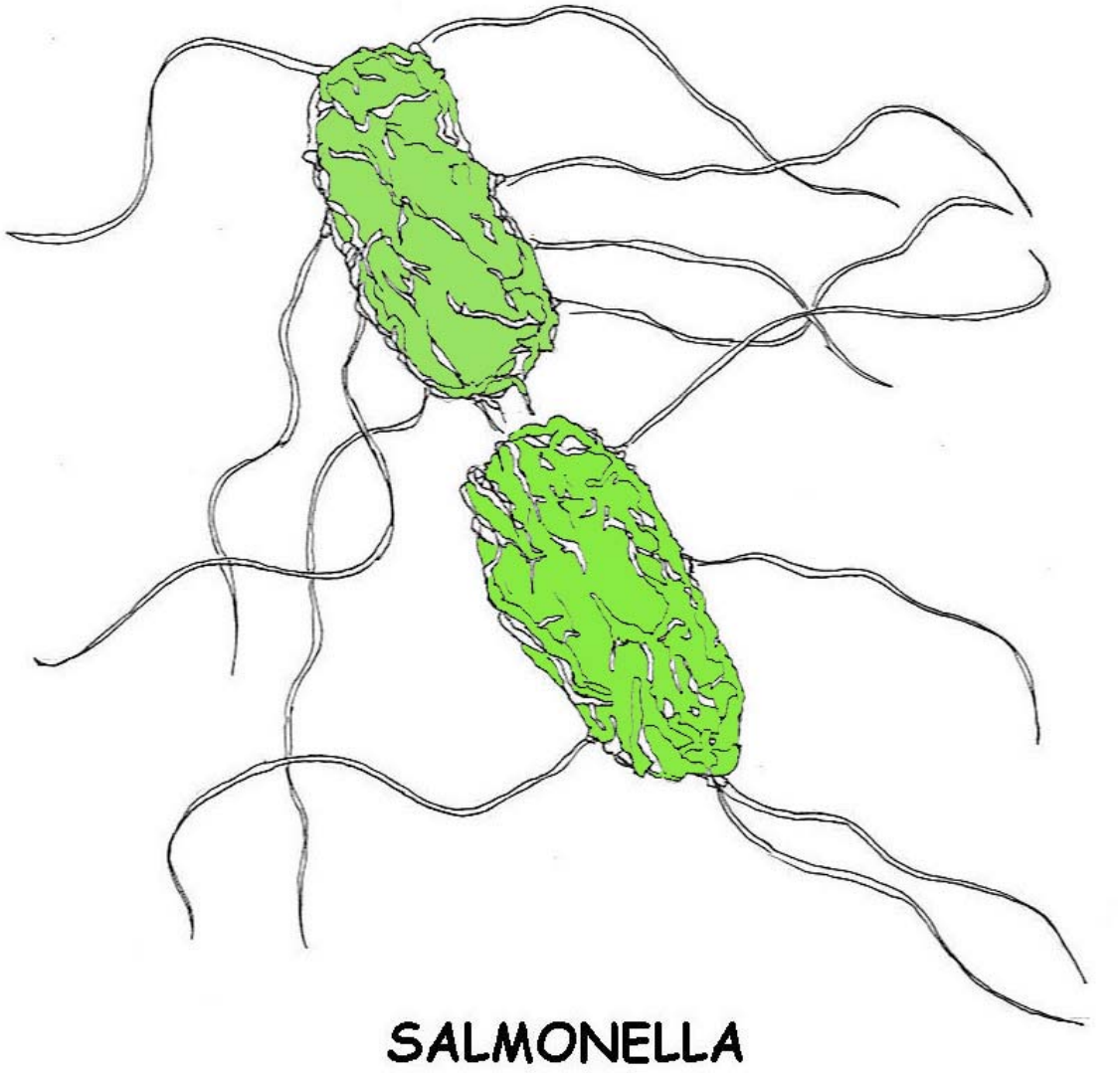
Type	Characteristics
Acetic acid	Rod-shaped, gram-negative, aerobic; highly tolerant of acidic conditions; generate organic acids
Actinomycete	Rod-shaped or filamentous, gram-positive, aerobic; common in soils; essential to growth of many plants; source of much of original antibiotic production in pharmaceutical industry
Coccoid	Spherical, sometimes in clusters or strings, gram-positive, aerobic and anaerobic; resistant to drying and high-salt conditions; *Staphylococcus* species common on human skin, certain strains associated with toxic shock syndrome
Coryneform	Rod-shaped, form club or V shapes, gram-positive, aerobic; found in wide variety of habitats, particularly soils; highly resistant to drying; include *Arthrobacter,* among most common forms of life on earth
Endospore-forming	Usually rod-shaped, can be gram-positive or gram-negative; have highly adaptable, heat-resistant spores that can go dormant for long periods, possibly thousands of years; include *Clostridium* (anaerobic) and *Bacillus* (aerobic)
Enteric	Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include *Escherichia coli, Salmonella* (over 1000 types), and *Shigella*
Gliding	Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures
Lactic acid	Gram-positive, anaerobic; produce lactic acid through fermentation; include *Lactobacillus*, essential in dairy product formation, and *Streptococcus*, common in humans
Mycobacterium	Pleomorphic, spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include *Mycobacterium tuberculosis,* cause of tuberculosis
Mycoplasma	Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (*myco-* means 'fungus')
Nitrogen-fixing	Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include *Azotobacter,* a common genus
Propionic acid	Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide
Pseudomonad	Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy
Rickettsia	Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to *Agrobacterium,* a common gall-causing plant bacterium
Sheathed	Filamentous, gram-negative, aerobic; 'swarmer' (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment

Spirillum	Spiral-shaped, gram-negative, aerobic; include *Bdellovibrio,* predatory on other bacteria
Spirochete	Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis
Sulfate- and Sulfur-reducing	Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes
Sulfur- and iron-oxidizing	Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment
Vibrio	Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids





Salmonella



Salmonella is a Gram-negative bacterium. It is found in many turtles and other reptiles. In clinical laboratories, it is usually isolated on MacConkey agar, XLD agar, XLT agar, DCA agar, or Önöz agar. Because they cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium, so use of a relatively non-selective medium such as CLED agar is not often practiced. Numbers of salmonella may be so low in clinical samples that stools are routinely also subjected to "enrichment culture", where a small volume of stool is incubated in a selective broth medium, such as selenite broth or Rappaport Vassiliadis soya peptone broth, overnight. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing salmonellae to become enriched in numbers. Salmonellae may then be recovered by inoculating the enrichment broth on one or more of the primary selective media. On blood agar, they form moist colonies about 2 to 3 mm in diameter.

When the cells are grown for a prolonged time at a range of 25—28°C, some strains produce a biofilm, which is a matrix of complex carbohydrates, cellulose and proteins. The ability to produce biofilm (a.k.a. "rugose", "lacy", or "wrinkled") can be an indicator of dimorphism, which is the ability of a single genome to produce multiple phenotypes in response to environmental conditions. Salmonellae usually do not ferment lactose; most of them produce hydrogen sulfide which, in media containing ferric ammonium citrate, reacts to form a black spot in the centre of the creamy colonies.

Classification

Salmonella taxonomy is complicated. As of December 7, 2005, there are two species within the genus: S. bongori (previously subspecies V) and S. enterica (formerly called S. choleraesuis), which is divided into six subspecies:

* I—enterica
* II—salamae
* IIIa—arizonae
* IIIb—diarizonae
* IV—houtenae
* V—obsolete (now designated S. bongori)
* VI—indica



There are also numerous (over 2500) serovars within both species, which are found in a disparate variety of environments and which are associated with many different diseases. The vast majority of human isolates (>99.5%) are subspecies S. enterica. For the sake of simplicity, the CDC recommends that Salmonella species be referred to only by their genus and serovar, e.g.

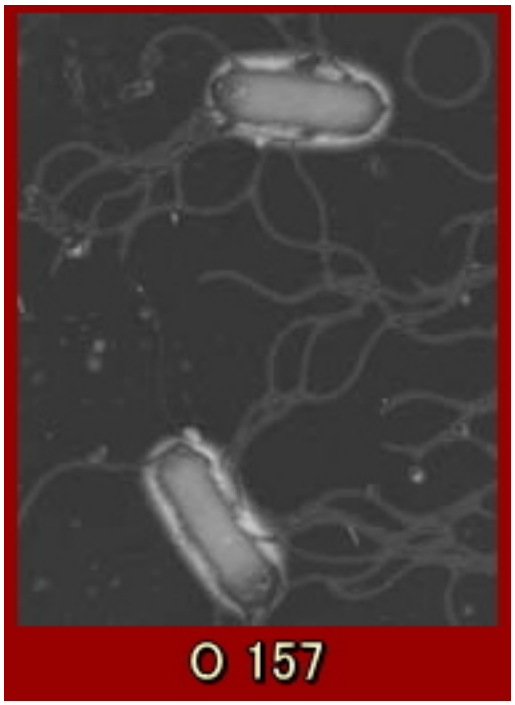
Salmonella Typhi instead of the more technically correct designation, Salmonella enterica subspecies enterica serovar Typhi.

Escherichia Coli Section

Fecal Coliform Bacteria
Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.

Reasons for Natural Variation
Unlike the other conventional water quality parameters, fecal coliform bacteria are living organisms. They do not simply mix with the water and float straight downstream. Instead they multiply quickly when conditions are favorable for growth, or die in large numbers when conditions are not. Because bacterial concentrations are dependent on specific conditions for growth, and these conditions change quickly, fecal coliform bacteria counts are not easy to predict. For example, although winter rains may wash more fecal matter from urban areas into a stream, cool water temperatures may cause a major die-off. Exposure to sunlight (with its ultraviolet disinfection properties) may have the same effect, even in the warmer water of summertime.

Expected Impact of Pollution
The primary sources of fecal coliform bacteria to fresh water are wastewater treatment plant discharges, failing septic systems, and animal waste. Bacteria levels do not necessarily decrease as a watershed develops from rural to urban. Instead, urbanization usually generates new sources of bacteria. Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in fecal coliform bacteria concentrations.



The presence of old, disintegrating storm and sanitary sewers, misplaced sewer pipes, and good breeding conditions are common explanations for the high levels measured.

Coliform Standards (in colonies/100ml)
Drinking water.....1FC
Total body contact (swimming).....200FC
Partial body contact (boating).....1000FC
Threatened sewage effluent .....not to exceed 200 FC

*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

Indicator Connection Varies
General coliforms, E. Coli, and Enterococcus bacteria are the "indicator" organisms generally measured to assess microbiological quality of water. However, these aren't generally what get people sick. Other bacteria, viruses, and parasites are what we are actually worried about.

Because it is so much more expensive and tedious to do so, actual pathogens are virtually never tested for. Over the course of a professional lifetime pouring over indicator tests, in a context where all standards are based on indicators, water workers tend to forget that the indicators are not the things we actually care about.

What are these indicators? More information in the Laboratory section.

- **General coliforms** indicate that the water has come in contact with plant or animal life. General coliforms are universally present, including in pristine spring water. They are of little concern at low levels, except to indicate the effectiveness of disinfection. Chlorinated water and water from perfectly sealed tube wells is the only water I've tested which had zero general coliforms. At very high levels they indicate there is what amounts to a lot of compost in the water, which could easily include pathogens (Ten thousand general coliform bacteria will get you a beach closure, compared to two or four hundred fecal coliforms, or fifty enterococcus).
- **Fecal coliforms**, particularly E. coli, indicate that there are mammal or bird feces in the water.
- **Enterococcus bacteria** also indicate that there are feces from warm blooded animals in the water. Enterococcus are a type of fecal streptococci. They are another valuable indicator for determining the amount of fecal contamination of water.
  According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater.

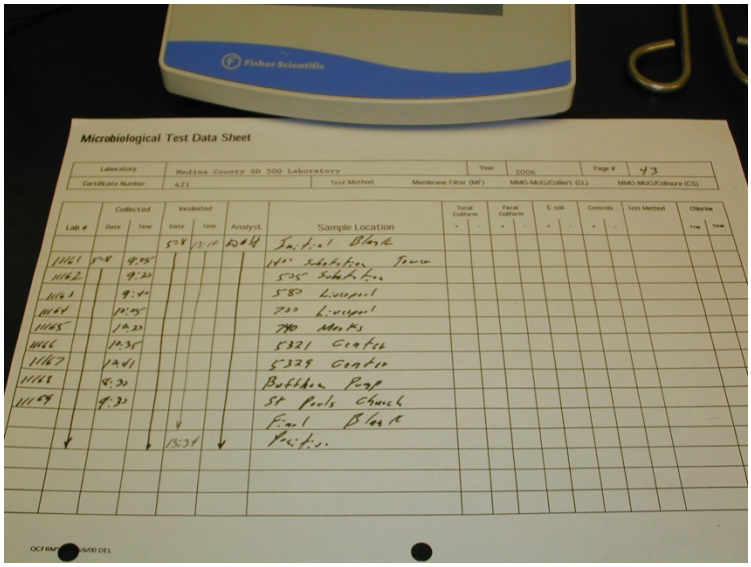
The more closely related the animal, the more likely pathogens excreted with their feces can infect us. Human feces are the biggest concern, because anything which infects one human could infect another. There isn't currently a quantitative method for measuring specifically human fecal bacteria (expensive genetic studies can give a presence/absence result). Ingesting a human stranger's feces via contaminated water supply is a classic means for infections to spread rapidly. The more pathogens an individual carries, the more hazardous their feces. Ingesting feces from someone who is not carrying any pathogens may gross you out, but it can't infect you. Infection rates are around 5% in the US, and approach 100% in areas with poor hygiene and contaminated water supplies. Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment factor for your best guess of the ratio between indicators and actual pathogens.

Membrane Filter Total Coliform Technique

The membrane filter total Coliform technique is used at Medina County for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers by Medina County.

The samples are taken in sterile 100 mL containers. These containers, when used for chlorinated water samples, have a sodium thiosulfate pill or solution to dechlorinate the sample.

The sample is placed in cold storage after proper sample taking procedures are followed. (See sample procedures below)



The samples are taken to the laboratory with a chain of custody to assure no tampering of samples can occur.

These samples are logged in at the laboratory.

No longer than 30 hours can lapse between the time of sampling and time of test incubation. (8 hours for heterotrophic, nonpotable 6 hours, others not longer than 24 hours)

All equipment is sterilized by oven and autoclave.
Glassware in oven at 170°C ± 10°C with foil (or other suitable wrap) loosely fitting and secured immediately after sterilization.



Filtration units in autoclave at 121°C for 30 minutes.

Use sterile petri dishes, grid, and pads bought from a reliable company – certified, quality assured - test for satisfactory known positive amounts.

Incubators – 35°C ± .5°C (60% relative humidity)
M-endo medium is prepared and heated to near boiling removed from heat cooled to 45°C pH adjusted to 7.2 ± .2 and immediately dispensed 8ml to plates. Keep refrigerated and discard after 2 weeks.

Plates can be stored in a dated box with expiration date and discarded if not used. No denatured alcohol should be used. Everclear or 95% proof alcohol or absolute methyl may be used for sterilizing forceps by flame.

Procedure:
Counters are alcohol wiped.
Bench sheets are filled out.
Samples are removed from refrigeration.
Sterile wrapped utensils are placed on counters.
Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
Sterile petri dishes are labeled.
The samples closures are clipped.
The sample is shaken 25 times 1 foot in length within 7 seconds.
100 mL is filtered and rinsed with sterile distilled water 3 times.
The membrane filter is aseptically removed from filter holder.
A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
The sterile labeled lid is placed on the petri dish.
2 blanks and a known is run with each series of samples.
The samples are placed in the 35^{o}C $\pm$ .5^{o}C incubator stacked no higher than 3 for 22 – 24 hours (Humidity can be maintained by saturated paper towels placed under containers holding petri dishes.)
After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
Count all colonies that appear pink to dark red with a metallic surface sheen – the sheen may vary in size from a pin head to complete coverage.
Report as Total Coliform per 100 mL.
If no colonies are present report as <1 coliform/100mL.

Anything greater than 1 is over the limit for drinking water for 2 samples taken 24 hours apart. Further investigation may be necessary – follow Standard Methods accordingly.



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Aran™ Aqua Analytical Laboratory Director.

Escherichia coli EPEC

Two types of pathogenic Escherichia coli, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC), cause diarrheal disease by disrupting the intestinal environment through the intimate attachment of the bacteria to the intestinal epithelium.

E. coli O157:H7
E. coli O157:H7 (bacterium) found in human feces. Symptoms vary with type caused gastroenteritis.

Escherichia coli O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illnesses have been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water.

Consumers can prevent *E. coli* O157:H7 infection by thoroughly cooking ground beef, avoiding unpasteurized milk, and washing hands carefully. Because the organism lives in the intestines of healthy cattle, preventive measures on cattle farms and during meat processing are being investigated.

What is *Escherichia coli* O157:H7?
E. coli O157:H7 is one of hundreds of strains of the bacterium Escherichia coli. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.

E. coli O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to contaminated hamburgers. Since then, most infections have come from eating undercooked ground beef.

The combination of letters and numbers in the name of the bacterium refers to the specific markers found on its surface and distinguishes it from other types of *E. coli.*

Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* is a normal inhabitant of the intestines of all animals, including humans. When aerobic culture methods are used, *E. coli* is the dominant species found in feces.

Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. A minority of *E. coli* strains are capable of causing human illness by several different mechanisms. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins [verotoxin (VT), shiga-like toxin] are closely related or identical to the toxin produced by *Shigella dysenteriae*.

How does *E. coli* or other fecal coliforms get in the water?
E. coli comes from human and animal wastes. During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, *E. coli* may end up in drinking water.

How is water treated to protect me from *E. coli*?
The water can be treated using chlorine, ultra-violet light, or ozone, all of which act to kill or inactivate *E. coli.* Systems using surface water sources are required to disinfect to ensure that all bacterial contamination such as *E. coli*. is inactivated. Systems using ground water sources are not required to disinfect, although many of them do.

How does the U.S. Environmental Protection Agency regulate *E. coli*?
According to EPA regulations, a system that operates at least 60 days per year, and serves 25 people or more or has 15 or more service connections, is regulated as a public water system under the Safe Drinking Water Act. If a system is not a public water system as defined by EPA regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities.

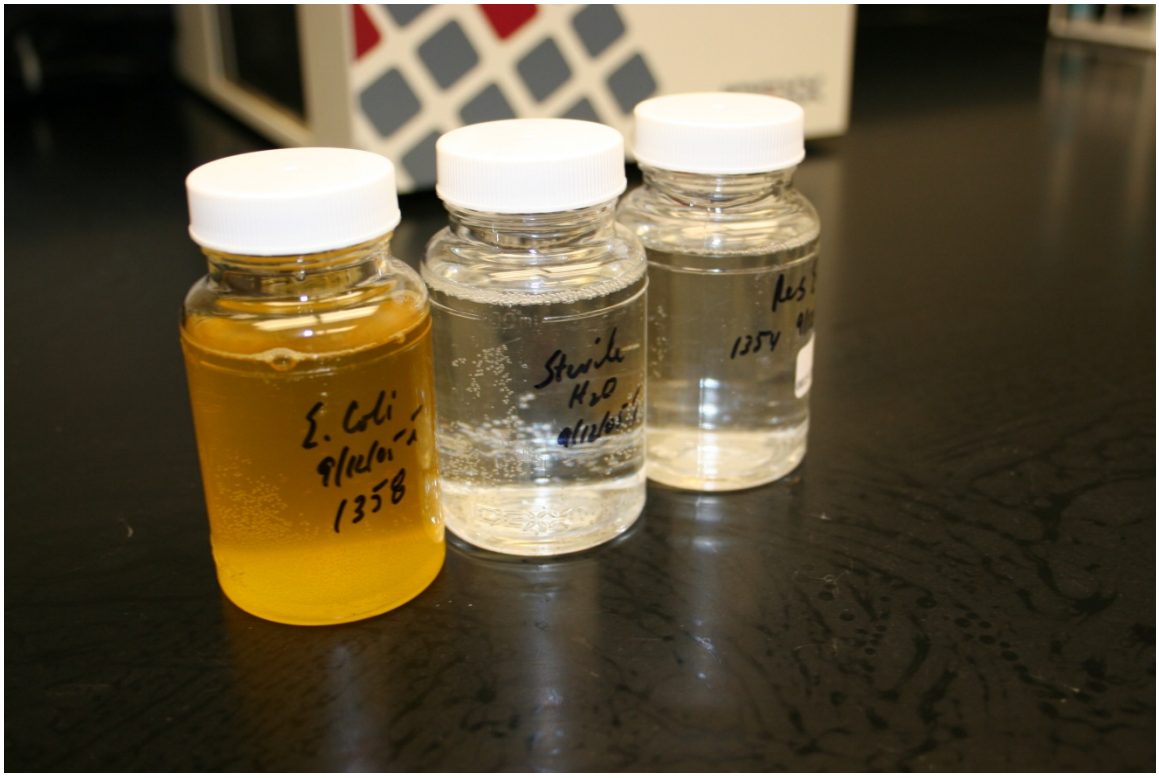
Under the Safe Drinking Water Act, the EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliform, because this test is faster to produce results. Any time that a sample is positive for total coliform, the same sample must be analyzed for either fecal coliform or *E. coli*. Both are indicators of contamination with animal waste or human sewage.

The largest public water systems (serving millions of people) must take at least 480 samples per month. Smaller systems must take at least five samples a month unless the state has conducted a sanitary survey – a survey in which a state inspector examines system components and ensures they will protect public health – at the system within the last five years.

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Some types of systems can qualify for annual monitoring. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection will kill *E. coli* O157:H7.

What can I do to protect myself from *E. coli* O157:H7 in drinking water?
Approximately 89 percent of Americans are receiving water from community water systems that meet all health-based standards. Your public water system is required to notify you if, for any reason, your drinking water is not safe. If you wish to take extra precautions, you can boil your water for one minute at a rolling boil, longer at higher altitudes. To find out more information about your water, see the Consumer Confidence Report from your local water supplier or contact your local water supplier directly. You can also obtain information about your local water system on the EPA's website at www.epa.gov/safewater/dwinfo.htm.

Positive Tests
If you draw water from a private well, you can contact your state health department to obtain information on how to have your well tested for total coliforms, and *E. coli* contamination. If your well tests positive for *E. coli*, there are several steps that you should take: (1) begin boiling all water intended for consumption, (2) disinfect the well according to procedures recommended by your local health department, and (3) monitor your water quality to make certain that the problem does not recur. If the contamination is a recurring problem, you should investigate the feasibility of drilling a new well or install a point-of-entry disinfection unit, which can use chlorine, ultraviolet light, or ozone.

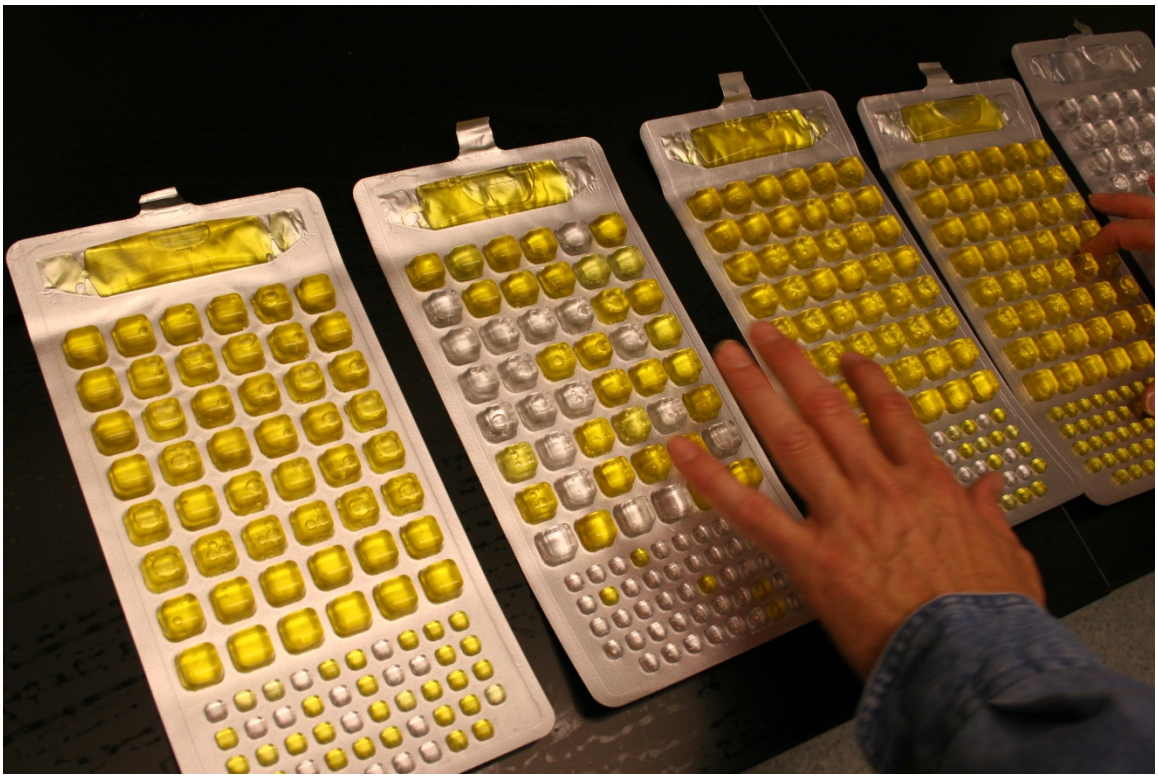


How is *E. coli* O157:H7 spread?
The organism can be found on a small number of cattle farms and can live in the intestines of healthy cattle. Meat can become contaminated during slaughter, and organisms can be thoroughly mixed into beef when it is ground. Bacteria present on a cow's udders or on equipment may get into raw milk. Eating meat, especially ground beef that has not been cooked sufficiently to kill *E. coli* O157:H7 can cause infection. Contaminated meat looks and smells normal. Although the number of organisms required to cause disease is not known, it is suspected to be very small.

Among other known sources of infection are consumption of sprouts, lettuce, salami, unpasteurized milk and juice, and swimming in or drinking sewage-contaminated water. Bacteria in diarrheal stools of infected persons can be passed from one person to another if hygiene or hand washing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected. Young children typically shed the organism in their feces for a week or two after their illness resolves. Older children rarely carry the organism without symptoms.

What illness does *E. coli* O157:H7 cause?
E. coli O157:H7 infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes non-bloody diarrhea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. Hemorrhagic colitis is the name of the acute disease caused by *E. coli* O157:H7.

In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7.



How is *E. coli* O157:H7 infection diagnosed?
Infection with *E. coli* O157:H7 is diagnosed by detecting the bacterium in the stool. Most laboratories that culture stool do not test for *E. coli* O157:H7, so it is important to request that the stool specimen be tested on sorbitol-MacConkey (SMAC) agar for this organism. All persons who suddenly have diarrhea with blood should get their stool tested for *E. coli* O157:H7.

How is the illness treated?
Most persons recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. Antidiarrheal agents, such as loperamide (Imodium), should also be avoided. Hemolytic uremic syndrome is a life-threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for hemolytic uremic syndrome is 3%-5%.

Legionnaire's Disease Legionella Section

Introduction **Genus: *Legionella* Species: *pneumophila***

The first discovery of bacteria from genus Legionella came in 1976 when an outbreak of pneumonia at an American Legion convention led to 29 deaths. The causative agent, what would come to be known as Legionella pneumophila, was isolated and given its own genus. The organisms classified in this genus are Gram-negative bacteria that are considered intracellular parasites. The disease has two distinct forms:

- Legionnaires' disease, the more severe form of infection which includes pneumonia, and
- Pontiac fever, a milder illness.



What have been the water sources for Legionnaires' disease?
The major source is water distribution systems of large buildings, including hotels and hospitals. Cooling towers have long been thought to be a major source for *Legionella*, but new data suggest that this is an overemphasized mode of transmission. Other sources include mist machines, humidifiers, whirlpool spas, and hot springs. Air conditioners are not a source for Legionnaires' disease. They were suspected to be the source in the original American Legion outbreak in a Philadelphia hotel, but new data now suggests that the water in the hotel was the actual culprit.



Legionnaire's disease is caused most commonly by the inhalation of small droplets of water or fine aerosol containing Legionella bacteria. Legionella bacteria are naturally found in environmental water sources such as rivers, lakes and ponds and may colonize man-made water systems that include air conditioning systems, humidifiers, cooling tower waters, hot water systems, spas and pools.

How do people contract Legionella?
The most popular theory is that the organism is aerosolized in water and people inhale the droplets containing *Legionella*. However, new evidence suggests that another way of contracting *Legionella* is more common. "Aspiration" is the most common way that bacteria enter into the lungs to cause pneumonia. Aspiration means choking such that secretions in the mouth get past the choking reflexes and instead of going into the esophagus and stomach, mistakenly, enter the lung. The protective mechanisms to prevent aspiration is defective in patients who smoke or have lung disease. Aspiration now appears to be the most common mode of transmission.

Legionella may multiply to high numbers in cooling towers, evaporative condensers, air washers, humidifiers, hot water heaters, spas, fountains, and plumbing fixtures. Within one month, Legionella can multiply, in warm water-containing systems, from less than 10 per milliliter to over 1,000 per milliliter of water. Once high numbers of Legionella have been found, a relatively simple procedure for disinfecting water systems with chlorine and detergent is available. This procedure is not part of a routine maintenance program because equipment may become corroded.

Property owners have been sued for the spread of Legionella, resulting in expensive settlements. Regular monitoring with a battery of DFA monoclonal antibodies for several serogroups and species of Legionella morphologically intact bacteria provides a means for exercising 'reasonable care' to deter potential litigation.

Currently, there are no United States government regulations concerning permissible numbers of legionella in water systems and there are no federal or state certification programs for laboratories that perform legionella testing of environmental samples.

Epifluorescence Microscopy DFA Method
The epifluorescence microscopy DFA method that most labs use was published in the British Journal, Water Research 19:839-848, 1985 "Disinfection of circulating water systems by ultraviolet light and halogenation", R. Gilpin, et al. so we can count viable-but-nonculturable (VBNC) legionella.

Most labs will provide a quantitative epifluorescence microscopic analysis of your cooling tower and potable water samples for 14 serogroups of Legionella pneumophila and 15 other Legionella species (listed below).

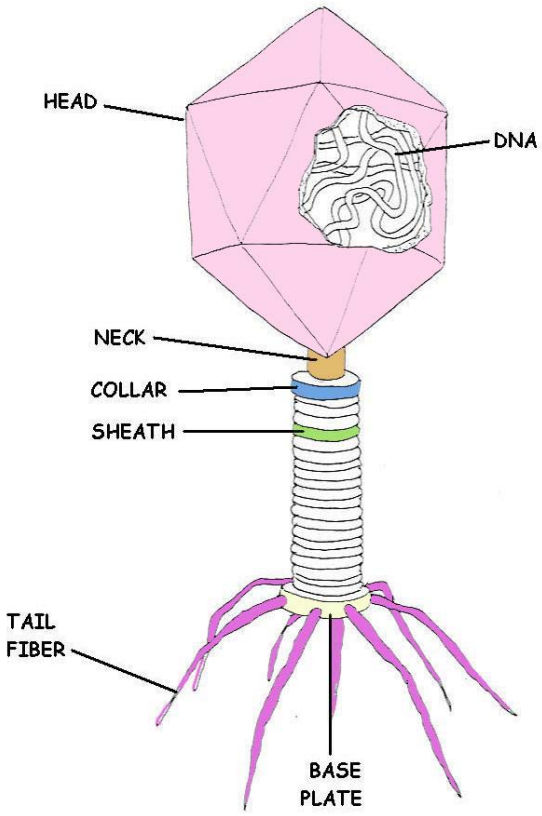
Legionella anisa	*Legionella bozemanii* sg 1 & 2
Legionella dumoffi	*Legionella feeleii* sg 1 & 2
Legionella gormanii	*Legionella hackeliae* sg 1 & 2
Legionella jordanis	*Legionella longbeachae* sg 1& 2
Legionella maceachernii	*Legionella micdadei*
Legionella oakridgensis	*Legionella parisiensis*
Legionella pneumophila sg 1-14	*Legionella sainthelensi*
Legionella santicrucis	*Legionella wadsworthii*

Heterotrophic bacterial CFU are often inversely proportional to numbers of Legionella in cooling tower samples, in our experience. Routine biocide treatments will not eradicate Legionella bacteria in the environment, only in laboratory studies.

Culture methods are good during outbreaks for bio-typing; but culture methods lack sensitivity for routine, quantitative monitoring. Many factors will inhibit growth or identification of legionella on BCYE with or without antimicrobial agents, heat or acid treatment.

Culture methods will not identify non-culturable legionella that can still cause outbreaks (non-culturable, viable legionella have been reported in several peer-reviewed journals). Only DFA tests performed by trained laboratory personnel can identify these legionella. Direct fluorescent antibody (DFA) tests using a battery of monoclonal antibodies provide more useful routine monitoring information than culture methods. Legionella species of bacteria cause Legionnaire's disease. They are gram negative (but stain poorly), strictly aerobic rods.

The U.S. Environmental Protection Agency and the U.S. Occupational Safety and Health Administration recommend routine maintenance of water-containing equipment. Most State health departments recommend monthly testing for Legionella as part of a routine maintenance program.



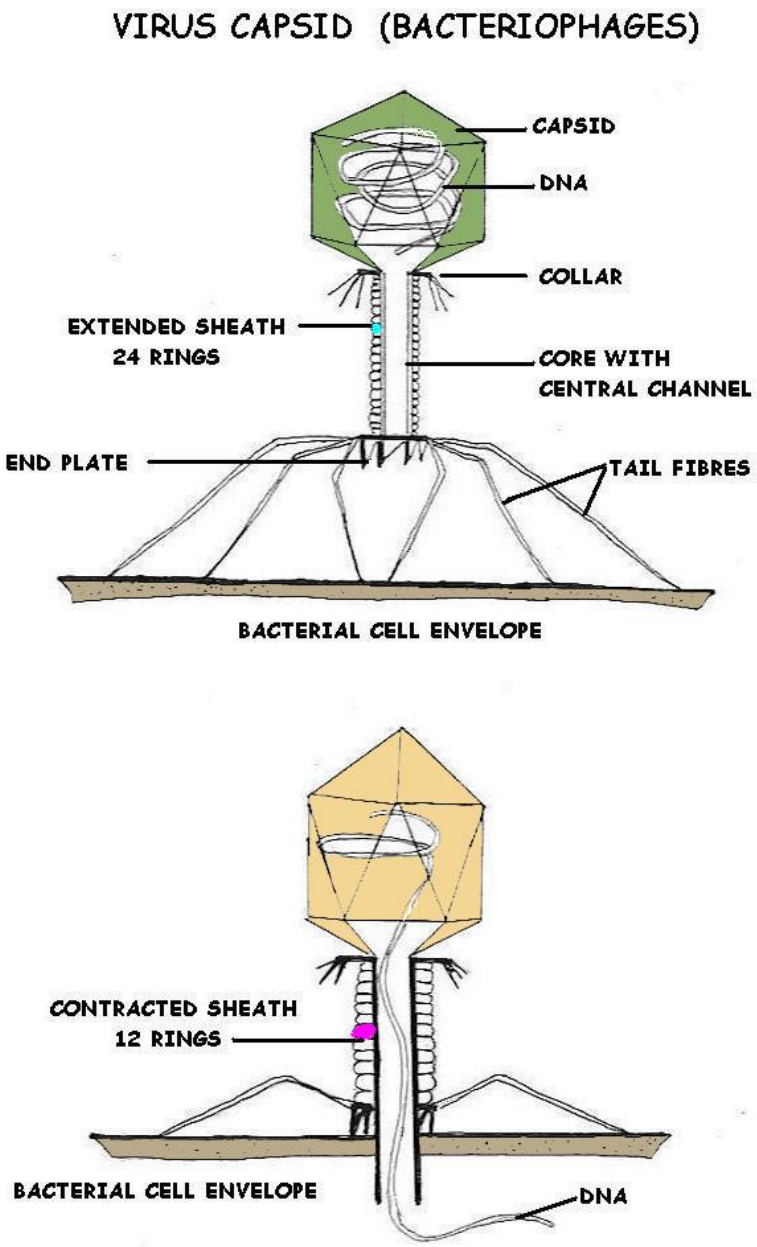
BACTERIOPHAGE

Bacteriophage

A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage.

Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestine of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×108 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.

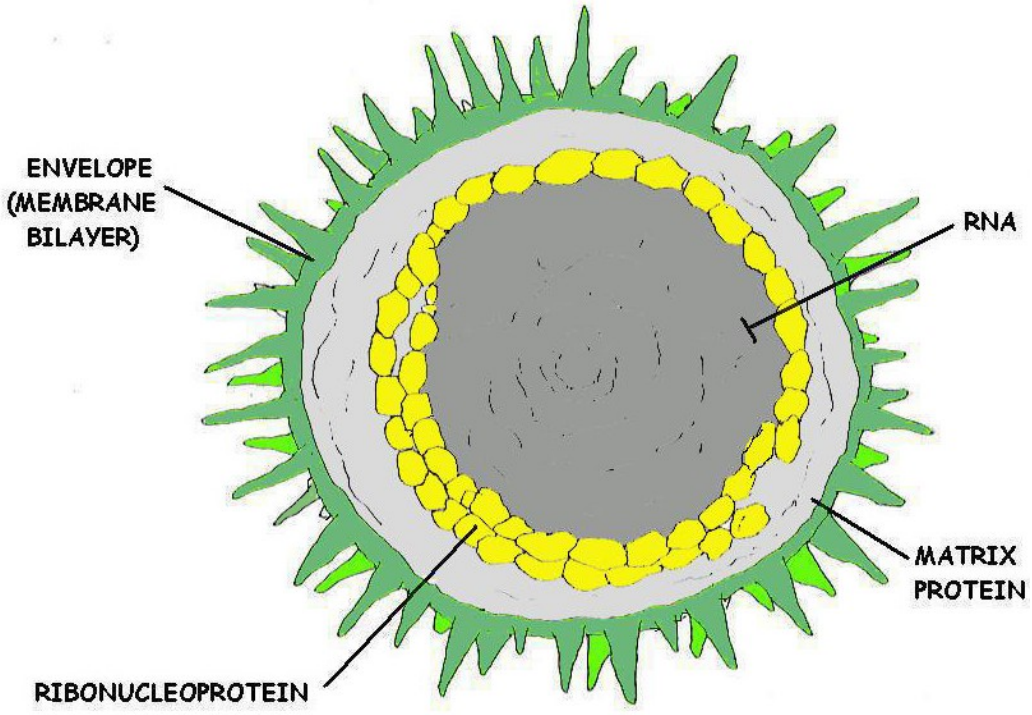


Release of Virions

Phages may be released via cell lysis or by host cell secretion. In the case of the T4 phage, in just over twenty minutes after injection upwards of three hundred phages will be released via lysis within a certain timescale. This is achieved by an enzyme called endolysin which attacks and breaks down the peptidoglycan. In contrast, "lysogenic" phages do not kill the host but rather become long-term parasites and make the host cell continually secrete more new virus particles. The new virions bud off the plasma membrane, taking a portion of it with them to become enveloped viruses possessing a viral envelope. All released virions are capable of infecting a new bacterium.

Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce. A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



CROSS SECTIONAL VIEW

Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication. Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.

Virions
Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA. Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or non-segmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

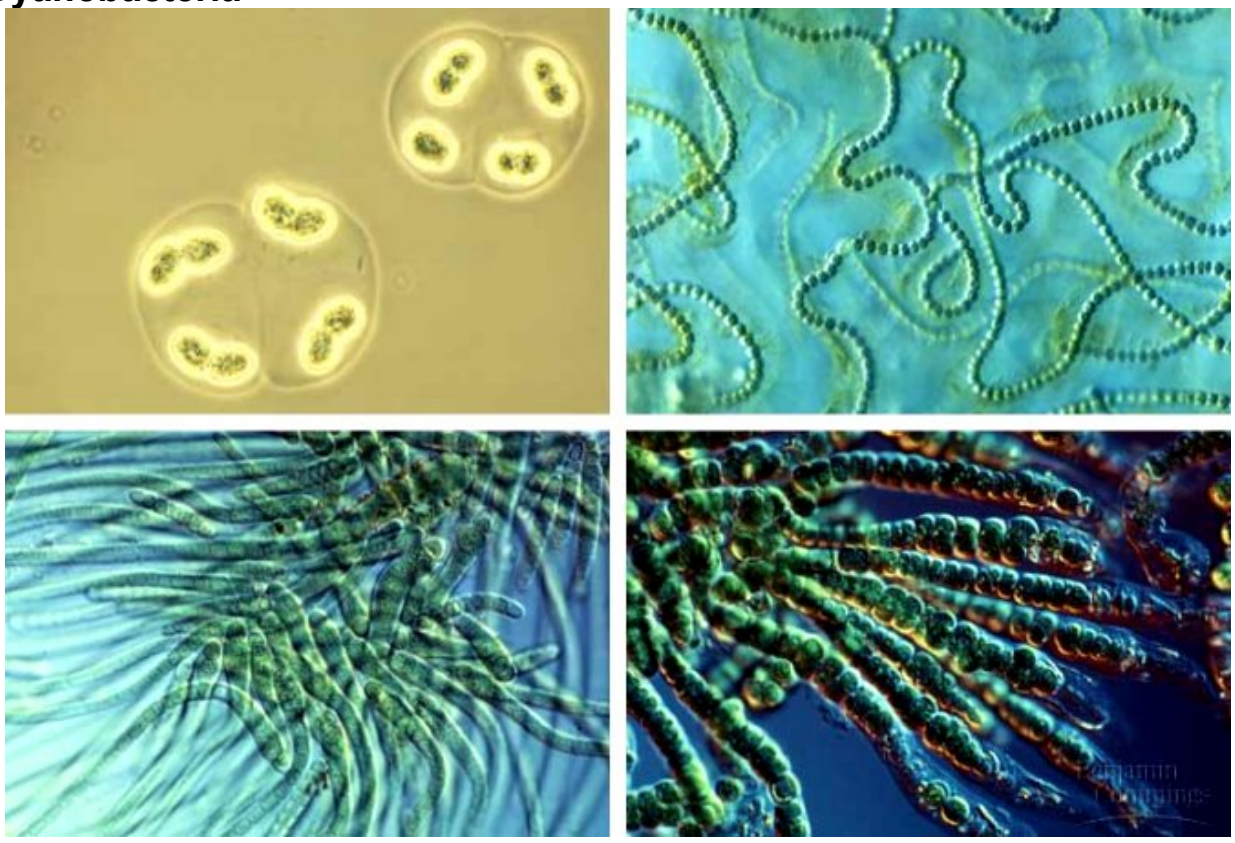
Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.

Bacteriophages
The most elaborate viruses are the bacteriophages, which use bacteria as their hosts. Some bacteriophages resemble an insect with an icosahedral head attached to a tubular sheath. From the base of the sheath extend several long tail fibers that help the virus attach to the bacterium and inject its DNA to be replicated, direct capsid production, and virus particle assembly inside the cell.

Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule. The single-stranded RNA circle collapses on itself to form a rod-like structure. The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

Cyanobacteria



Cyanobacteria

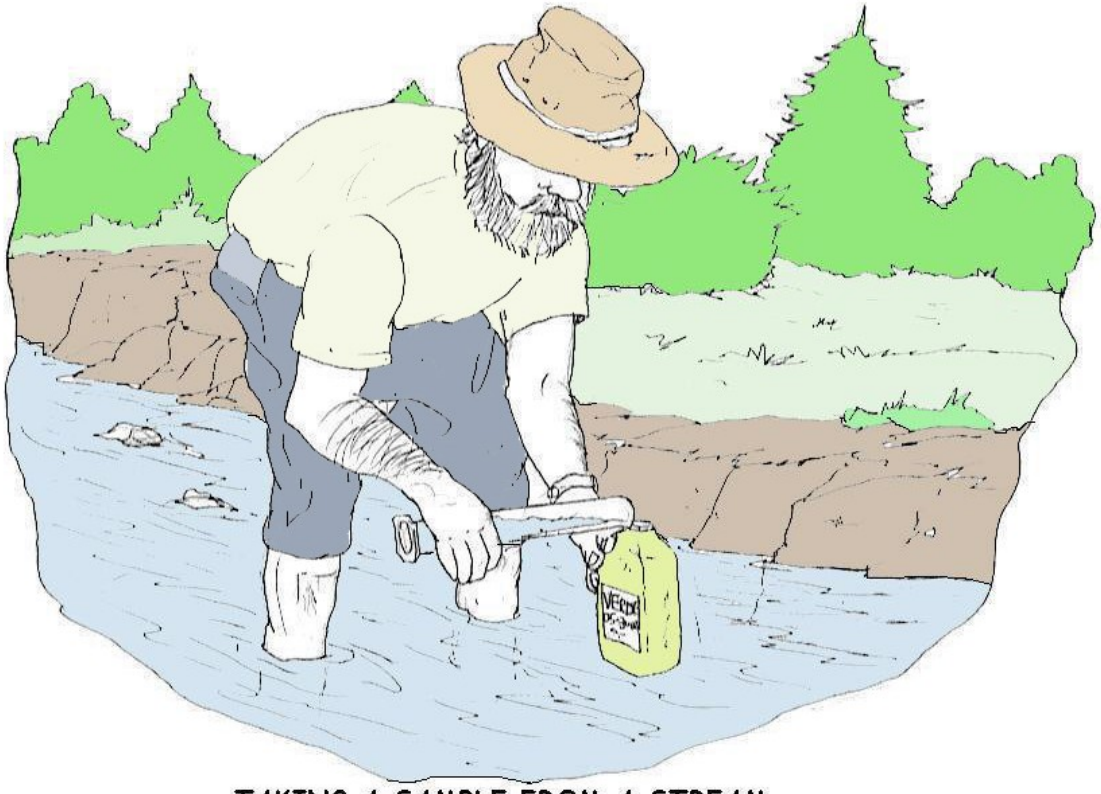
Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

Cyanobacteria include unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) wherever nitrogen is necessary. Heterocyst-forming species are specialized for nitrogen fixation and are able to fix nitrogen gas, which cannot be used by plants, into ammonia (NH_3), nitrites (NO_2) or nitrates (NO_3), which can be absorbed by plants and converted to protein and nucleic acids.

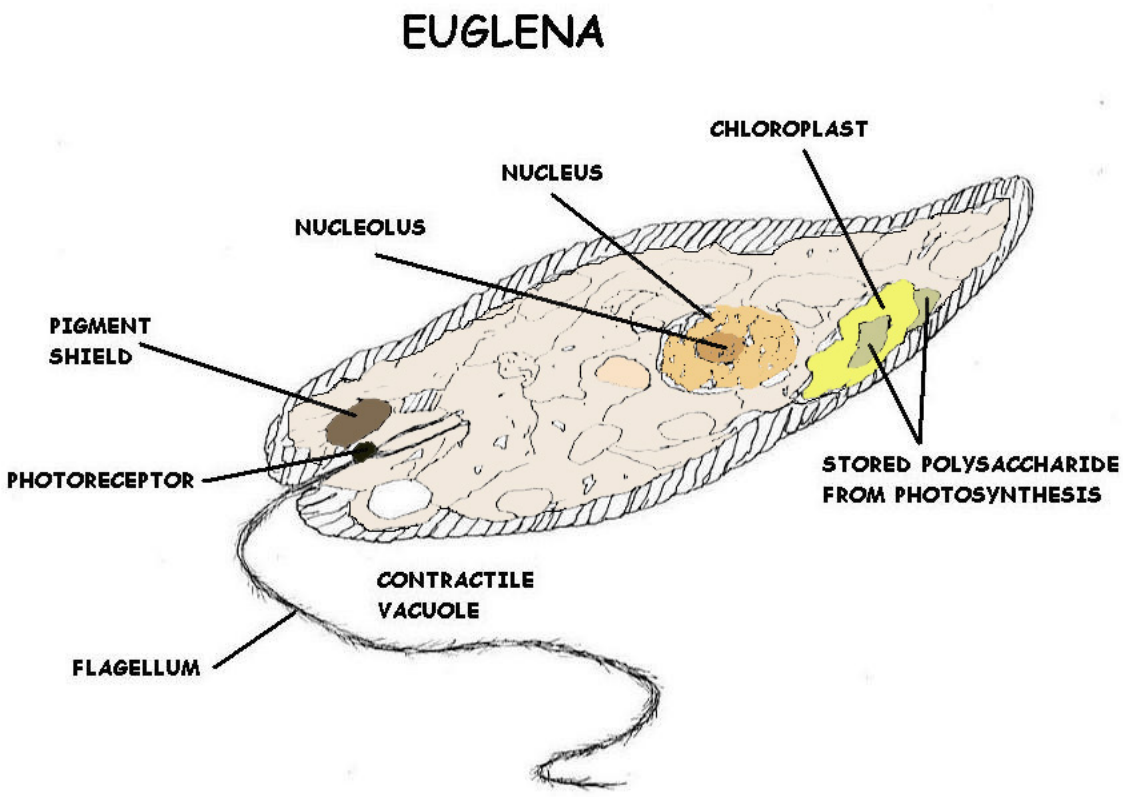
The rice paddies of Asia, which produce about 75% of the world's rice, could not do so were it not for healthy populations of nitrogen-fixing cyanobacteria in the rice paddy fertilizer too.

Many cyanobacteria also form motile filaments, called hormogonia, that travel away from the main biomass to bud and form new colonies elsewhere. The cells in a hormogonium are often thinner than in the vegetative state, and the cells on either end of the motile chain may be tapered. In order to break away from the parent colony, a hormogonium often must tear apart a weaker cell in a filament, called a necridium.

Each individual cell of a cyanobacterium typically has a thick, gelatinous cell wall. They differ from other gram-negative bacteria in that the quorum sensing molecules autoinducer-2[4] and acyl-homoserine lactones are absent. They lack flagella, but hormogonia and some unicellular species may move about by gliding along surfaces. In water columns some cyanobacteria float by forming gas vesicles, like in archaea.



TAKING A SAMPLE FROM A STREAM



Euglena

Euglenas are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so, and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features.

Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food. In order for Euglena gracilis to make more Euglena gracilis it will complete a process called mitosis. That means it can split itself in half and become two Euglena gracilis. It can only do this if it is well-fed and if the temperature is right. Euglena gracilis can reproduce better in warm temperatures.

Euglena gracilis, and other euglena, are harmless to people, but they are often signs that water is polluted, since they do well where there is a lot of green algae to eat. Green algae does well where there is a lot of nitrogen (comes from waste) in the water. If you don't clean your swimming pool, leaves and twigs get in the water and turn into waste. Then algae and euglena show up.

KINGDOM: Protist, PHYLUM: Euglenophyta, CLASS: Euglenophyceae, ORDER: Euglenales, FAMILY: Euglenidae, GENUS: Euglena, SPECIES: Euglena gracilis

Peptidoglycan

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of eubacteria. The sugar component consists of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid residues. Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer.



Hepatitis



There are five types of hepatitis -- A through E -- all of which cause inflammation of the liver. Type D affects only those who also have hepatitis B, and hepatitis E is extremely rare in the United States.

- Type A hepatitis is contracted through anal-oral contact, by coming in contact with the feces of someone with hepatitis A, or by eating or drinking hepatitis A contaminated food or water.
- Type B hepatitis can be contracted from infected blood, seminal fluid, vaginal secretions, or contaminated drug needles, including tattoo or body-piercing equipment. It can also be spread from a mother to her newborn.
- Type C hepatitis is not easily spread through sex. You're more likely to get it through contact with infected blood, contaminated razors, needles, tattoo and body-piercing equipment, or manicure or pedicure tools that haven't been properly sanitized, and a mother can pass it to her baby during delivery.
- Type D hepatitis can be passed through contact with infected blood, contaminated needles, or by sexual contact with an HIV-infected person.
- Type E hepatitis is most likely to be transmitted in feces, through oral contact, or in water that's been contaminated.


COMBS
BOMBS
MIKE ROBES'
ICE CREAM

Disinfection Rules Chapter 2

Disinfection Byproduct Regulations

In December 1998, the EPA established the Stage 1 Disinfectants/Disinfection Byproducts Rule that requires public water systems to use treatment measures to reduce the formation of disinfection byproducts and to meet the following specific standards:

Total Trihalomethanes (TTHM)	80 parts per billion (ppb)
Haloacetic Acids (HAA5)	60 ppb
Bromate	10 ppb
Chlorite	1.0 parts per million (ppm)

Currently trihalomethanes are regulated at a maximum allowable annual average level of 100 parts per billion for water systems serving over 10,000 people under the Total Trihalomethane Rule finalized by the EPA in 1979. The Stage 1 Disinfectant/Disinfection Byproduct Rule standards became effective for trihalomethanes and other disinfection byproducts listed above in December 2001 for large surface water public water systems. Those standards became effective in December 2003 for small surface water and all ground water public water systems.

Disinfection byproducts are formed when disinfectants used in water treatment plants react with bromide and/or natural organic matter (i.e., decaying vegetation) present in the source water. Different disinfectants produce different types or amounts of disinfection byproducts. Disinfection byproducts for which regulations have been established have been identified in drinking water, including trihalomethanes, haloacetic acids, bromate, and chlorite.

Trihalomethanes (**THM**) are a group of four chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The trihalomethanes are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. The EPA has published the **Stage 1 Disinfectants/Disinfection Byproducts Rule** to regulate total trihalomethanes (**TTHM**) at a maximum allowable annual average level of **80 parts per billion**. This new standard replaced the old standard of a maximum allowable annual average level of 100 parts per billion back in December 2001 for large surface water **public water systems**. The standard became effective for the first time back in December 2003 for small surface water and all ground water systems.

Haloacetic Acids (**HAA5**) are a group of chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The regulated haloacetic acids, known as HAA5, are: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. EPA has published the **Stage 1 Disinfectants/Disinfection Byproducts Rule** to regulate HAA5 at **60 parts per billion** annual average.

This standard became effective for large surface water **public water systems** back in December 2001 and for small surface water and all ground water public water systems back in December 2003.

Bromate

Bromate is a chemical that is formed when ozone used to disinfect drinking water reacts with naturally occurring bromide found in source water. The EPA has established the **Stage 1 Disinfectants/Disinfection Byproducts Rule** to regulate bromate at annual average of **10 parts per billion** in drinking water.

This standard became effective for large **public water systems** by December 2001 and for small surface water and all ground public water systems back in December 2003.

Chlorite is a byproduct formed when chlorine dioxide is used to disinfect water. EPA has published the **Stage 1 Disinfectants/Disinfection Byproducts Rule** to regulate chlorite at a monthly average level of 1 part per million in drinking water. This standard became effective for large surface water public water systems back in December 2001 and for small surface water and all ground water public water systems back in December 2003.

Microbial Regulations

One of the key regulations developed and implemented by the United States Environmental Protection Agency (**USEPA**) to counter pathogens in drinking water is the **Surface Water Treatment Rule**. Among its provisions, the rule requires that a public water system, using surface water (or ground water under the direct influence of surface water) as its source, have sufficient treatment to reduce the source water concentration of ***Giardia*** and viruses by at least 99.9% and 99.99%, respectively. The Surface Water Treatment Rule specifies treatment criteria to assure that these performance requirements are met; they include turbidity limits, disinfectant residual, and disinfectant contact time conditions.

The **Interim Enhanced Surface Water Treatment Rule** was established in December 1998 to control ***Cryptosporidium***, and to maintain control of pathogens while systems lower disinfection byproduct levels to comply with the **Stage 1 Disinfectants/Disinfection Byproducts Rule**. The EPA established a Maximum Contaminant Level Goal (MCLG) of zero for all public water systems and a 99% removal requirement for Cryptosporidium in filtered public water systems that serve at least 10,000 people. The new rule will tighten turbidity standards by December 2001. Turbidity is an indicator of the physical removal of particulates, including pathogens.

The EPA is also planning to develop other rules to further control pathogens. The EPA has promulgated a Long Term 1 Enhanced Surface Water Treatment Rule, for systems serving fewer than 10,000 people. This is to improve physical removal of *Cryptosporidium*, and to maintain control of pathogens while systems comply with **Stage 1 Disinfectants/Disinfection Byproducts Rule**.



Microbes **More Information in the water Monitoring Section**

Coliform bacteria are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease.

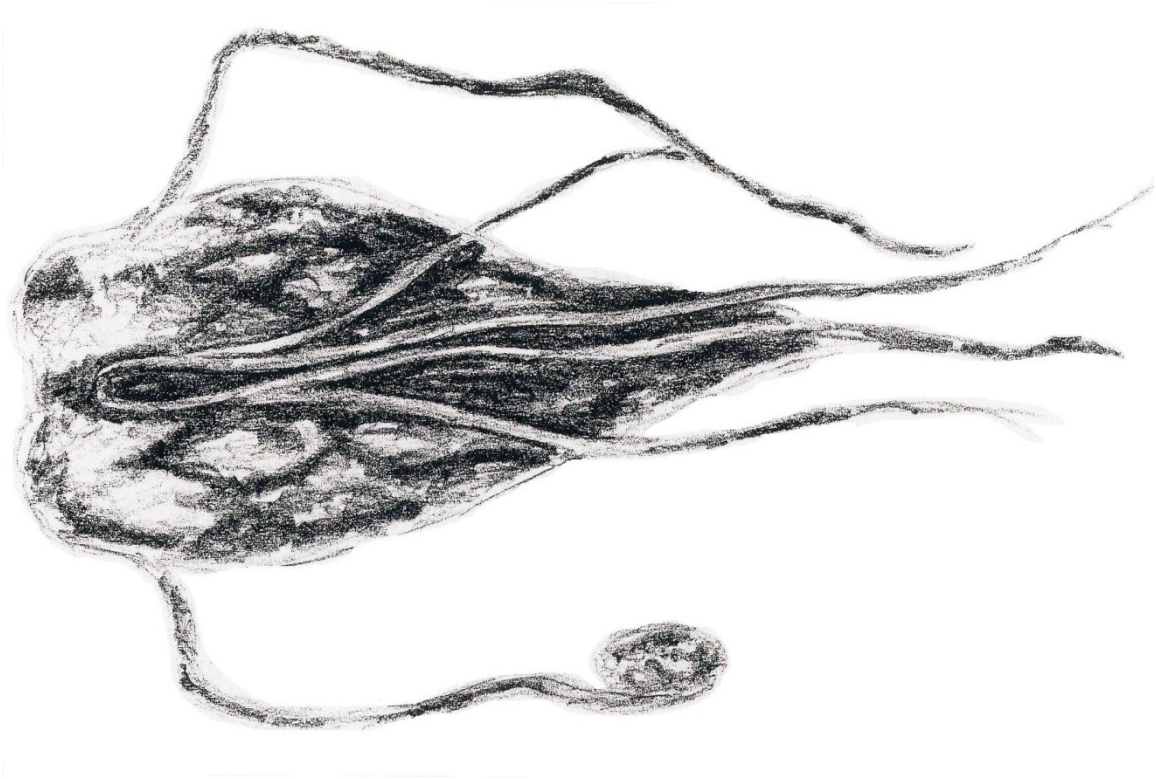
Fecal Coliform and E coli are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms.

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems. The EPA and CDC have prepared advice for those with severely compromised immune systems who are concerned about *Cryptosporidium*.

Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhea, vomiting, and cramps).



Heterotrophic Plate Count Bacteria: A broad group of bacteria including non-pathogens, pathogens, and opportunistic pathogens; they may be an indicator of poor general biological quality of drinking water. Often referred to as HPC. The above photo is of a SimPlate for HPC multi-dose used for the quantification of HPC in water.



Giardia



Cryptosporidium

Chlorine (DDBP)

Today, most of our drinking water supplies are free of the micro-organisms — viruses, bacteria, and protozoa — that cause serious and life-threatening diseases, such as cholera and typhoid fever. This is largely due to the introduction of water treatment, particularly chlorination, at the turn of the century. Living cells react with chlorine and reduce its concentration while they die. The organic matter and other substances that are present, convert to chlorinated derivatives, some of which are effective killing agents. Chlorine present as Cl, HOCl, and OCl^- is called ***free available chlorine***, and that which is bound but still effective is ***combined chlorine***. A particularly important group of compounds with combined chlorine is the chloramines formed by reactions with ammonia.

One especially important feature of disinfection using chlorine is the ease of overdosing to create a "**residual**" concentration. There is a constant danger that safe water leaving the treatment plant may become contaminated later. There may be breaks in water mains, loss of pressure that permits an inward leak, or plumbing errors. This residual concentration of chlorine provides some degree of protection right to the water faucet. With free available chlorine, a typical residual is from 0.1 to 0.5 ppm. Because chlorinated organic compounds are less effective, a typical residual is 2 ppm for combined chlorine. There will be no chlorine residual unless there is an excess over the amount that reacts with the organic matter present. However, reaction kinetics complicates interpretation of chlorination data. The correct excess is obtained in a method called ***"Break Point Chlorination ".***

Chlorine By-Products
Chlorination by-products are the chemicals formed when the chlorine used to kill disease- causing micro-organisms reacts with naturally occurring organic matter (e.g., decay products of vegetation) in the water. The most common chlorination by-products found in U.S. drinking water supplies are the trihalomethanes (**THMs**).

The Principal Trihalomethanes are:
Chloroform, bromodichloromethane, chlorodibromomethane, and bromoform. Other less common chlorination by-products includes the haloacetic acids and haloacetonitriles.

The amount of THMs formed in drinking water can be influenced by a number of factors, including the season and the source of the water. For example, THM concentrations are generally lower in winter than in summer, because concentrations of natural organic matter are lower and less chlorine is required to disinfect at colder temperatures. THM levels are also low when wells or large lakes are used as the drinking water source, because organic matter concentrations are generally low in these sources. The opposite — high organic matter concentrations and high THM levels — is true when rivers or other surface waters are used as the source of the drinking water.

Health Effects
Laboratory animals exposed to very high levels of THMs have shown increased incidences of cancer. Also, several studies of cancer incidence in human populations have reported associations between long-term exposure to high levels of chlorination by-products and an increased risk of certain types of cancer.

For instance, a recent study conducted in the Great Lakes basin reported an increased risk of bladder and possibly colon cancer in people who drank chlorinated surface water for 35 years or more.

Possible relationships between exposure to high levels of THMs and adverse reproductive effects in humans have also been examined recently. In a California study, pregnant women who consumed large amounts of tap water containing elevated levels of THMs were found to have an increased risk of spontaneous abortion.

The available studies on health effects do not provide conclusive proof of a relationship between exposure to THMs and cancer or reproductive effects, but indicate the need for further research to confirm their results and to assess the potential health effects of chlorination by-products other than THMs.



Chlorine cylinder storage room, notice the vents at the bottom and top. The bottom vent will allow the gas to ventilate because Cl_2 gas is heavier than air.

Chlorine Disinfectants/Disinfectant By-Products (DBPs)

- Chlorine is a naturally existing element that has been used to disinfect drinking water supplies in America for most of the 20th Century.
- Chlorine disinfection has been extremely effective in protecting drinking water resources from bacterial and viral contamination. It has virtually wiped out instances of water-borne diseases like typhoid fever, cholera and dysentery in America and other developed countries.
- Over 200 million Americans currently drink water that has been disinfected.
- The three primary chemical agents used in chlorine disinfection are: free chlorine, chloramine (chlorine and ammonia bonded together) and chlorine dioxide (chlorine and oxygen bonded together).
- Ozone is also used to disinfect water.
- Disinfectants are very active compounds. When added to a water supply, disinfectants not only kill bacteria and viruses, but also react with other chemicals present in the water. These chemicals generally enter the water supply through natural plant and soil breakdown.
- When disinfectants react with other chemicals, new compounds known as disinfectant by-products or "DBPs", are created. DBPs associated with chlorine disinfection include trihalomethanes (THMs), such as chloroform.
- Because chlorination has been used for almost 100 years to disinfect water supplies, approximately 40 percent of the DBPs from chlorination have been identified and researched. Much less is known about the kind of DBPs produced by other disinfectants because of their relatively recent emergence.
- Use of chloramine or chlorine dioxide in chlorine disinfection produces fewer DBPs than chlorine, but each has associated risks. Chloramine is not as strong a disinfectant as chlorine, and disinfection with chlorine dioxide produces its own DBPs.
- Animal research using high concentration of DBPs found increased occurrence of cancer development, although why this occurs has not yet been determined. Research on the relationship between DBPs and cancer and other health risks is ongoing.
- American drinking water has **very low** concentrations of DBPs.
- The U.S. Environmental Protection Agency (USEPA) has **not** been able to link exposure to DBPs at low concentration levels and the health risks associated with high concentration level exposure.
- Since 1984, American drinking water utilities have spent almost $23 million researching the production of DBPs, the risks posed by them and methods to treat them. These research efforts are ongoing. In addition, the 300 largest drinking water utilities have spent more than $150 million to conduct the information gathering required by the Information Collection Rule (ICR). The ICR is the largest study to date pertaining to the occurrence of DBPs and associated treatment practices.
- Since 1979, the U.S. Environmental Protection Agency (USEPA), under the authority of the Safe Drinking Water Act, has regulated the acceptable levels of some DBPs. USEPA cites the large population of Americans potentially at-risk from low-level DPB exposure as the impetus for regulation.

- The Safe Drinking Water Act Amendments of 1996 required USEPA to comply with the regulatory timeline it set forth in its initial Disinfectant and Disinfectant-By-Product (DDPB) rule and Interim Enhanced Surface Water Treatment Rule (IESWTR). USEPA proposed both in 1994.

The research on DBPs and their impact on public health continues and serious questions about the actual health risks posed by DBPs still remain.

Risks and Benefits of Chlorine

Current evidence indicates that the benefits of chlorinating our drinking water — reduced incidence of water-borne diseases — are much greater than the risks of health effects from THMs. Although other disinfectants are available, chlorine continues to be the choice of water treatment experts. When used with modern water filtration practices, chlorine is effective against virtually all infective agents — bacteria, viruses, and protozoa. It is easy to apply, and, most importantly, small amounts of chlorine remain in the water and continue to disinfect throughout the distribution system. This ensures that the water remains free of microbial contamination on its journey from the treatment plant to the consumer's tap.

A number of cities use ozone to disinfect their source water and to reduce THM formation. Although ozone is a highly effective disinfectant, it breaks down quickly, so that small amounts of chlorine or other disinfectants must be added to the water to ensure continued disinfection as the water is piped to the consumer's tap. Modifying water treatment facilities to use ozone can be expensive, and ozone treatment can create other undesirable by-products that may be harmful to health if they are not controlled (e.g., bromate).

Examples of other disinfectants include chloramines and chlorine dioxide. Chloramines are weaker disinfectants than chlorine, especially against viruses and protozoa; however, they are very persistent and, as such, can be useful for preventing re-growth of microbial pathogens in drinking water distribution systems.

Chlorine dioxide can be an effective disinfectant, but it forms chlorate and chlorite, compounds whose toxicity has not yet been fully determined. Assessments of the health risks from these and other chlorine-based disinfectants and chlorination by-products are currently under way.

In general, the preferred method of controlling chlorination by-products is removal of the naturally occurring organic matter from the source water so it cannot react with the chlorine to form by-products. THM levels may also be reduced through the replacement of chlorine with alternative disinfectants. A third option is removal of the by-products by adsorption on activated carbon beds. It is extremely important that water treatment plants ensure that methods used to control chlorination by-products do not compromise the effectiveness of water disinfection.

Halogen Section Chapter 3

Halogens

Before we get started, let's review the halogens. The halogens are a chemical series. They are the elements in Group 17 (old-style: VII or VIIA) of the periodic table: fluorine (F), chlorine (Cl), bromine (Br), iodine (I), astatine (At) and the as yet undiscovered ununseptium (Uus). The periodic table is the single most unifying concept in chemistry. It is a structured listing of all known elements, or substances, that consist of one type of atom. Elements cannot be reduced to simpler substances.

The term "*halogen*" means "*salt-former*" and compounds containing halogens are called "salts". The word halogen was coined to mean elements which produce salt in union with a metal. It comes from 18th c. scientific French nomenclature based on erring adaptations of Greek roots.

Halogens are highly reactive, and as such can be harmful or lethal to biological organisms in sufficient quantities. Chlorine and iodine are both used as disinfectants for such things as drinking water, swimming pools, fresh wounds, dishes, and surfaces. They kill bacteria and other potentially harmful microorganisms, a process known as sterilization. Their reactive properties are also put to use in bleaching. Chlorine is the active ingredient of most fabric bleaches and is used in the production of most paper products.

Halides

These elements are diatomic molecules in their natural form. They require one more electron to fill their outer electron shells, and so have a tendency to form a singly-charged negative ion. This negative ion is referred to as a halide ion; salts containing these ions are known as halides.

Halide ions combined with single hydrogen atoms form the hydrohalic acids (i.e., HF, HCl, HBr, HI), a series of particularly strong acids. (HAt, or "hydrastatic acid", should also qualify, but it is not typically included in discussions of hydrohalic acid due to astatine's extreme instability toward alpha decay.)They react with each other to form interhalogen compounds. Diatomic interhalogen compounds (BrF, ICl, ClF, etc.) bear strong superficial resemblance to the pure halogens. Many synthetic organic compounds such as plastic polymers, and a few natural ones, contain halogen atoms; these are known as halogenated compounds or organic halides.

Chlorine

Chlorine is by far the most abundant of the halogens, and the only one needed in relatively large amounts (as chloride ions) by humans. For example, chloride ions play a key role in brain function by mediating the action of the inhibitory transmitter GABA and are also used by the body to produce stomach acid. Iodine is needed in trace amounts for the production of thyroid hormones such as thyroxine.

On the other hand, neither fluorine nor bromine are believed to be really essential for humans, although small amounts of fluoride can make tooth enamel resistant to decay.

Halogens

All halogens have 7 electrons in their outer shells, giving them an oxidation number of -1. The halogens exist, at room temperature, in all three states of matter:

- **Solid**- Iodine, Astatine
- **Liquid**- Bromine
- **Gas**- Fluorine, Chlorine

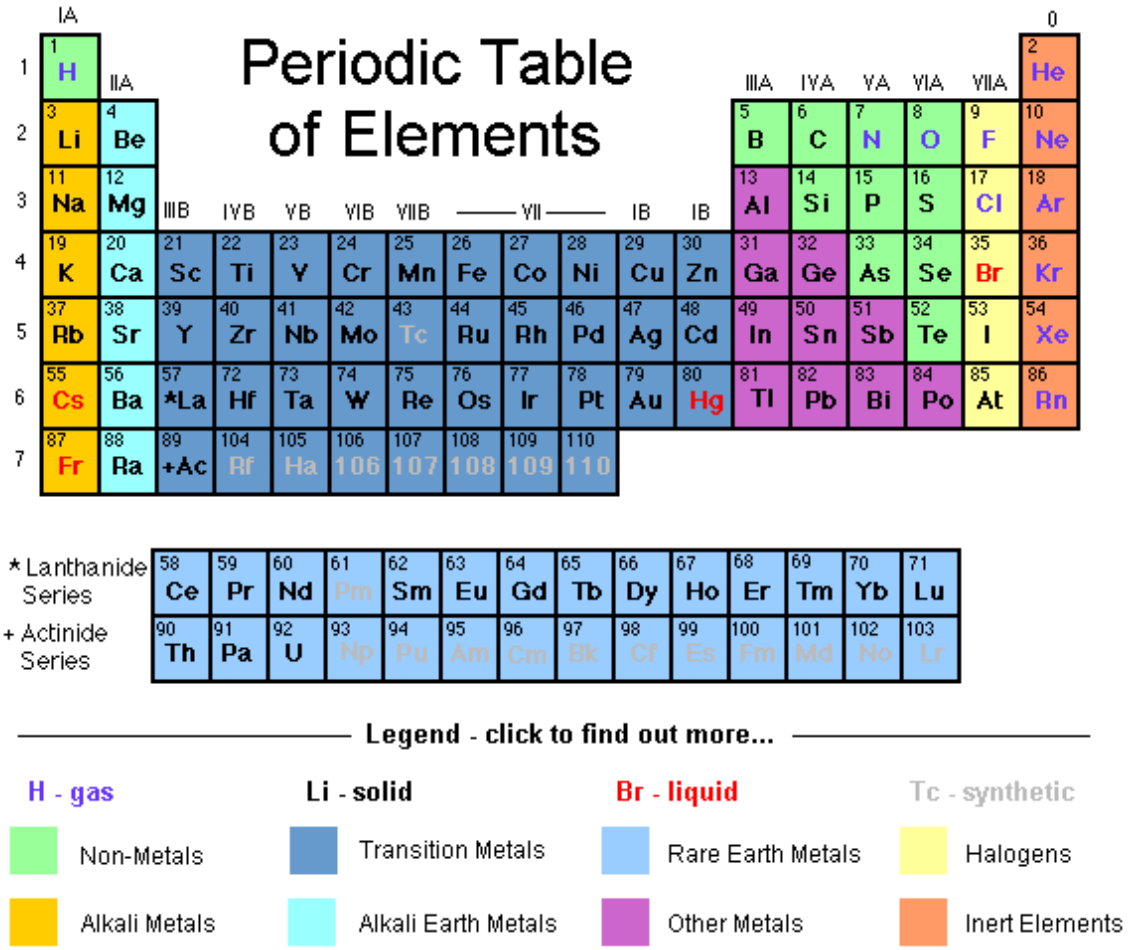
The Halogens are:

Halogen	Atomic Mass	Melting Point k	Boiling Point k	Electronegativity
Fluorine	19	53.53	85.03	3.98
Chlorine	35.5	171.6	239.11	3.16
Bromine	80	265.8	332.0	2.96
Iodine	127	396.85	457.4	2.66
Astatine	210	575	610	2.2
Ununseptium	291*	*	*	*

- Ununseptium has not yet been discovered; values are either unknown if no value appears, or are estimates based on other similar chemicals.



Chlorine gas rotometer



How is the Periodic Table Organized?
The periodic table is organized with eight principal vertical columns called groups and seven horizontal rows called periods. (The groups are numbered I to VIII from left to right, and the periods are numbered 1 to 7 from top to bottom.)

All the metals are grouped together on the left side of the periodic table, and all the nonmetals are grouped together on the right side of the periodic table. Semimetals are found in between the metals and nonmetals.

What are the Eight Groups of the Periodic Table?

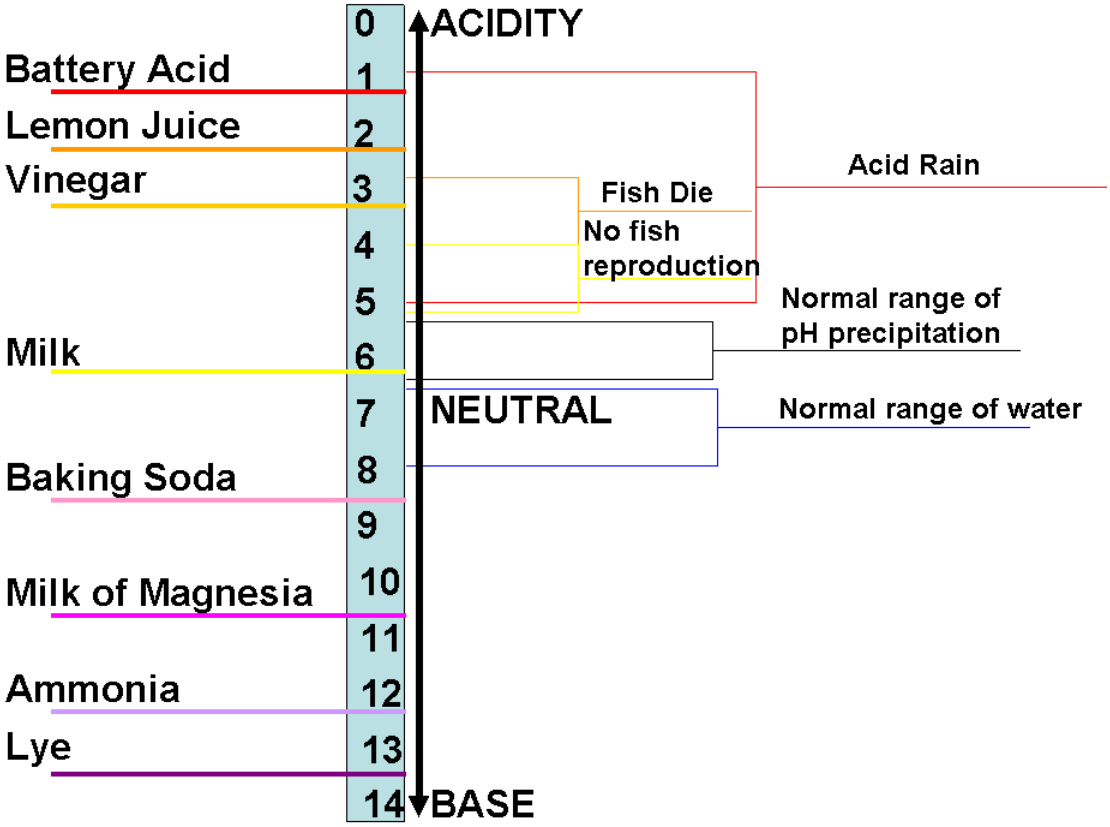
- ✓ **Group I: Alkali Metals - Li, Na, K, Rb, Cs, Fr**
  known as alkali metals
  most reactive of the metals
  react with all nonmetals except the noble gases
  contain typical physical properties of metals (ex. shiny solids and good conductors of heat and electricity)
  softer than most familiar metals; can be cut with a knife

- ✓ **Group II: Alkaline Earth Metals-Be, Mg, Ca, Sr, Ba, Ra**
  known as alkaline earth metals
  react with nonmetals, but more slowly than the Group I metals
  solids at room temperature
  have typical metallic properties
  harder than the Group I metals
  higher melting points than the Group I metals
- ✓ **Group III: B, Al, Ga, In, Tl**
  boron is a semimetal; all the others are metals
- ✓ **Group IV: C, Si, Ge, Sn, Pb**
  carbon is a nonmetal; silicon and germanium are semimetals; tin and lead are metals
- ✓ **Group V: N, P, As, Sb, Bi**
  nitrogen and phosphorus are nonmetals; arsenic and antimony are semimetals; bismuth is a metal
- ✓ **Group VI: O, S, Se, Te, Po**
  oxygen, sulfur, and selenium are nonmetals; tellurium and polonium are semimetals
- ✓ **Group VII: Halogens-F, Cl, Br, I, At**
  very reactive nonmetals
- ✓ **Group VIII: Noble Gases-He, Ne, Ar, Kr, Xe, Rn**
  very unreactive

Properties of Metals
Solids at room temperature
Conduct heat very well
Have electrical conductivities that increase with decreasing temperature
Have a high flexibility and a shiny metallic luster
Are malleable-can be beaten out into sheets or foils
Are ductile-can be pulled into thin wires without breaking
Emit electrons when they are exposed to radiation of sufficiently high energy or when
They are heated (known as photoelectric effect and thermionic effect)

Properties of Nonmetals
May be gases, liquids, or solids at room temperature
Poor conductors of heat
Are insulators-very poor conductors of electricity
Do not have a high reflectivity or a shiny metallic appearance
In solid form generally brittle and fracture easily under stress
Do not exhibit photoelectric or thermionic effects

The pH Scale



pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity.

pH = (**Power of Hydroxyl Ion Activity**).

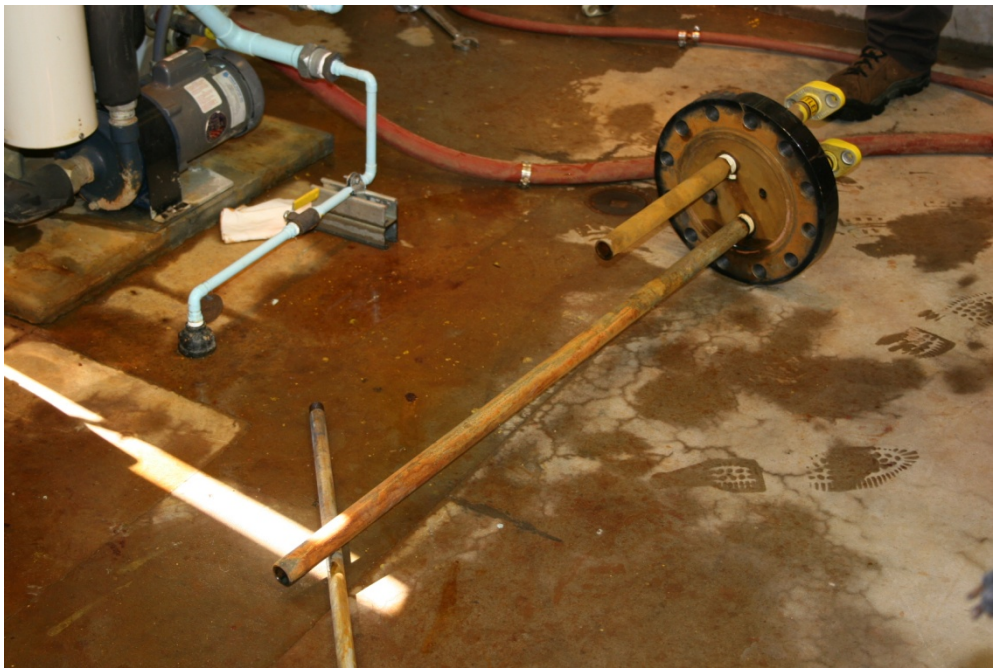
The acidity of a water sample is measured on a pH scale. This scale ranges from **0** (maximum acidity) to **14** (maximum alkalinity). The middle of the scale, **7**, represents the neutral point. The acidity increases from neutral toward **0**.

Because the scale is logarithmic, a difference of one pH unit represents a tenfold change. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on.

Normal rain has a pH of **5.6** – slightly acidic because of the carbon dioxide picked up in the earth's atmosphere by the rain.



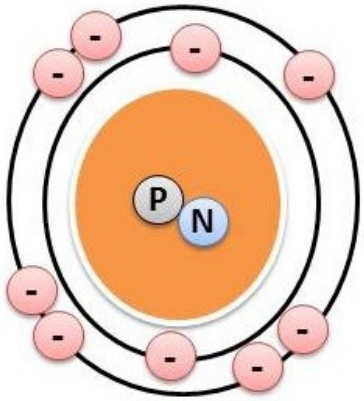
Maintenance crews are required to perform PM on chlorine evaporator units. The photograph on the bottom is of the heating elements from an Evaporator that are being cleaned. This facility will use approximately 200 tons of Chlorine per day for disinfection and has 4-5 personnel working around the clock to keep the chlorine flowing.

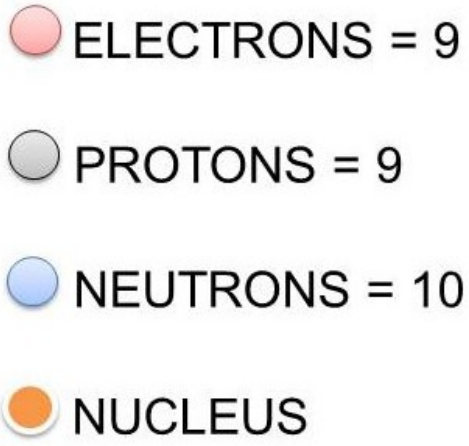


Fluorine Section

Name: Fluorine
Symbol: F
Atomic Number: 9
Atomic Mass: 18.998404 amu
Melting Point: -219.62 °C (53.530006 K, -363.31598 °F)
Boiling Point: -188.14 °C (85.01 K, -306.652 °F)
Number of Protons/Electrons: 9
Number of Neutrons: 10
Classification: Halogen
Crystal Structure: Cubic
Density @ 293 K: 1.696 g/cm^3
Color: Greenish

Atomic Structure





Isotopes

Isotope	Half Life
F-18	1.8 hours
F-19	Stable

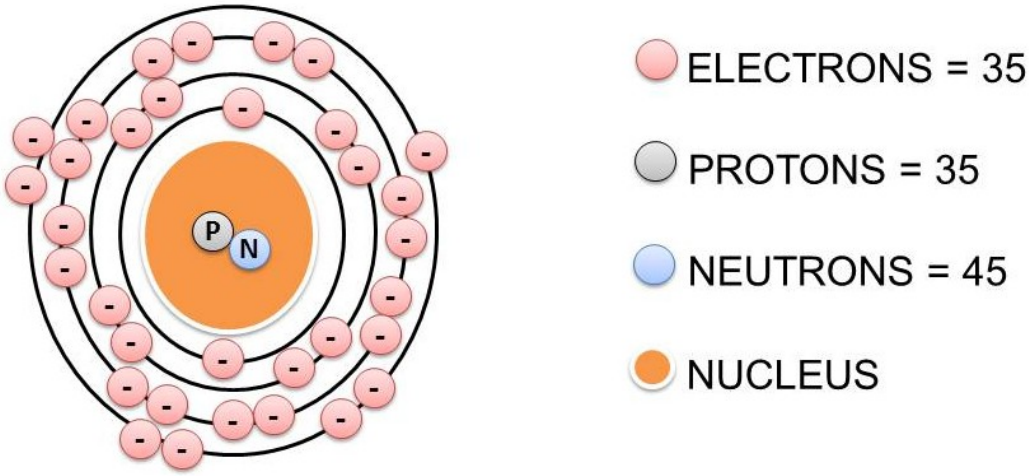
Facts
Date of Discovery: 1886
Discoverer: Joseph Henri Moissan
Name Origin: From the Latin word *fluo* (flow)
Uses: Refrigerants
Obtained From: Mineral fluorite

Bromine Section

Name: Bromine
Symbol: Br
Atomic Number: 35
Atomic Mass: 79.904 amu
Melting Point: -7.2 °C (265.95 K, 19.04 °F)
Boiling Point: 58.78 °C (331.93 K, 137.804 °F)
Number of Protons/Electrons: 35
Number of Neutrons: 45

Classification: Halogen
Crystal Structure: Orthorhombic
Density @ 293 K: 3.119 g/cm^3
Color: Red
Date of Discovery: 1826
Discoverer: Antoine J. Balard
Name Origin: From the Greek word *brômos* (stench)
Uses: Poisonous
Obtained From: Sea Water

Atomic Structure



Isotopes

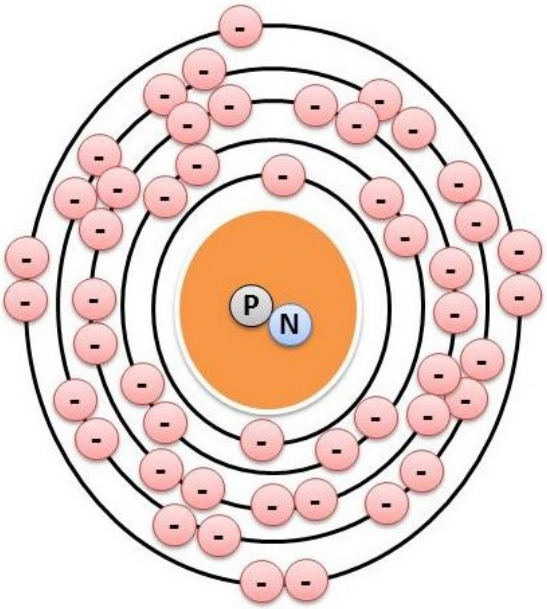
Isotope	Half Life
Br-76	16.0 hours
Br-77	2.4 days
Br-79	Stable
Br-80	17.7 minutes
Br-80m	4.42 hours
Br-81	Stable
Br-82	1.5 days
Br-83	2.4 hours
Br-84	31.8 minutes
Br-85	2.9 minutes

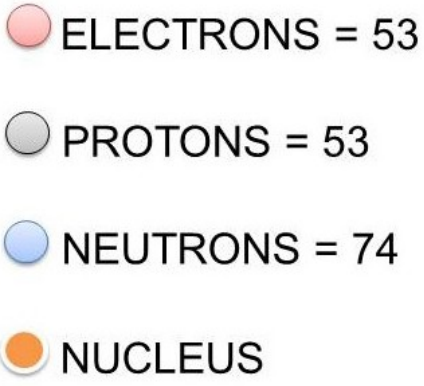
Iodine Section

Name: Iodine
Symbol: I
Atomic Number: 53
Atomic Mass: 126.90447 amu
Melting Point: 113.5 °C (386.65 K, 236.3 °F)
Boiling Point: 184.0 °C (457.15 K, 363.2 °F)
Number of Protons/Electrons: 53
Number of Neutrons: 74
Classification: Halogen

Crystal Structure: Orthorhombic
Density @ 293 K: 4.93 g/cm^3
Color: Blackish
Facts:
Date of Discovery: 1811
Discoverer: Bernard Courtois
Name Origin: From the Greek word *iôdes* (violet)
Uses: Required in humans
Obtained From: Sodium and potassium compounds

Atomic Structure





Isotopes

Isotope	Half Life
I-122	3.6 minutes
I-123	13.2 hours
I-124	4.2 days
I-125	60.1 days
I-126	13.0 days
I-127	Stable
I-128	25.0 minutes

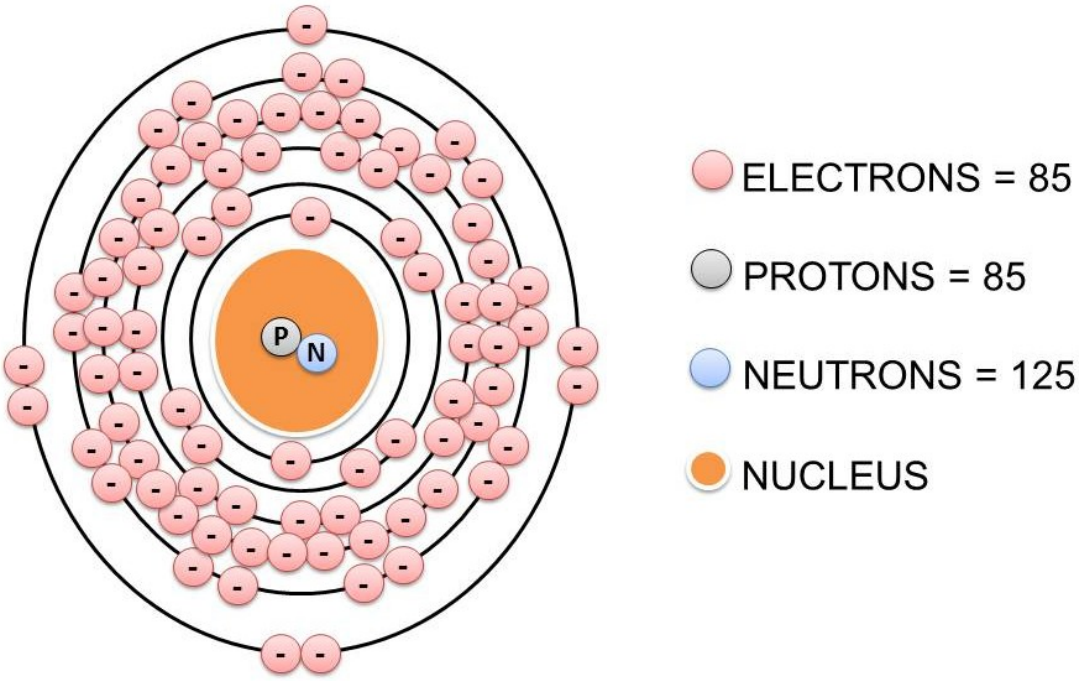
I-129	1.57E7 years
I-130	12.4 hours
I-131	8.0 days
I-132	2.3 hours
I-133	20.8 hours
I-134	52.6 minutes
I-135	6.6 hours
I-136	1.4 minutes

Astatine Section

Name: Astatine
Symbol: At
Atomic Number: 85
Atomic Mass: (210.0) amu
Melting Point: 302.0 °C (575.15 K, 575.6 °F)
Boiling Point: 337.0 °C (610.15 K, 638.6 °F)
Number of Protons/Electrons: 85
Number of Neutrons: 125

Classification: Halogen
Crystal Structure: Unknown
Density @ 293 K: Unknown
Color: Unknown
Date of Discovery: 1940
Discoverer: D.R. Corson
Name Origin: From the Greek word *astatos* (unstable)
Uses: No uses known
Obtained From: Man-made

Atomic Structure



Isotopes

Isotope	Half Life
At-206	29.4 minutes
At-208	1.6 hours
At-211	7.2 hours
At-215	0.1 milliseconds
At-217	32.0 milliseconds
At-218	1.6 seconds
At-219	50.0 seconds



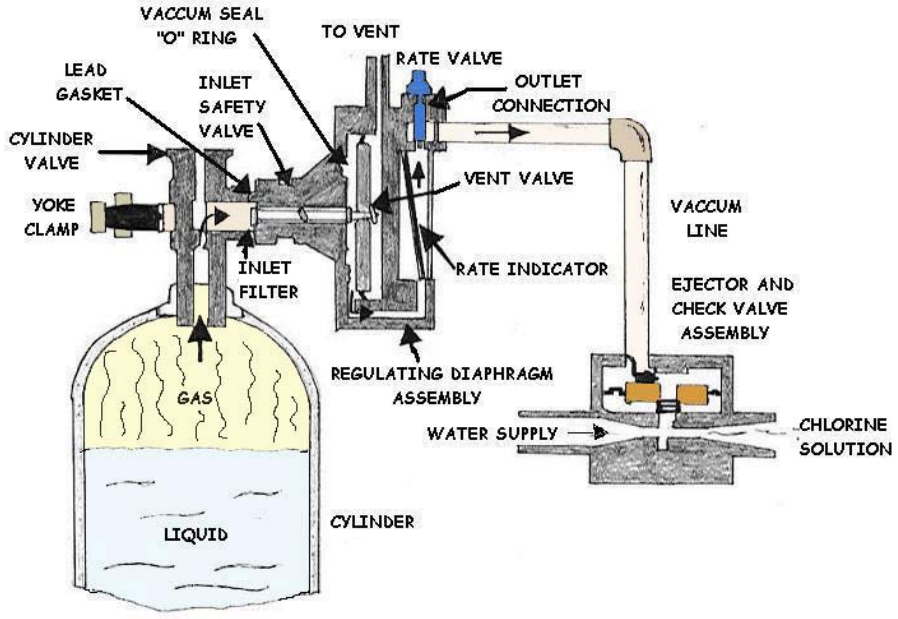
1 Ton Containers

The top line or valve is for extracting the gas, and the bottom line is for extracting the Cl2 liquid. Never place water on a leaking metal cylinder. The water will help create acid which will make the leak larger.





150 Pound Chlorine Cylinder



Chlorine Exposure Limits and Related Information

This information is necessary to pass your pre-test.

*** OSHA PEL 1 PPM - IDLH 10 PPM and Fatal Exposure Limit 1,000 PPM**

The current Occupational Safety and Health Administration (**OSHA**) permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level. *** IDLH 10 PPM**

Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Monochloramine, dichloramine, and trichloramine are also known as Combined Available Chlorine. $Cl_2 + NH_4$.

HOCl and OCl-; The **OCL-** is the hypochlorite ion and both of these species are known as free available chlorine. These are the two main chemical species formed by chlorine in water and they are known collectively as hypochlorous acid and the hypochlorite ion. When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equation that best describes this reaction is **$Cl2 + H_2O$ --> H+ + Cl- + HOCl.** Hypochlorous acid is the most germicidal of the chlorine compounds with the possible exception of chlorine dioxide.

Yoke-type connectors should be used on a chlorine cylinder's valve, assuming that the threads on the valve may be worn.

The connection from a chlorine cylinder to a chlorinator should be replaced by using a new, approved gasket on the connector. Always follow your manufacturer's instructions.

On 1 ton Chlorine gas containers, the chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator. This is the liquid chlorine supply line and it is going to be made into Chlorine gas.

In water treatment, chlorine is added to the effluent before the contact chamber (before the clear well) for complete mixing. One reason for not adding it directly to the chamber is that the chamber has very little mixing due to low velocities.

Here are several safety precautions when using chlorine gas. In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate. Emergency procedures in the case of a large uncontrolled chlorine leak are as follows: Notify local emergency response team, warn and evacuate people in adjacent areas, and be sure that no one enters the leak area without adequate self-contained breathing equipment.

Here are several symptoms of chlorine exposure. Burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting, headaches and dizziness, fatal pulmonary edema, pneumonia, and skin blisters. A little Cl_2 will corrode the teeth and then progress to throat cancer.

Approved method for storing a 150 - 200 pound chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve and firmly secure each cylinder. Never store near heat. Always store the empty in an upright, secure position with proper signage.



ABC Repair Kit



Rail Car Tanker

Chlorine Timeline

1879
- This marked the first time that chlorine was applied as a disinfectant. William Soper of England treated the feces of typhoid patients before disposal into the sewer. He used chlorinated lime, which was a common form of chlorine used initially. (White, 1972)

1893
- This date was the first time that chlorine was applied as a disinfectant on a plant scale basis. This application was made at Hamburg, Germany. (White, 1972)

1903
- This marked the first time chlorine gas was used as a disinfectant in drinking water. This took place in Middlekerke, Belgium. Prior to this date, chlorine was applied through the use of hydrated lime, chloride of lime, or bleaching powder. The use of chlorine gas was designed by Maurice Duyk, a chemist for the Belgian Ministry of Public Works. (Pontius, 1990)

1908
- The first full scale chlorine installation at a drinking water plant in the United States was initiated in this year. This installation took place at the Bubbly Creek Filter Plant in Chicago. This plant served the Chicago Stockyards and was designed by George A. Johnson. The raw water contained a large amount of sewage which was causing sicknesses in the livestock. Johnson implemented chlorine through chloride of lime, and the bacterial content of the water dropped drastically. (Pontius, 1990)

1910
- C. R. Darnall became the first to use compressed chlorine gas from steel cylinders, which is an approach still commonly used today. His installation was in Youngstown, Ohio. His implementation used a pressure-reducing mechanism, a metering device, and an absorption chamber. It was moderately successful, but his setup was only used once.

1912
- John Kienle, chief engineer of the Wilmington, Delaware water department, invented another way to apply chlorine to drinking water. He developed a way to push compressed chlorine from cylinders into an absorption tower in which water was flowing opposite the flow of the chlorine. Because the gas flow was opposite the water flow, the chlorine was able to disinfect the water. (Pontius, 1990)

1913
- An Ornstein chlorinator was installed at Kienle's Wilmington, Delaware water treatment plant. This marked the first time a commercial chlorination system was installed at a municipal water treatment plant. The chlorinator used the same basic premise that Kienle's previous installation did, but the Ornstein chlorinator used both a high and low pressure gauge to more accurately control the amount of chlorine added to the system. (Pontius, 1990)

1914
- On October 14, 1914, the Department of the Treasury enacted the first set of standards that required the use of disinfection for drinking water. These standards called for a maximum level of bacterial concentration of 2 coliforms per 100 milliliters. Because chlorination was the main disinfectant at the time, these standards dramatically increased the number of treatment plants using chlorine. (White, 1972)

1919
- Two important discoveries were made during this year. Wolman and Enslow discovered the concept of chlorine demand which states that the amount of chlorine needed to disinfect the water is related to the concentration of the waste and the amount of time the chlorine has to contact the water. The other important discovery of 1919 was by Alexander Houston. He discovered that chlorine can also eliminate taste and odor problems in water. (Pontius, 1990)

1925
- New drinking water standards were enacted that reduced the maximum permissible limit of coliforms from 2 to 1 coliform per 100 milliliters. This increased the amount and frequency of chlorination again. (White, 1972)

1939
- The theory of the chlorine breakpoint was discovered in this year. Chlorine breakpoint theory is discussed in the following section. (White, 1972)

1960
- A new implementation practice was discovered in this year. The compound loop principle of chlorinator control was implemented, which is the most recent major discovery in chlorine application. (White, 1972)

1972
- A report entitled "Industrial Pollution of the Lower Mississippi River in Louisiana" was published containing the first evidence of disinfection byproducts in drinking water resulting from organic pollution in source water. (Pontius, 1990)

As is evident by the dates in the timeline, most of the innovation in chlorination occurred over 70 years ago. Very few innovations or discoveries have been made recently. Most of the current research is being performed in other areas of disinfection. These areas include ozone, chlorine dioxide, and UV radiation. Chlorine is still the most widely used disinfectant in the United States, but other areas of the world are beginning to use other methods of disinfection with increasing frequency. Since chlorine is still widely used, a thorough understanding of how it disinfects and is implemented is important to those interested in water treatment.

Chlorine Supplement **Pre-Quiz**

1. How should the connection from a chlorine cylinder to a chlorinator be replaced?

2. How many turns should a chlorine gas cylinder be initially opened?

3. If the temperature of a full chlorine cylinder is increased by 50°F or 30°C, what is the most likely result?

4. What is meant by the specific gravity of a liquid?

5. Which metals are the only metals that are ***TOTALLY*** inert to moist chlorine gas?

6. What will be discharged when opening the top valve on a one-ton chlorine cylinder?

7. What are the approved methods for storing a chlorine cylinder?

8. What are normal conditions for a gas chlorination start-up?

9. Name a safety precaution when using chlorine gas?

10. What compounds are formed in water when chlorine gas is introduced?

11. Why should roller bearings not be used to rotate a one-ton chlorine cylinder?

12. What are the physical and chemical properties of chlorine?

13. What are the necessary emergency procedures in the case of a large uncontrolled chlorine leak?

14. Name several symptoms of chlorine exposure.

15. 5 lbs. of a 70% concentration sodium hypochlorite solution is added to a tank containing 650 gallons of water. What is the chlorine dosage?

16. As soon as Cl_2 gas enters the throat area, a victim will sense a sudden stricture in this area - nature's way of signaling to prevent passage of the gas to the lungs. At this point, the victim must attempt to do two things. Name them.

17. Positive pressure SCBAs and full face piece SARs can be used in oxygen deficient atmospheres containing less than what percentage of oxygen in the atmosphere?

18. Death is possible from asphyxia, shock, reflex spasm in the larynx, or massive pulmonary edema. Populations at special risk from chlorine exposure are individuals with pulmonary disease, breathing problems, bronchitis, or chronic lung conditions.
A. TRUE
B. FALSE

19. Chlorine gas reacts with water producing a strongly oxidizing solution causing damage to the moist tissue lining the respiratory tract when the tissue is exposed to chlorine. The respiratory tract is rapidly irritated by exposure to 10-20 ppm of chlorine gas in air, causing acute discomfort that warns of the presence of the toxicant.
A. TRUE
B. FALSE

20. Even brief exposure to 1,000 ppm of Cl_2 can be fatal.
A. TRUE
B. FALSE

21. What are the two main chemical species formed by chlorine in water and what name are they are known collectively as?

22. When chlorine gas is added to water, it rapidly hydrolyzes according to the reaction:

23. Which chemical reaction equation represents the dissociation of hypochlorous acid?

24. This species of chlorine is the most germicidal of all chlorine compounds with the possible exception of chlorine dioxide.



Here are both half ton container and 150 pound chlorine gas cylinders.
Answers in rear section before the final assignment.



Chlorine evaporators next to a rail tank chlorine cylinder. Bottom photograph, SCBA should always be stored on the outside of the chlorine storage room.





Chlorine C repair kit, used for the tank cars and tankers.



Chlorine Introduction



Name: Chlorine
Symbol: Cl
Atomic Number: 17
Atomic Mass: 35.4527 amu
Melting Point: -100.98 °C (172.17 K, -149.764 °F)
Boiling Point: -34.6 °C (238.55 K, -30.279997 °F)
Number of Protons/Electrons: 17
Number of Neutrons: 18
Classification: Halogen
Crystal Structure: Orthorhombic
Density @ 293 K: 3.214 g/cm^3
Color: Green
Uses: Water purification, bleaches
Obtained From: Salt
Date of Discovery: 1774
Discoverer: Carl Wilhelm Scheele
Name Origin: From the Greek word *khlôros* (green)

Chlorine Gas Information
Identifiers
1. CAS No.: 7782-50-5
2. RTECS No.: FO2100000
3. DOT UN: 1017 20
4. DOT label: Poison gas



Chlorinators

Safety Data
NIOSH IDHL: 25 ppm
NIOSH Ceiling: **0.5ppm/15 minutes**
PEL/TWA: **1 ppm**
TLV/TWA: **1 ppm**
TLV/STEL: **3 ppm**
TLV/IDLH: **25 ppm**

Physical Data
1. **Molecular weight:** 70.9
2. **Boiling point (at 760 mm Hg):** -34.6 degrees C (-30.28 degrees F)
3. **Specific gravity (liquid):** 1.41 at 20 degrees C (68 degrees F) and a pressure of 6.86 atm
4. **Vapor density**: 2.5
5. **Melting point:** -101 degrees C (-149.8 degrees F)
6. **Vapor pressure at 20 degrees C (68 degrees F):** 4,800 mm Hg
7. **Solubility**: Slightly soluble in water; soluble in alkalis, alcohols, and chlorides.
8. **Evaporation rate:** Data not available.

Chlorine's Appearance and Odor
Chlorine is a greenish-yellow gas with a characteristic pungent odor. It condenses to an amber liquid at approximately -34 degrees C (-29.2 degrees F) or at high pressures. Odor thresholds ranging from 0.08 to part per million (ppm) parts of air have been reported. Prolonged exposures may result in olfactory fatigue.

Reactivity
1. **Conditions Contributing to Instability**: Cylinders of chlorine may burst when exposed to elevated temperatures. Chlorine in solution forms a corrosive material.
2**. Incompatibilities**: Flammable gases and vapors form explosive mixtures with chlorine. Contact between chlorine and many combustible substances (such as gasoline and petroleum products, hydrocarbons, turpentine, alcohols, acetylene, hydrogen, ammonia, and sulfur), reducing agents, and finely divided metals may cause fires and explosions. Contact between chlorine and arsenic, bismuth, boron, calcium, activated carbon, carbon disulfide, glycerol, hydrazine, iodine, methane, oxomonosilane, potassium, propylene, and silicon should be avoided. Chlorine reacts with hydrogen sulfide and water to form hydrochloric acid, and it reacts with carbon monoxide and sulfur dioxide to form phosgene and sulfuryl chloride. Chlorine is also incompatible with moisture, steam, and water.
3. **Hazardous Decomposition Products:** None reported.
4**. Special Precautions:** Chlorine will attack some forms of plastics, rubber, and coatings.

Flammability
Chlorine is a non-combustible gas.
The National Fire Protection Association has assigned a flammability rating of 0 (no fire hazard) to chlorine; however, most combustible materials will burn in chlorine.
1. **Flash point:** Not applicable.
2. **Autoignition temperature**: Not applicable.
3. **Flammable limits in air:** Not applicable.
4. **Extinguishant:** For small fires use water only; do not use dry chemical or carbon dioxide. Contain and let large fires involving chlorine burn. If fire must be fought, use water spray or fog.

Fires involving chlorine should be fought upwind from the maximum distance possible.

Keep unnecessary people away; isolate the hazard area and deny entry. For a massive fire in a cargo area, use unmanned hose holders or monitor nozzles; if this is impossible, withdraw from the area and let the fire burn. Emergency personnel should stay out of low areas and ventilate closed spaces before entering.

Containers of chlorine may explode in the heat of the fire and should be moved from the fire area if it is possible to do so safely. If this is not possible, cool fire exposed containers from the sides with water until well after the fire is out. Stay away from the ends of containers. Firefighters should wear a full set of protective clothing and self-contained breathing apparatus when fighting fires involving chlorine.

Chlorine Exposure Limits

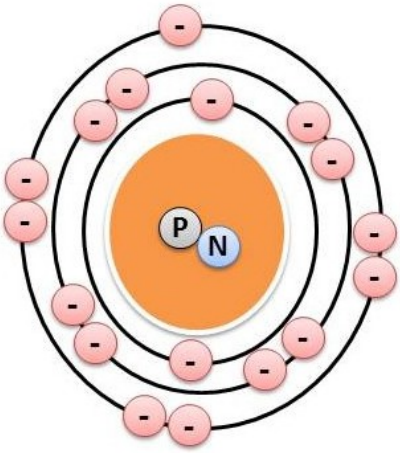
*** OSHA PEL**
The current **OSHA** permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level [29 CFR 1910.1000, Table Z-1].

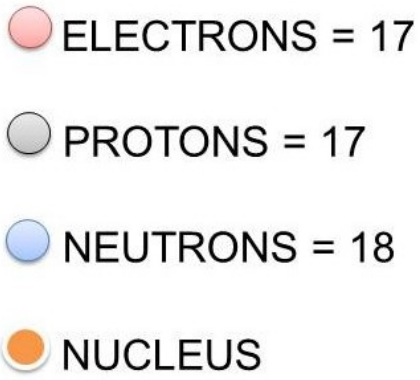
*** NIOSH REL**
The National Institute for Occupational Safety and Health (**NIOSH**) has established a recommended exposure limit (**REL**) for chlorine of 0.5 ppm $mg/m^{(3)}$) as a TWA for up to a 10-hour workday and a 40-hour workweek and a short-term exposure limit (**STEL**) of 1 ppm (3 $mg/m^{(3)}$)[NIOSH 1992].

*** ACGIH TLV**
The American Conference of Governmental Industrial Hygienists (**ACGIH**) has assigned chlorine a threshold limit value (**TLV**) of 0.5 ppm (1.5 $mg/m^{(3)}$) as a TWA for a normal 8-hour workday and a 40-hour workweek and a **STEL** of 1 ppm (2.9 $mg/m^{(3)}$) for periods not to exceed 15 minutes. Exposures at the STEL concentration should not be repeated more than four times a day and should be separated by intervals of at least 60 minutes [ACGIH 1994, p. 15].

*** Rationale for Limits**
The NIOSH limits are based on the risk of severe eye, mucous membrane and skin irritation [NIOSH 1992]. The ACGIH limits are based on the risk of eye and mucous membrane irritation [ACGIH 1991, p. 254].

Chlorine's Atomic Structure





Isotopes

Isotope	Half Life
Cl-35	Stable
Cl-36	301000.0 years
Cl-37	Stable
Cl-38	37.2 minutes



Top photograph, this blue device prevents the liquid from being pulled and freezing the lines. Bottom photograph, the application of an ammonia mist to detect a chlorine gas leak.



Chlorine Basics

Chlorine is one of 90 natural elements, the basic building blocks of our planet. To be useful, an element must be relatively abundant or have extremely desirable properties. Chlorine has both characteristics. As a result -- over the course of many decades of careful research and development -- scientists have learned to use chlorine and the products of chlorine chemistry to make drinking water safe, destroy life-threatening germs, produce life-saving drugs and medical equipment, shield police and fire fighters in the line of duty, and ensure a plentiful food supply.

In 1774, in his small experimental laboratory, Swedish pharmacist Carl Wilhem Scheele released a few drops of hydrochloric acid onto a piece of manganese dioxide. Within seconds, a greenish-yellow gas arose. Although he had no idea at the time, he had just discovered chlorine.

The fact that the greenish-yellow gas was actually an element was only recognized several decades later by English chemist Sir Humphrey Davy. Until that time, people were convinced that the gas was a compound of oxygen. Davy gave the element its name on the basis of the Greek word khloros, for greenish-yellow. In 1810 he suggested the name "*chloric gas*" or "*chlorine*."

One of the most effective and economical germ-killers, chlorine also destroys and deactivates a wide range of dangerous germs in homes, hospitals, swimming pools, hotels, restaurants, and other public places. Chlorine's powerful disinfectant qualities come from its ability to bond with and destroy the outer surfaces of bacteria and viruses. First used as a germicide to prevent the spread of "child bed fever" in the maternity wards of Vienna General Hospital in Austria in 1846, chlorine has been one of society's most potent weapons against a wide array of life-threatening infections, viruses, and bacteria for 150 years.

When the first men to set foot on the moon returned to earth (Apollo 11 mission: 24.7.69) a hypochlorite solution was chosen as one of the disinfectants for destroying any possible moon germs.

What Happens to Chlorine When it Enters the Environment?

- When released to air, chlorine will react with water to form hypochlorous acid and hydrochloric acid, which are removed from the atmosphere by rainfall.

- Chlorine is slightly soluble in water. It reacts with water to form hypochlorous acid and hydrochloric acid. The hypochlorous acid breaks down rapidly. The hydrochloric acid also breaks down; its breakdown products will lower the pH of the water (makes it more acidic).

- Since chlorine is a gas it is rarely found in soil. If released to soil, chlorine will react with moisture forming hypochlorous acid and hydrochloric acid. These compounds can react with other substances found in soil.

- Chlorine does not accumulate in the food chain.

Disinfectant Qualities

Restaurants and meat and poultry processing plants rely on chlorine bleach and other chlorine-based products to kill harmful levels of bacteria such as *Salmonella* and *E. coli* on food preparation surfaces and during food processing. Chlorine is so important in poultry processing that the US Department of Agriculture requires an almost constant chlorine rinse for much of the cutting equipment. In fact, no proven economical alternative to chlorine disinfection exists for use in meat and poultry processing facilities.

Properties

Because it is highly reactive, chlorine is usually found in nature bound with other elements like sodium, potassium, and magnesium. When chlorine is isolated as a free element, chlorine is a greenish yellow gas, which is 2.5 times heavier than air. It turns to a liquid state at -34°C (-29°F), and it becomes a yellowish crystalline solid at -103°C (-153°F). Chemists began experimenting with chlorine and chlorine compounds in the 18th century. They learned that chlorine has an extraordinary ability to extend a chemical bridge between various elements and compounds that would not otherwise react with each other. Chlorine has been especially useful in studying and synthesizing organic compounds -- compounds that have at least one atom of the element carbon in their molecular structure. All living organisms, including humans, are composed of organic compounds.

Chlorine is one of the most abundant chemical elements on Earth. It is ubiquitous in soils, minerals, plants and animals. Seawater is a huge reservoir of dissolved chlorine weathered from the continents and transported to the oceans by Earth's rivers.

Chlorine is also one of the most useful chemical elements. Each chemical element has its own set of unique properties and chlorine is known as a very reactive element--so reactive, in fact, that it is usually found combined with other elements in the form of compounds. More than 3,500 naturally occurring chlorinated organic (associated with living organisms) compounds alone have been identified.

Chlorine's chemical properties have been harnessed innovatively for good use. For example, this element plays a huge role in public health. Chlorine-based disinfectants are capable of removing a wide variety of disease-causing germs from drinking water and wastewater as well as from hospital and food production surfaces. Additionally, chlorine plays an important role in the manufacture of thousands of products we depend upon every day, including such diverse items as cars, computers, pharmaceuticals and military flak jackets. As the ninth largest chemical produced in the U.S. by volume, chlorine is truly a "workhorse chemical."

Released From the Salt of the Earth

Chlorine is produced industrially from the compound sodium chloride, one of the many salts found in geologic deposits formed from the slow evaporation of ancient seawater. When electricity is applied to a brine solution of sodium chloride, chlorine gas (Cl2), caustic soda (NaOH) and hydrogen gas (H_2) are generated according to the following reaction:

Salt	+	Water	→	Chlorine gas	+	Caustic soda	+	Hydrogen gas
$2\ NaCl$	+	$2\ H_2O$	→	Cl_2	+	$2\ NaOH$	+	H_2

Co-Products
As the reaction demonstrates, chlorine gas cannot be produced without producing caustic soda, so chlorine and caustic soda are known as "co-products," and their economics are inextricably linked. Caustic soda, also called "alkali," is used to produce a wide range of organic and inorganic chemicals and soaps. In addition, the pulp and paper, alumina and textiles industries use caustic soda in their manufacturing processes. Thus, the "chlor-alkali" industry obtains two very useful chemicals by applying electrical energy to sea salt.



Definitions

Chlorine Gas Feed Room
A chlorine gas feed room, for the purposes of this document, is a room that contains the chlorinator(s) and active cylinder(s) used to apply chlorine gas at a water or wastewater facility.

Chlorine Gas Storage Room
A chlorine gas storage room, for the purposes of this document, is a room other than a chlorine gas feed room, in which full, partial, or empty chlorine gas cylinders or ton containers are stored at a water or wastewater facility.

Gas Chlorinator
A gas chlorinator is a device used to meter and control the application rate of chlorine gas into a liquid. There is the danger of the gas escaping at a water or wastewater treatment facility. The gas chlorinator should be isolated from a water or wastewater treatment plant.

Chlorine Cabinet
A chlorine cabinet is a pre-assembled or factory built unit that contains the equipment used to apply chlorine gas at a water or wastewater treatment facility. It is isolated from a water or wastewater treatment plant.



Top photograph, a view of the top of a 150 gas cylinder. Bottom, always work in pairs when working around Chlorine. Here the hoist is being used to move the container.



Chemical Equations, Oxidation States, and Balancing of Equations

Before we breakdown chlorine and other chemicals, let's start with this review of basic chemical equations.

Beginning
The common chemical equation could be A + B --> C + D. This is chemical A + chemical B, the two reacting chemicals will go to products C + D, etc.

Oxidation
The term "oxidation" originally meant a reaction in which oxygen combines chemically with another substance, but its usage has long been broadened to include any reaction in which electrons are transferred.

Oxidation and reduction always occur simultaneously (redox reactions), and the substance which gains electrons is termed the oxidizing agent. For example, cupric ion is the oxidizing agent in the reaction: Fe (metal) + Cu++ --> Fe++ + Cu (metal); here, two electrons (negative charges) are transferred from the iron atom to the copper atom; thus the iron becomes positively charged (is oxidized) by loss of two electrons, while the copper receives the two electrons and becomes neutral (is reduced).

Electrons may also be displaced within the molecule without being completely transferred away from it. Such partial loss of electrons likewise constitutes oxidation in its broader sense and leads to the application of the term to a large number of processes, which at first sight might not be considered to be oxidation. Reaction of a hydrocarbon with a halogen, for example, CH_4 + 2 Cl --> CH_3Cl + HCl, involves partial oxidation of the methane; halogen addition to a double bond is regarded as an oxidation.

Dehydrogenation is also a form of oxidation; when two hydrogen atoms, each having one electron, are removed from a hydrogen-containing organic compound by a catalytic reaction with air or oxygen, as in oxidation of alcohol to aldehyde.

Oxidation Number
The number of electrons that must be added to or subtracted from an atom in a combined state to convert it to the elemental form; i.e., in barium chloride ($BaCl_2$) the oxidation number of barium is +2 and of chlorine is -1. Many elements can exist in more than one oxidation state.

Now, let us look at some common ions. An ion is the reactive state of the chemical, and is dependent on its place within the periodic table.

Have a look at the "periodic table of the elements". It is arranged in columns of elements, there are 18 columns. You can see column one, H, Li, Na, K, etc. These all become ions as H^+, Li^+, K^+, etc. The next column, column 2, Be, Mg, Ca etc. become ions Be^{2+}, Mg^{2+}, Ca^{2+}, etc. Column 18, He, Ne, Ar, Kr are inert gases. Column 17, F, Cl, Br, I, ionize to a negative F^-, Cl^-, Br^-, I^-, etc.

What you now need to do is memorize the table of common ions, both positive ions and negative ions.

Table of Common Ions

Positive Ions

Valency 1		Valency 2		Valency 3	
lithium	Li^+	magnesium	Mg_2^+	aluminum	Al_3^+
sodium	Na^+	calcium	Ca_2^+	iron III	Fe_3^+
potassium	K^+	strontium	Sr_2^+	chromium	Cr_3^+
silver	Ag^+	barium	Ba_2^+		
hydronium	H_3O^+	copper II	Cu_2^+		
(or hydrogen)	H^+	lead II	Pb_2^+		
ammonium	NH_4^+	zinc	Zn_2^+		
copper I	Cu^+	manganese II	Mn_2^+		
mercury I	Hg^+	iron II	Fe_2^+		
		tin II	Sn_2^+		

Negative Ions

Valency 1		Valency 2		Valency 3	
fluoride	F^-	oxide	O_2^-	phosphate	PO_4^{3-}
chloride	Cl^-	sulfide	S_2^-		
bromide	Br^-	carbonate	CO_3^{2-}		
iodide	I^-	sulfate	SO_4^{2-}		
hydroxide	OH^-	sulfite	SO_3^{2-}		
nitrate	NO_3^-	dichromate	$Cr_2O_7^-$		
bicarbonate	HCO_3^-	chromate	CrO_4^{2-}		
bisulphate	HSO_4^-	oxalate	$C_2O_4^{2-}$		
nitrite	NO_2^-	thiosulfate	$S_2O_3^{2-}$		
chlorate	ClO_3^-	tetrathionate	$S_4O_6^{2-}$		
permanganate	MnO_4^-	monohydrogen phosphate	HPO_4^{2-}		
hypochlorite	OCl^-				
dihydrogen phosphate	$H_2PO_4^-$				

Positive ions will react with negative ions, and vice versa. This is the start of our chemical reactions. For example:

$Na^+ + OH^- \rightarrow NaOH$ (sodium hydroxide)

$Na^+ + Cl^- \rightarrow NaCl$ (salt)

$3H^+ + PO_4^{3-} \rightarrow H_3PO_4$ (phosphoric acid)

$2Na^+ + S_2O_3^{2-} \rightarrow Na_2S_2O_3$

You will see from these examples, that if an ion of one (+), reacts with an ion of one (-) then the equation is balanced. However, an ion like PO_4^{3-} (phosphate) will require an ion of 3+ or an ion of one (+) (but needs three of these) to neutralize the 3- charge on the phosphate. So, what you are doing is balancing the charges (+) or (-) to make them zero, or cancel each other out.

For example, since aluminum exists in its ionic state as Al^{3+}, it will react with many negatively charged ions; for example: Cl^-, OH^-, SO_4^{2-}, PO_4^{3-}.

Let us do these examples and balance them.
$Al^{3+} + Cl^- \rightarrow AlCl$ **(incorrect)**
$Al^{3+} + 3Cl^- \rightarrow AlCl_3$ **(correct)**

How did we work this out?
Al^{3+} has three positives (3+)
Cl^- has one negative (-)
It will require **3 negative charges** to cancel out the **3 positive charges** on the aluminum (Al^{3+}).

When the **left hand side** of the equation is written, to balance the number of chlorine's (Cl^-) required, the number 3 is placed in front of the ion concerned, in this case Cl^-, becomes $3Cl^-$.

On the **right hand side** of the equation, where the ions have become a compound (a chemical compound), the number is transferred to after the relevant ion, Cl_3.

Another example:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ (**incorrect**)
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$ (**correct**)

Let me give you an easy way of balancing:
Al is 3+
SO_4 is 2-
Simply transpose the number of positives (or negatives) for each ion, to the other ion, by placing this value of one ion, in front of the other ion. That is, Al^{3+} the 3 goes in front of the SO_4^{2-} as $3SO_4^{2-}$, and SO_4^{2-}, the 2 goes in front of the Al^{3+} to become $2Al^{3+}$. Then on the **right hand side** of the equation, this same number (now in front of each ion on the **left side** of the equation), is placed after each "ion" entity.

Let us again look at:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ **(incorrect)**
$Al^{3+} + SO_4^{2-} \rightarrow Al_2(SO_4)_3$ **(correct)**
Put the three from the Al in front of the SO_4^{2-} and the 2 from the SO_4^{2-} in front of the Al^{3+}.
Equation becomes:
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$. You simply place the valency of one ion, as a whole number, in front of the other ion, and vice versa.

Remember to encase the SO_4 in brackets. **Why?** Because we are dealing with the sulfate ion, SO_4^{2-}, and it is this ion that is 2- charged (not just the O_4), so we have to ensure that the "ion" is bracketed. Now to check, the 2 times $3^+ = 6^+$, and 3 times $2^- = 6^-$. We have equal amounts of positive ions, and equal amounts of negative ions.

Another example:
NaOH + HCl --> ?
Na is Na^+, OH is OH^-, so this gave us NaOH. Originally, the one positive canceled the one negative.

HCl is $H^+ + Cl^-$, this gave us HCl.

Reaction is going to be the Na^+ reacting with a negatively charged ion. This will have to be the chlorine, Cl^-, because at the moment the Na^+ is tied to the OH^-. **So:** $Na^+ + Cl^-$ --> NaCl
The H+ from the HCl will react with a negative (-) ion this will be the OH^- from the NaOH.
So: $H^+ + OH^-$ --> H_2O (water).

The complete reaction can be written:
NaOH + HCl --> NaCl + H_2O. We have **equal amounts** of all atoms **each side** of the equation, so the equation is **balanced**.
or
$Na^+OH^- + H^+Cl^-$ --> $Na^+Cl^- + H^+OH^-$

Something More Difficult:
$Mg(OH)_2 + H_3PO_4$ --> ? (equation on left **not** balanced)
Mg^{2+} $2OH^- + 3H^+PO_4^{3-}$ --> ? (equation on left **not** balanced), so let us rewrite the equation in **ionic form**.

The Mg^{2+} **needs to react with a negatively charged ion**, this will be the PO_4^{3-},
so: $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$
(**Remember** the **swapping** of the positive or negative charges on the ions in the **left side** of the equation, and placing it in front of each ion, and then placing this number after each ion on the **right side** of the equation)

What is left is the H^+ from the H_3PO_4 and this will react with a negative ion, we only have the OH^- from the $Mg(OH)_2$ left for it to react with.
$6H^+ + 6OH^-$ --> $6H_2O$

Where did I get the 6 from? When I balanced the Mg^{2+} with the PO_4^{3-}, the equation became $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$

Therefore, I must have required $3Mg(OH)_2$ to begin with, and $2H_3PO_4$, (because we originally had $(OH)_2$ attached to the Mg, and H_3 attached to the PO_4. I therefore have $2H_3$ reacting with $3(OH)_2$. We have to write this, on the **left side** of the equation, as $6H^+ + 6OH^-$ because we need it in ionic form.
The equation becomes:
$6H^+ + 6OH^-$ --> $6H_2O$

The full equation is now balanced and is:
$3Mg(OH)_2 + 2H_3PO_4$ --> $Mg_3(PO_4)_2 + 6H_2O$
I have purposely split the equation into segments of reactions. This is showing you which ions are reacting with each other. Once you get the idea of equations you will not need this step.

The balancing of equations is simple. You need to learn the valency of the common ions (see tables). The rest is pure mathematics; you are balancing valency charges, positives versus

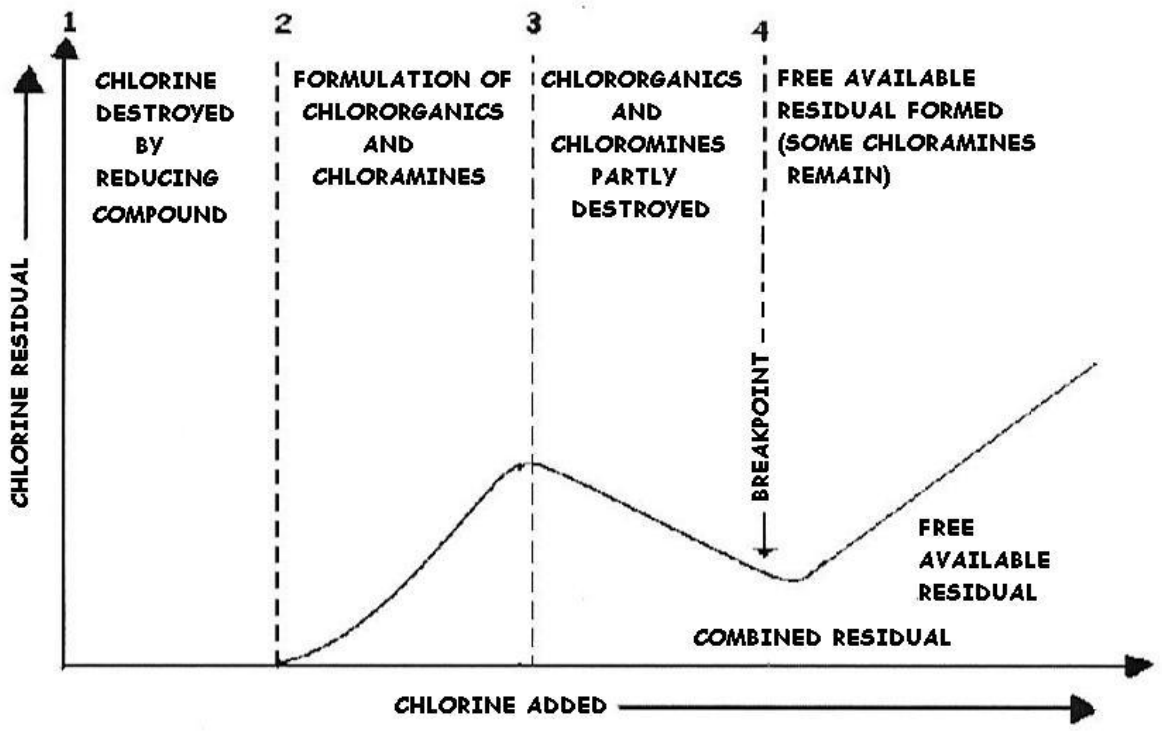
negatives. You have to have the **same number** of **negatives**, or **positives**, each side of the equation, and the **same number** of **ions** or **atoms** each side of the equation.

If one ion, example Al^{3+}, (3 positive charges) reacts with another ion, example OH^- (one negative ion) then we require 2 more negatively charged ions (in this case OH^-) to counteract the 3 positive charges the Al^{3+} contains.

Take my earlier hint, place the 3 from the Al^{3+} in front of the OH^-, now reads $3OH^-$, place the 1 from the hydroxyl OH^- in front of the Al^{3+}, now stays the same, Al^{3+} (the 1 is **never** written in chemistry equations).

$Al^{3+} + 3OH^- \rightarrow Al(OH)_3$

The 3 is simply written in front of the OH^-, a recognized ion, there are no brackets placed around the OH^-. On the right hand side of the equation, all numbers in front of each ion on the left hand side of the equation are placed after each same ion on the right side of the equation. Brackets are used in the right side of the equation because the result is a compound. Brackets are also used for compounds (reactants) in the left side of equations, as in $3Mg(OH)_2 + 2H_3PO_4 \rightarrow ?$





Hard to tell, but these are one ton chlorine gas containers. Notice the five gallon bucket of motor oil in the bottom photograph. Also notice that this photograph is the only eye wash station that we found during our inspection of 10 different facilities. Do you have an eye wash and emergency shower?



Chemistry of Chlorination

Chlorine can be added as sodium hypochlorite, calcium hypochlorite or chlorine gas. When any of these is added to water, chemical reactions occur as these equations show:

Cl 2 + H 2 O → HOCl + HCl
(chlorine gas) (water) (hypochlorous acid) (hydrochloric acid)

CaOCl + H 2 O → 2HOCl + Ca(OH)
(calcium hypochlorite) (water) (hypochlorous acid) (calcium hydroxide)

NaOCl + H 2 O → HOCl + Na(OH)
(sodium hypochlorite) (water) (hypochlorous acid) (sodium hydroxide)

All three forms of chlorine produce hypochlorous acid (HOCl) when added to water. Hypochlorous acid is a weak acid but a strong disinfecting agent. The amount of hypochlorous acid depends on the pH and temperature of the water. Under normal water conditions, hypochlorous acid will also chemically react and break down into a hypochlorite. ion

(OCl -): HOCl H + + OCl – Also expressed HOCl → H + + OCl –
(hypochlorous acid) (hydrogen) (hypochlorite ion)
The hypochlorite ion is a much weaker disinfecting agent than hypochlorous acid, about 100 times less effective.

Let's now look at how pH and temperature affect the ratio of hypochlorous acid to hypochlorite ions. As the temperature is decreased, the ratio of hypochlorous acid increases. Temperature plays a small part in the acid ratio. Although the ratio of hypochlorous acid is greater at lower temperatures, pathogenic organisms are actually harder to kill. All other things being equal, higher water temperatures and a lower pH are more conducive to chlorine disinfection.

Types of Residual

If water were pure, the measured amount of chlorine in the water should be the same as the amount added. But water is not 100% pure. There are always other substances (interfering agents) such as iron, manganese, turbidity, etc., which will combine chemically with the chlorine.

This is called the ***chlorine demand.*** Naturally, once chlorine molecules are combined with these interfering agents, they are not capable of disinfection. It is free chlorine that is much more effective as a disinfecting agent.

So let's look now at how free, total and combined chlorine are related. When a chlorine residual test is taken, either a total or a free chlorine residual can be read.

Total residual is all chlorine that is available for disinfection.

Total chlorine residual = free + combined chlorine residual.

Free chlorine residual is a much stronger disinfecting agent. Therefore, most water regulating agencies will require that your daily chlorine residual readings be of free chlorine residual.

Break-point chlorination is where the chlorine demand has been satisfied, and any additional chlorine will be considered ***free chlorine*.**

Residual Concentration/Contact Time (CT) Requirements

Disinfection to eliminate fecal and coliform bacteria may not be sufficient to adequately reduce pathogens such as Giardia or viruses to desired levels. Use of the ***"CT"*** disinfection concept is recommended to demonstrate satisfactory treatment, since monitoring for very low levels of pathogens in treated water is analytically very difficult.

The CT concept, as developed by the United States Environmental Protection Agency (Federal Register, 40 CFR, Parts 141 and 142, June 29, 1989), uses the combination of disinfectant residual concentration (mg/L) and the effective disinfection contact time (in minutes) to measure effective pathogen reduction. The residual is measured at the end of the process, and the contact time used is the T10 of the process unit (time for 10% of the water to pass).

CT = Concentration (mg/L) x Time (minutes)

The effective reduction in pathogens can be calculated by reference to standard tables of required CTs.

Required Giardia/Virus Reduction
All surface water treatment systems shall ensure a minimum reduction in pathogen levels: 3-log reduction in Giardia; and 4-log reduction in viruses.

These requirements are based on unpolluted raw water sources with Giardia levels of = 1 cyst/100 L, and a finished water goal of 1 cyst/100,000 L (equivalent to 1 in 10,000 risk of infection per person per year). Higher raw water contamination levels may require greater removals as shown on Table 4.1.

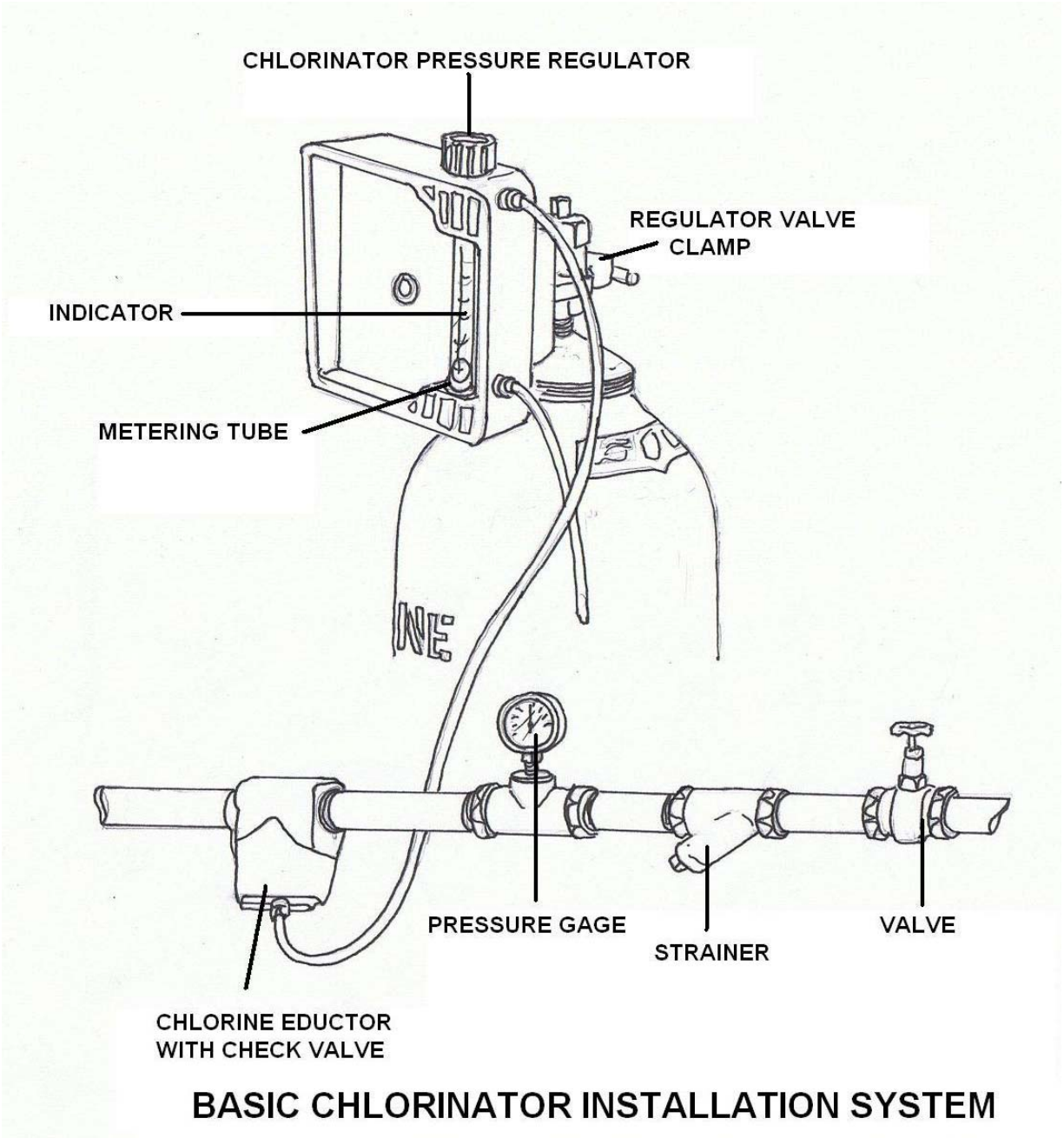
TABLE 4.1
Level of Giardia Reduction
Raw Water Giardia Levels*
Recommended Giardia Log Reduction
< 1 cyst/100 L 3-log
1 cyst/100 L - 10 cysts/100 L 3-log - 4-log
10 cysts/100 L - 100 cysts/100 L 4-log - 5-log
> 100 cysts/100 L > 5-log
*Use geometric means of data to determine raw water Giardia levels for compliance.

Required CT Value
Required CT values are dependent on pH, residual concentration, temperature, and the disinfectant used. The tables attached to Appendices A and B shall be used to determine the required CT.

Calculation and Reporting of CT Data
Disinfection CT values shall be calculated daily, using either the maximum hourly flow and the disinfectant residual at the same time, or by using the lowest CT value if it is calculated more frequently. Actual CT values are then compared to required CT values.

Results shall be reported as a reduction Ratio, along with the appropriate pH, temperature, and disinfectant residual. The reduction Ratio must be greater than 1.0 to be acceptable.

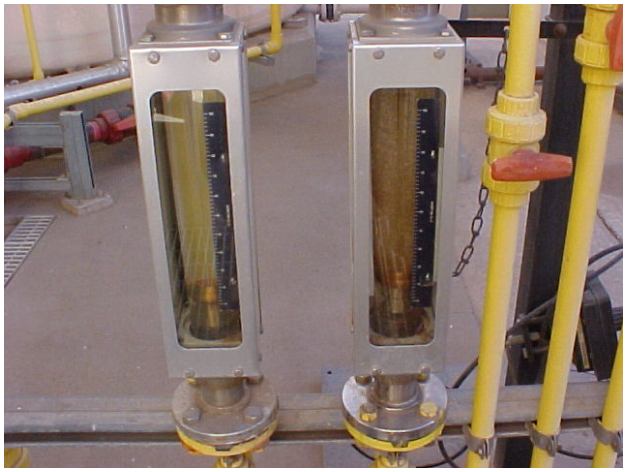
Users may also calculate and record actual log reductions.
Reduction Ratio = CT actual divide by CT required.



BASIC CHLORINATOR INSTALLATION SYSTEM

Chlorinator Components

A. **Ejector**
B. **Check Valve Assembly**
C. **Rate Valve**
D. **Diaphragm Assembly**
E. **Interconnection Manifold**
F. **Rotometer Tube and Float**
G. **Pressure Gauge**
H. **Gas Supply**



Chlorine measurement devices or Rotometers.



Chlorine Safety Information: There is a fusible plug on every chlorine tank. This metal plug will melt at 158 to 165° F. This is to prevent a build-up of excessive pressure and the possibility of cylinder rupture due to fire or high temperatures.

Chlorine Review

Chlorine Demand: The minimum amount of chlorine needed to react in a water purification system; used as a monitoring measurement by system operators.

Chlorine Residual: The concentration of chlorine in the water after the chlorine demand has been satisfied. The concentration is normally expressed in terms of total chlorine residual, which includes both the free and combined or chemically bound chlorine residuals.

Combined Chlorine Residual: The amount of chlorine used up in a water purification system; used as a monitoring measurement by system operators. Combined chlorine is defined as the residual chlorine existing in water in chemical combination with ammonia or organic amines which can be found in natural or polluted waters. Ammonia is sometimes deliberately added to chlorinated public water supplies to provide inorganic chloramines.

Free Chlorine: Free chlorine is defined as the concentration of residual chlorine in water present as dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl-). The three forms of free chlorine exist together in equilibrium.

$$Cl_2 + H_2O \quad HOCl + H^+ + Cl^-$$

$$HOCl \quad H^+ + OCl^-$$

Their relative proportions are determined by the pH value and temperature. Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination and .2 mg/L in the distribution system to guard against backflow.

Total Chlorine Residual: The total of free residual and combined residual chlorine in a water purification system; used as a monitoring measurement by system operators. Total chlorine is the sum of free and combined chlorine. When chlorinating most potable water supplies, total chlorine is essentially equal to free chlorine since the concentration of ammonia or organic nitrogen compounds (needed to form combined chlorine) will be very low. When chloramines are present in the municipal water supply, then total chlorine will be higher than free chlorine.

Pre-chlorination: The addition of chlorine at the plant headworks or prior to other water treatment or groundwater production processes and mainly used for disinfection and control of tastes, odors, and aquatic growths.

Post-chlorination: The addition of chlorine after a process or adding chlorine downstream to meet a demand in the system.

Breakpoint chlorination: Breakpoint chlorination means adding Cl_2 to the water until the Cl_2 demand is satisfied. Until all the microorganisms are killed.

What is the process of chlorination called as a treatment process and how does it differ from sterilization?
Chlorination: A method of water disinfection where gaseous, liquid, or dissolved chlorine is added to a water supply system. Water which has been treated with chlorine is effective in preventing the spread of disease. The chlorination of public drinking supplies was originally met with resistance, as people were concerned about the health effects of the practice. The use of chlorine has greatly reduced the prevalence of waterborne disease as it is effective against almost all bacteria and viruses, as well as amoeba. Sterilization kills everything.

What are the physical properties of chlorine, what hazards does it present, what advantages does it have over most other disinfectants, and how does it react with bacteria?
Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Chlorine reacts with bacteria as if it was very corrosive and burns the skin or covering killing the bacteria.

What is the purpose of a fusible plug, at what temperature does it melt, and where is it located on 150-lb. and 1-ton cylinders?
Fusible plug is a safety device that melts. If the temperature of a full Cl2 cylinder is increased by 50^{o} F or 30^{o} C, a rupture may occur. It will melt at 158 to 165 degrees F. It is found on the side of a 1 ton container and on top of the 150 pound cylinder and is located in the valve below the valve seat.

What is the correct procedure to follow in changing a chlorine cylinder and what item should always be replaced with a new one in doing so?
Hook up the chlorinator to the container or cylinder with the chlorine valve turned off. Use the gas side not the liquid if using a 1 ton container. Remove the cylinder valve outlet cap and check the valve face or damage. Clean with wire brush if necessary. If the valve face is smooth, clean proceed with hooking up the cylinder. Check the inlet face of the chlorinator and clean if necessary. Place a new lead gasket on the chlorinator inlet, place the chlorinator on the cylinder valve, install the yoke clamp and slowly tighten the yoke clamp until the two faces are against the lead gasket. Tighten the yoke, compressing the gasket one half to three quarters turn, do not over tighten. Replace the lead gasket with every change out.

How, when and where should chlorine residuals be taken and what information do they provide? The sample must be taken within the distribution system of your PWS. If you take it before the distribution system you will not get an accurate reading. The sample must be taken at the same tap that you take the Bac-t sample.

Chlor-Alkali Membrane Process

The electrolysis occurs in a cell containing electrodes submerged in solutions called electrolytes. One electrode is referred to as the anode and is submerged in a salt water solution. The second electrode is the cathode and is submerged in a sodium hydroxide (caustic soda) solution. A membrane is used to keep the two different solutions from mixing. This particular method of producing chlorine is called the chlor-alkali membrane process.

When a low voltage direct current (DC) power supply is applied to the electrodes in the cell, the sodium and chlorine ions in the brine are attracted in opposite directions to the polarized electrodes. The sodium ion passes across an ion selective membrane leaving the chlorine ion to combine with a second chlorine ion, which makes a chlorine gas bubble at the anode (electrode).

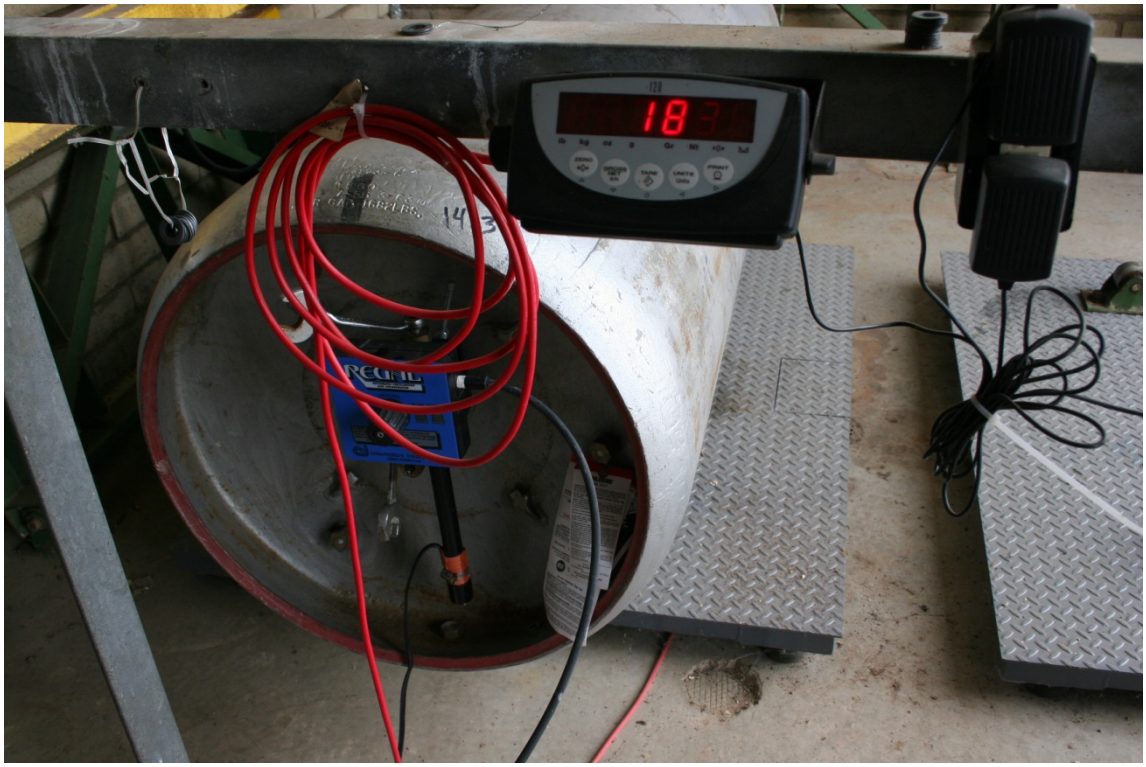
When the sodium crosses the membrane, it combines with a hydroxyl ion at the cathode (electrode) making sodium hydroxide, or caustic soda (NaOH). The hydroxyl ion originates from the dissolution of water at the cathode where hydrogen gas also develops. The membrane in the cell keeps the two solutions separate; otherwise, the chlorine gas bubble would immediately combine with the caustic soda forming sodium hypochlorite, or bleach. This process, which uses a membrane to separate the two solutions, is called the chlor-alkali process. The chemical equation for the chlor-alkali process is illustrated in the following equation:

2NaCl + 2H2O + electric current (2NaOH + Cl2- +- H2)





Top photograph, adjusting the Chlorine leak alarm sensor. Bottom photograph, Chlorine container weight scales.



Chlorine's Effectiveness

The effectiveness of chlorination depends on the chlorine demand of the water, the concentration of the chlorine solution added, the time that chlorine is in contact with the organism, and water quality. These effects can be summarized in the following manner:

- As the concentration of the chlorine increases, the required contact time to disinfect decreases.
- Chlorination is more effective as water temperature increases.
- Chlorination is less effective as the water's pH increases (becomes more alkaline).
- Chlorination is less effective in cloudy (turbid) water.
- When chlorine is added to the water supply, part of it combines with other chemicals in water (like iron, manganese, hydrogen sulfide, and ammonia) and is not available for disinfection. The amount of chlorine that reacts with the other chemicals plus the amount required to achieve disinfection is the **chlorine demand** of the water.



The safest way to be sure that the amount of chlorine added is sufficient is to add a little more than is required. This will result in a free chlorine residual that can be measured easily. This chlorine residual must be maintained for several minutes depending on chlorine level and water quality. Table 4 lists the free chlorine residual level needed for different contact times, water temperatures and pH levels.

Kits are available for measuring the chlorine residual by looking for a color change after the test chemical is added. The test is simple and easy for a homeowner to perform. If chlorination is required for the water supply, the chlorine residual should be tested regularly to make sure the system is working properly. The kit should specify that it measures the free chlorine residual and not the total chlorine. Once chlorine has combined with other chemicals it is not effective as a disinfectant. If a test kit does not distinguish between free chlorine and chlorine combined with other chemicals, the test may result in an overestimation of the chlorine residual.

Chlorine will kill bacteria in water, but it takes some time (Table 4) . The time needed depends on the concentration of chlorine. Two methods of chlorination are used to disinfect water: **simple chlorination** and **superchlorination**.

Table 4. Necessary chlorine residual to disinfect water for various contact times, water temperatures and pH			
Water Temp. 50 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.2	0.3	0.4
30	0.3	0.4	0.5
20	0.4	0.6	0.8
10	0.8	1.2	1.6
5	1.6	2.4	3.2
2	4.0	6.0	8.0
1	8.0	12.0	16.0
Water Temp. 32 - 40 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.3	0.5	0.6
30	0.4	0.6	0.8
20	0.6	0.9	1.2
10	1.2	1.8	2.4
5	2.4	3.6	4.8
2	6.0	9.0	12.0
1	12.0	18.0	24.0

Example: ***What is the necessary chlorine residual for well water with pH 7.5?***

The well water is 38 degrees F when it enters the house. The pump delivers 7 gallons per minute and after the chlorine is added it is held in a 100 gallon holding tank.

1. Contact time (from Table 5) - gallons per minute for 50 gallon tank = 5 minutes
2. Multiply by 2 for a 100 gallon tank = 10 minutes.
3. Necessary chlorine residual (from Table 4)- for water at 38 degrees F and pH 7.5 = **1.8 mg/l.**

Simple chlorination involves maintaining a low level of free residual chlorine at a concentration between 0.30 to .5 mg/l for at least 30 minutes. The residual is measured at the faucet most distant from the where chlorine is added to the water supply.

To ensure the proper contact time of at least 30 minutes, a holding tank can be installed (Table 5). Pressure tanks, while often thought to be sufficient, are usually too small to always provide 30 minutes of contact time.

Table 5. Available contact time from a 50-gallon holding tank	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	7
7	5
10	3.5

Another way to maintain necessary contact time is to run the chlorinated water through a coil of pipe (Table 6).

Table 6. Available contact time from 1000 feet of 1-1/4 inch pipe	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	9.2
7	6.6
10	4.6

When the water cannot be held for at least 30 minutes before it is used, super chlorination is an alternative. For **superchlorination**, a chlorine solution is added to the water to produce a chlorine residual of between 3.0 and 5.0 mg/l, which is about ten times stronger than for simple chlorination.

The necessary contact time for this concentration is reduced to less than five minutes (Table 4). The water will have a very strong chlorine smell. If this is not desirable, the chlorine can be removed just before it is used with a carbon filter (Note: may not be currently allowed under your Department of Health for private water supplies).

Oxidation Chemistry

Oxidation chemistry has long been an accepted and effective part of many water treatment programs. Oxidizing chemicals used in today's water treatment programs include: chlorine, chlorine dioxide, bromine, bromine/chlorine releasing compounds, ozone and hydrogen peroxide.

Oxidizing microbiocides are often found at the forefront of many cooling water treatment programs. In large volume or once-through cooling systems they are usually the primary biocide and often are the most cost-effective programs available to a plant. When selecting these economical and versatile chemicals, several factors should be considered before a technically sound program is implemented. Environmental and regulatory impact, system pH, process contamination, and equipment capital and maintenance expense all play a role in the decision-making process.

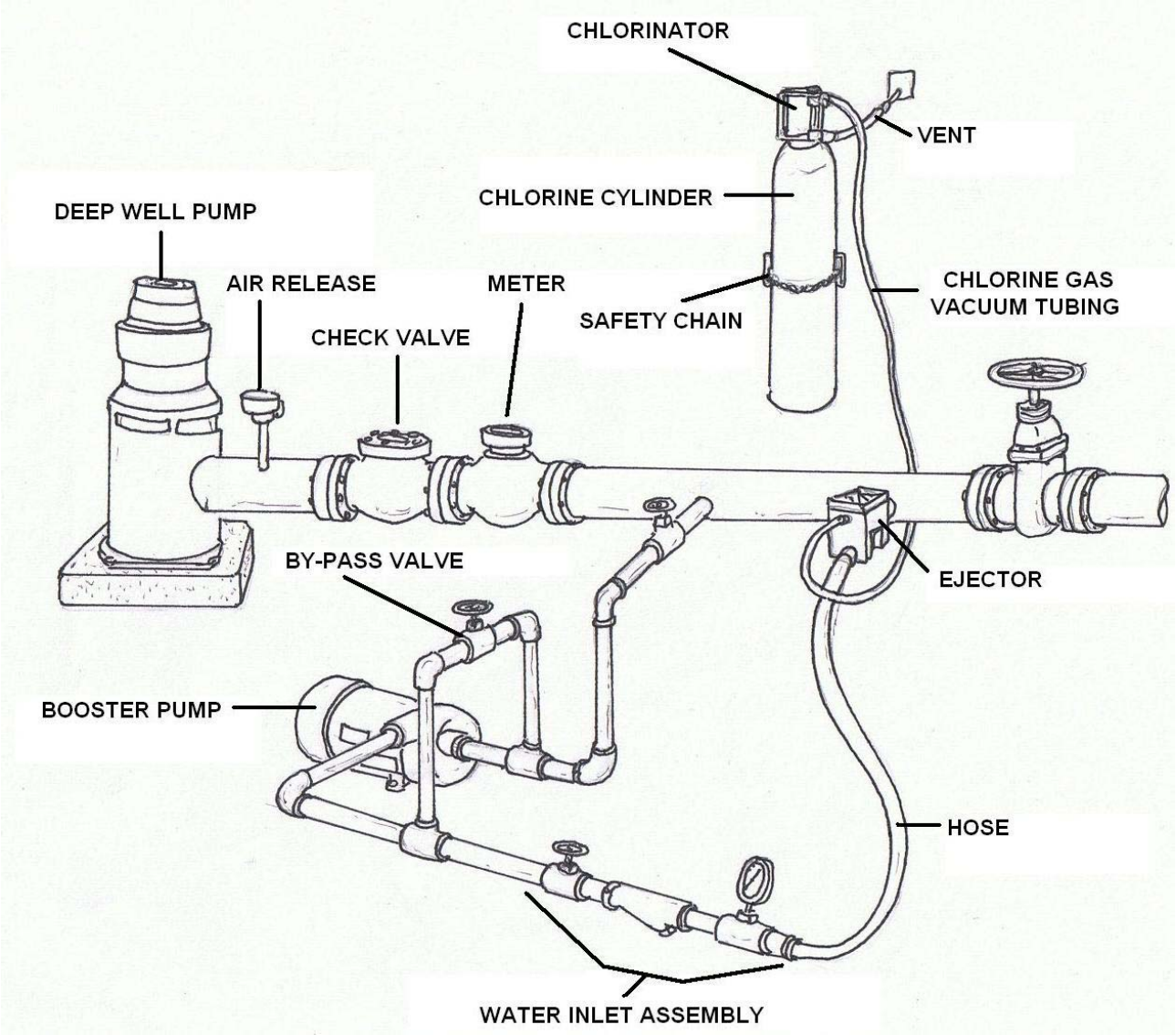
The primary killing mechanism these types of microbiocides use is oxidizing protein groups within a microorganism. Proteins are the basic components of essential cellular enzymes that are necessary for life-sustaining cellular processes such as respiration. The destruction of these proteins deprives the cell of its ability to carry out fundamental life functions and quickly kills it. One oxidant is chlorine dioxide, which appears to provide an additional killing mechanism. Chlorine dioxide is able to diffuse readily through hydrophobic lipid layers of an organism, allowing it to react with cellular amino acids, which directly inhibits protein synthesis. Since amino acids are the basic building blocks of all cellular proteins, destruction of these molecules has a devastating effect on the microorganism.

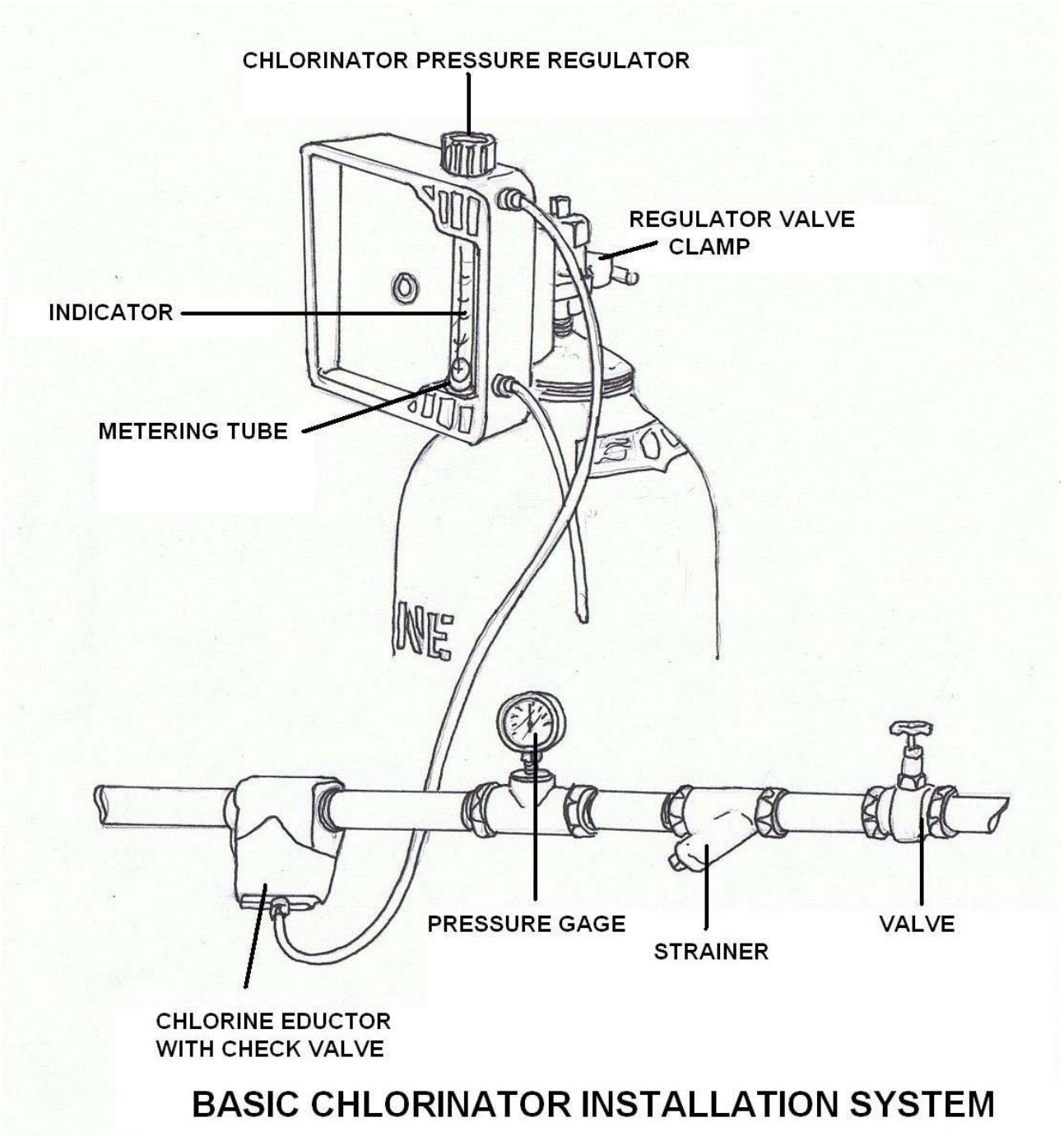


Staff shall be familiar with the locations of the chemical feed building as indicated by a posted site plan. Self-contained breathing apparatus (SCBA) and personal protective equipment should be facing the chemical feed building. Emergency repair kits “B” and “C” should be stored on site close to the chemical feed building.



Chlorine scrubber


CHLORINATOR
VENT
CHLORINE CYLINDER
DEEP WELL PUMP
AIR RELEASE
METER
CHLORINE GAS
VACUUM TUBING
SAFETY CHAIN
CHECK VALVE
BY-PASS VALVE
EJECTOR
BOOSTER PUMP
HOSE
WATER INLET ASSEMBLY



BASIC CHLORINATOR INSTALLATION SYSTEM

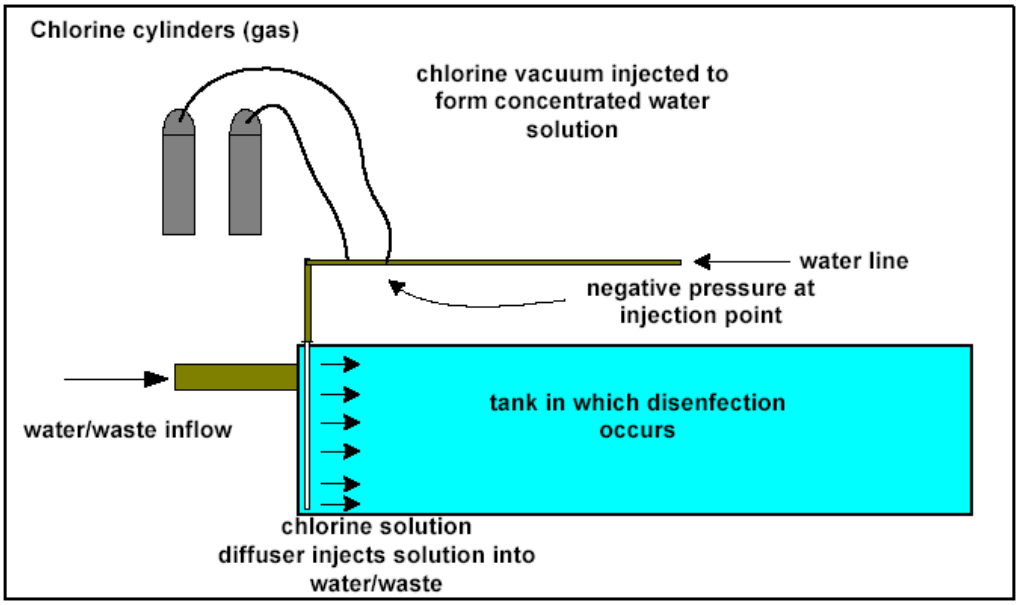
Chlorine Gas Section

Chlorine Gas



Background: Chlorine gas is a pulmonary irritant with intermediate water solubility that causes acute damage in the upper and lower respiratory tract. Chlorine gas was first used as a chemical weapon at Ypres, France in 1915. Of the 70,552 American soldiers poisoned with various gasses in World War I, 1843 were exposed to chlorine gas. Approximately 10.5 million tons and over 1 million containers of chlorine are shipped in the U.S. each year.

Chlorine is the most common chemical used to disenfect water and wastewater. It is added as a gas to most large installations. At very small facilities sodium hypochlorite (bleach) is added as a powder (tablets).



Chlorine is a yellowish-green gas at standard temperature and pressure. It is extremely reactive with most elements. Because its density is greater than that of air, the gas settles low to the ground. It is a respiratory irritant, and it burns the skin. Just a few breaths of it are fatal. Cl2 gas does not occur naturally, although Chlorine can be found in a number of compounds.

Chlorine gas is likely the most widely used oxidizing microbiocide. It has traditionally been the biocide of choice in many cooling water treatment systems. It is a strong oxidizer that is relatively easy to feed and is quite inexpensive. Upon introduction into the water stream, chlorine hydrolyzes into hypochlorous acid (HOCl) and hydrochloric acid (HCl).

This hydrolization provides the active toxicant, HOCl, which is pH-dependent. In alkaline cooling systems, it readily dissociates to form the hypochlorite ion (OCl-). This dissociation phenomenon is important to remember when working with systems that will operate at a higher pH. In alkaline conditions, OCl- becomes the predominant species and lacks the biocidal efficacy of the non-dissociated form. Considerably more HOCl is present at a pH of 7.0 than at pH 8.5.

It is also widely known that chlorine is non-selective, making it very sensitive to contamination from either cooling water makeup or from in-plant process leaks. Ammonia, organic acids and organic compounds, sulfides, iron and manganese all easily react with HOCl. The amount of chlorine needed to react with these contamination species is referred to as chlorine demand and it must be satisfied before active HOCl is available to provide a free chlorine residual.

The combination of high chlorine demand in process-contaminated systems and the dissociation process in alkaline systems creates the need for greater chlorine feed to obtain the same microbial efficacy. This results in a higher concentration of HCl in the cooling system. Since HCl removes alkalinity, pH depression and system corrosion could occur. In low pH water the passive metal oxide layers protecting the metal may resolubulize, exposing the surface to corrosion. At free mineral acidity (pH <4.3), many passivating inhibitors become ineffective, and corrosion will proceed rapidly. Increased chloride may also have a negative impact on system corrosion. The chloride ion (Cl^-) can damage or penetrate the passive oxide layer, leading to localized damage of the metal surface.

High chlorine concentrations have also been shown to directly attack traditional organic-based corrosion inhibitors. When these inhibitors are "deactivated," the metal surface would then be susceptible to corrosion. Process Safety Management (PSM) guidelines dictated by the U.S. Occupational Safety and Health Administration (OSHA), discharge problems related to chlorinated organic compounds such as trihalomethane (THM), dezincification of admiralty brass and delignification of cooling tower wood are other significant concerns associated with the use of chlorine.

Pathophysiology
Chlorine is a greenish-yellow, noncombustible gas at room temperature and atmospheric pressure. The intermediate water solubility of chlorine accounts for its effect on the upper airway and the lower respiratory tract.

Exposure to chlorine gas may be prolonged because its moderate water solubility may not cause upper airway symptoms for several minutes. In addition, the density of the gas is greater than that of air, causing it to remain near ground level and increasing exposure time.



The odor threshold for chlorine is approximately 0.3-0.5 parts per million (ppm); however, distinguishing toxic air levels from permissible air levels may be difficult until irritative symptoms are present.

Mechanism of Activity
The mechanisms of the above biological activity are poorly understood and the predominant anatomic site of injury may vary, depending on the chemical species produced. Cellular injury is believed to result from the oxidation of functional groups in cell components, from reactions with tissue water to form hypochlorous and hydrochloric acid, and from the generation of free oxygen radicals.

Although the idea that chlorine causes direct tissue damage by generating free oxygen radicals was once accepted, this idea is now controversial. The cylinders on the right contain chlorine gas.

The gas comes out of the cylinder through a gas regulator. The cylinders are on a scale that operators use to measure the amount used each day. The chains are used to prevent the tanks from falling over. Chlorine gas is stored in vented rooms that have panic bar equipped doors. Operators have the equipment necessary to reduce the impact of a gas leak, but rely on trained emergency response teams to contain leaks.

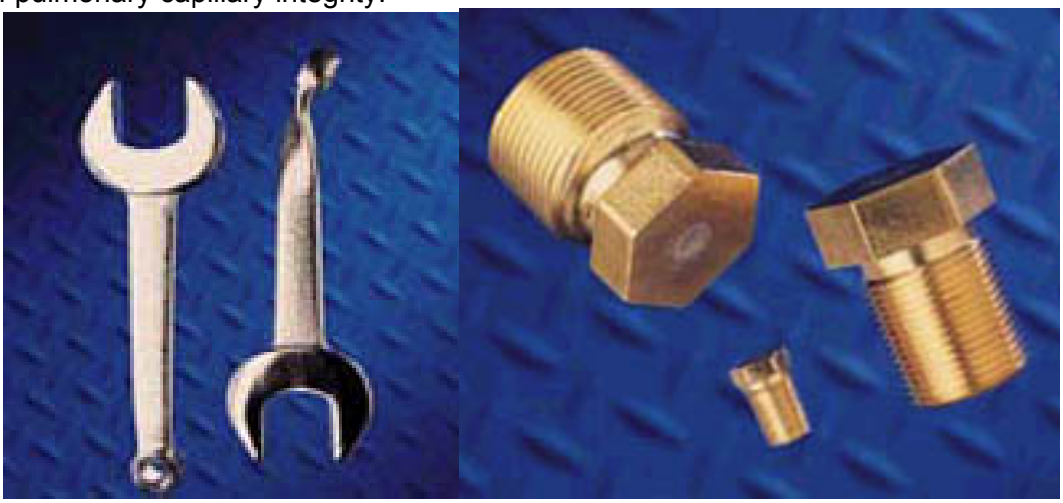
Solubility Effects
Hydrochloric acid is highly soluble in water. The predominant targets of the acid are the epithelia of the ocular conjunctivae and upper respiratory mucus membranes. Hypochlorous acid is also highly water soluble with an injury pattern similar to hydrochloric acid. Hypochlorous acid may account for the toxicity of elemental chlorine and hydrochloric acid to the human body.

Early Response to Chlorine Gas
Chlorine gas, when mixed with ammonia, reacts to form chloramine gas. In the presence of water, chloramines decompose to ammonia and hypochlorous acid or hydrochloric acid. The early response to chlorine exposure depends on the (1) concentration of chlorine gas, (2) duration of exposure, (3) water content of the tissues exposed, and (4) individual susceptibility.

Immediate Effects
The immediate effects of chlorine gas toxicity include acute inflammation of the conjunctivae, nose, pharynx, larynx, trachea, and bronchi. Irritation of the airway mucosa leads to local edema secondary to active arterial and capillary hyperemia. Plasma exudation results in filling the alveoli with edema fluid, resulting in pulmonary congestion.

Pathological Findings
Pathologic findings are nonspecific. They include severe pulmonary edema, pneumonia, hyaline membrane formation, multiple pulmonary thromboses, and ulcerative tracheobronchitis.

The hallmark of pulmonary injury associated with chlorine toxicity is pulmonary edema, manifested as hypoxia. Noncardiogenic pulmonary edema is thought to occur when there is a loss of pulmonary capillary integrity.



Chlorine Gas Cylinder Wrenches and Fusible Plugs.



Simple DPD Chlorine Field Test Kit.



Using DPD Method for Chlorine Residuals

N, N – diethyl-p-phenylenediame



Small portable chlorine measuring kit. The redder the mixture the "hotter" or stronger the chlorine in solution.

Measuring Chlorine Residual

Chlorine residual is the amount of chlorine remaining in water that can be used for disinfection. A convenient, simple and inexpensive way to measure chlorine residual is to use a small portable kit with pre-measured packets of chemicals that are added to water.

(Make sure you buy a test kit using the ***DPD method***, and not the outdated orthotolodine method.)

Chlorine test kits are very useful in adjusting the chlorine dose you apply. You can measure what chlorine levels are being found in your system (especially at the far ends).

Free chlorine residuals need to be checked and recorded daily. These results should be kept on file for a health or regulatory agency inspection during a regular field visit.

The most accurate method for determining chlorine residuals to use the laboratory amperometric titration method.

Amperometric Titration

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloro-organic compounds such as chloroform may be formed.

Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.



Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride.

The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts.

Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically the principal analytical problem has been to distinguish between free and combined forms of chlorine.

Hach's AutoCAT 9000™ Automatic Titrator is the newest solution to hit the disinfection industry – a comprehensive, benchtop chlorine-measurement system that does it all: calibration, titration, calculation, real-time graphs, graphic print output, even electrode cleaning. More a laboratory assistant than an instrument, the AutoCAT 9000 gives you:

- High throughput, performs the titration and calculates concentration, all automatically.
- Forward titration, USEPA-accepted methods for free and total chlorine and chlorine dioxide with chlorite.
- Back titration, USEPA-accepted method for total chlorine in wastewater.
- Accurate, yet convenient: the easiest way to complete ppb-level amperometric titration.

Sodium Hypochlorite Section

Physical Properties - Sodium Hypochlorite
Description: Clear greenish yellow liquid.
Warning properties: Chlorine odor; inadequate warning of hazardous concentrations.
Molecular weight: 74.44 daltons
Boiling point (760 mm Hg): Decomposes above 40ºC (HSDB 2001)
Freezing point: 6ºC (21ºF)
Specific gravity: 1.21 (14% NAOCl solution) (water=1)
Water solubility: 29.3 g/100 g at 32ºF (0ºC)
Flammability: Not flammable

Alternative Names
Bleach; Clorox; Carrel-Dakin solution

Incompatibilities
Calcium or sodium hypochlorite react explosively or form explosive compounds with many common substances such as ammonia, amines, charcoal, or organic sulfides

Introduction
The world's most universal and reliable means of water and wastewater disinfection is chlorination. Two fundamental methods include gas chlorination (Cl2) and liquid chlorination (NaOCl) otherwise known as Sodium Hypochlorite. Sodium hypochlorite (NaOCl) is a solution made from reacting chlorine with a sodium hydroxide solution. These two reactants are the major co-products from most chlor-alkali cells. Sodium hypochlorite has a variety of uses and is an excellent disinfectant/antimicrobial agent. Sodium hypochlorite also significantly increases the pH of the water. When sodium hypochlorite is used, it must be counterbalanced by a strong acid like sodium bisulfate or muriatic acid to keep the pH within the ideal range.

The hypochlorite form of chlorine has been used since 1850. The most widely used form of hypochlorite is the liquid, sodium hypochlorite (NaOCl), with more than 150 tons per day consumed in the United States. Sodium hypochlorite application in cooling water is essentially the same as with gas chlorine; HOCl is produced as the active toxicant. The HOCl is equally susceptible to process contamination, has the same chlorine demand as gas chlorine and displays the same tendency to dissociate.

Sodium hypochlorite differs from chlorine gas in two respects: method of feed and hydrolization properties. Sodium hypochlorite can either be gravity-fed or applied with a metering pump. The latter is generally recognized as a consistently more accurate method. The second difference, in hydrolysis, lies in the end products. The NaOCl reaction with water liberates sodium hydroxide (NaOH).

The addition of NaOH differs in that it tends to add alkalinity to the water. In large concentrations it may artificially elevate pH, leading to precipitation of calcium carbonate. While NaOCl eliminates low pH corrosion as a concern, the use of large quantities in contaminated systems still introduces a high concentration of the chloride ion, which can be very aggressive to cooling system metals. Many of the other problems associated with chlorine remain present with sodium hypochlorite.

When was Sodium Hypochlorite Discovered?
Sodium hypochlorite has a long history. Around 1785 the Frenchman Berthollet developed liquid bleaching agents based on sodium hypochlorite. The Javel company introduced this product and called it 'liqueur de Javel'. At first, it was used to bleach cotton. Because of its specific characteristics it soon became a popular compound. Hypochlorite can remove stains from clothes at room temperature. In France, sodium hypochlorite is still known as 'eau de Javel'.

Characteristics of Sodium hypochlorite
Sodium hypochlorite is a clear, slightly yellowish solution with a characteristic odor.

Sodium hypochlorite has a relative density of is 1.1 (5.5% watery solution).

As a bleaching agent for domestic use it usually contains 5% sodium hypochlorite (with a pH of around 11, it is irritating). If it is more concentrated, it contains a concentration 10-15% sodium hypochlorite (with a pH of around 13, it burns and is corrosive).

Sodium hypochlorite is unstable. Chlorine evaporates at a rate of 0,75 gram active chlorine per day from the solution. Then heated sodium hypochlorite disintegrates. This also happens when sodium hypochlorite comes in contact with acids, sunlight, certain metals and poisonous and corrosive gasses, including chlorine gas. Sodium hypochlorite is a strong oxidator and reacts with flammable compounds and reductors. Sodium hypochlorite solution is a weak base that is inflammable. These characteristics must be kept in mind during transport, storage and use of sodium hypochlorite.

pH value When Sodium Hypochlorite is Added to Water
Due to the presence of caustic soda in sodium hypochlorite, the pH of the water is increased. When sodium hypochlorite dissolves in water, two substances form, which play a role in oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl^-). The pH of the water determines how much hypochlorous acid is formed. While sodium hypochlorite is used, hydrochloric (HCl) is used to lower the pH. Sulfuric acid (H_2SO_4) can be used as an alternative for acetic acid. Less harmful gasses are produced when sulfuric acid is used. Sulfuric acid is a strong acid that strongly reacts with bases and is very corrosive.

How Can Sodium Hypochlorite be produced?
Sodium hypochlorite can be produced in two ways:
- By dissolving salt in softened water, which results in a concentrated brine solution. The solution is electrolyzed and forms a sodium hypochlorite solution in water. This solution contains 150 g active chlorine (Cl_2) per liter. During this reaction the explosive hydrogen gas is also formed.
- By adding chlorine gas (Cl_2) to caustic soda (NaOH). When this is done, sodium hypochlorite, water (H_2O) and salt (NaCl) are produced according to the following reaction:
$Cl_2 + 2NaOH \rightarrow NaOCl + NaCl + H_2O$

Applications of Sodium Hypochlorite
Sodium hypochlorite is used on a large scale; for example agriculture, chemical industries, paint- and lime industries, food industries, glass industries, paper industries, pharmaceutical industries, synthetics industries and waste disposal industries all use it. In the textile industry sodium hypochlorite is used to bleach textile. It is sometimes added to industrial waste water--this is done to reduce odors.

Hypochlorite neutralizes sulphur hydrogen gas (SH) and ammonia (NH3). It is also used to detoxify cyanide baths in metal industries. Hypochlorite can be used to prevent algae and shellfish growth in cooling towers. In water treatment, hypochlorite is used to disinfect water. In households, hypochlorite is used frequently for the purification and disinfection of the house.

How does Sodium Hypochlorite Disinfection Work?
By adding hypochlorite to water, hypochlorous acid (HOCl) is formed:
$NaOCl + H_2O \rightarrow HOCl + NaOH$

Hypochlorous acid is divided into hydrochloric acid (HCl) and oxygen (O). The oxygen atom is a very strong oxidant.

Sodium hypochlorite is effective against bacteria, viruses and fungi. Sodium hypochlorite disinfects the same way as chlorine does.

There are various ways to use sodium hypochlorite. For on-site salt electrolysis, a solution of salt (NaCl) in water is applied. Sodium (Na^+) and chloride (Cl^-) ions are produced.
$4NaCl \rightarrow 4Na^+ + 4Cl^-$

Subsequently, chlorine and hydroxide react to form hypochlorite:
$OH^- + Cl_2 \rightarrow HOCl + Cl^-$

You can work these problems in this section.

Salt Electrolysis System
The advantage of the salt electrolysis system is that no transport or storage of sodium hypochlorite is required. When sodium hypochlorite is stored for a long time, it becomes inactive. Another advantage of the onsite process is that chlorine lowers the pH and no other acid is required to lower pH.

The hydrogen gas that is produced is explosive and as a result ventilation is required for explosion prevention. This system is slow and a buffer of extra hypochlorous acid needs to be used. The maintenance and purchase of the electrolysis system is much more expensive than sodium hypochlorite.

When sodium hypochlorite is used, acetic or sulfuric acid are added to the water. An overdose can produce poisonous gasses. If the dosage is too low, the pH becomes too high and can irritate the eyes.

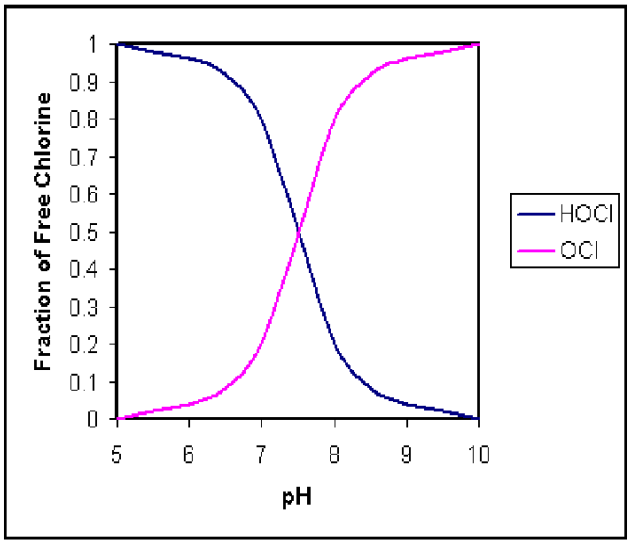
Because sodium hypochlorite is used both to oxidize pollutants (urine, sweat, cosmetics) and to remove pathogenic microorganisms, the required concentration of sodium hypochlorite depends on the concentrations of these pollutions. Especially the amount of organic pollutants helps determine the required concentration. If the water is filtered before sodium hypochlorite is applied, less sodium hypochlorite is needed.

Theory

Disinfection with chlorine is very popular in water and wastewater treatment because of its low cost, ability to form a residual, and its effectiveness at low concentrations. Although it is used as a disinfectant, it is a dangerous and potentially fatal chemical if used improperly.

Despite the fact the disinfection process may seem simple, it is actually a quite complicated process. Chlorination in wastewater treatment systems is a fairly complex science which requires knowledge of the plant's effluent characteristics.

When free chlorine is added to the wastewater, it takes on various forms depending on the pH of the wastewater. It is important to understand the forms of chlorine which are present because each has a different disinfecting capability. The acid form, HOCL, is a much stronger disinfectant than the hypochlorite ion, OCL-. The graph below depicts the chlorine fractions at different pH values (Drawing by Erik Johnston).



Ammonia present in the effluent can also cause problems as chloramines are formed, which have very little disinfecting power. Some methods to overcome the types of chlorine formed are to adjust the pH of the wastewater prior to chlorination or to simply add a larger amount of chlorine. An adjustment in the pH would allow the operators to form the most desired form of chlorine, hypochlorus acid, which has the greatest disinfecting power.

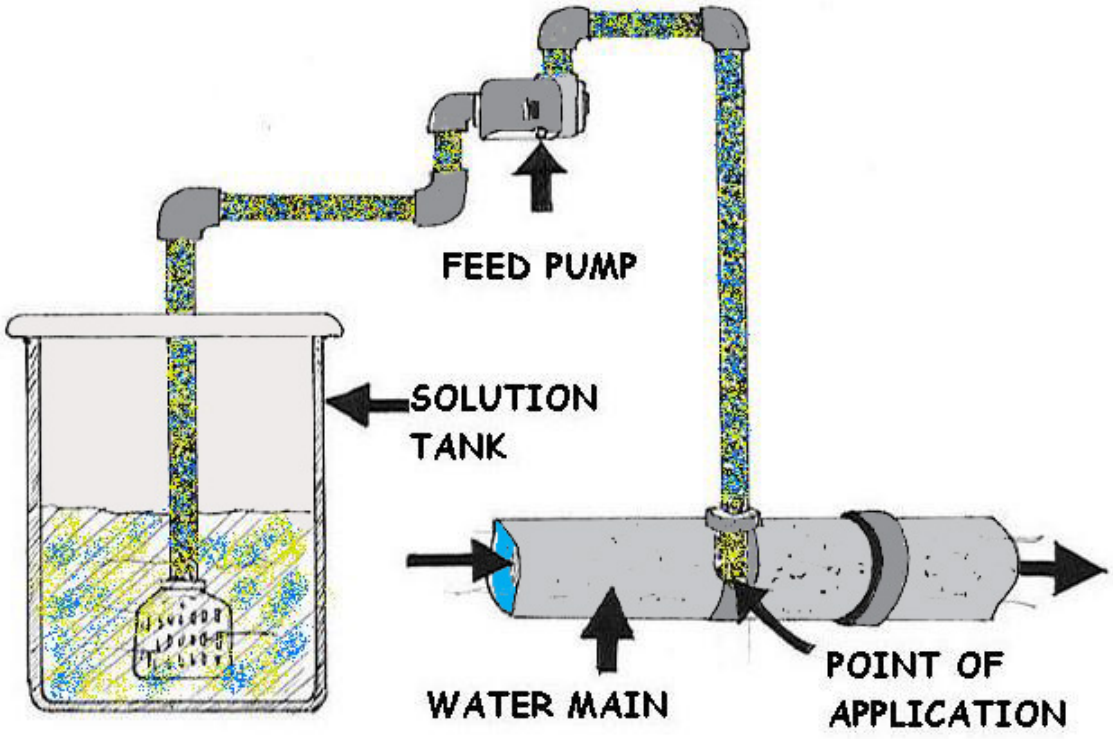
Adding larger amounts of chlorine would be an excellent method to combat the chloramines because the ammonia present would bond to the chlorine but further addition of chlorine would stay in the hypochlorus acid or hypochlorite ion state.

a) Chlorine gas, when exposed to water reacts readily to form hypochlorus acid, HOCl, and hydrochloric acid. Cl2 + H_2O -> HOCl + HCl

b) If the pH of the wastewater is greater than 8, the hypochlorus acid will dissociate to yield hypochlorite ion. HOCl <-> H^+ + OCl^-- If however, the pH is much less than 7, and then HOCl will not dissociate.

c) If ammonia is present in the wastewater effluent, then the hypochlorus acid will react to form one three types of chloramines depending on the pH, temperature, and reaction time.

Recommendations for Preparing/Handling/Feeding Sodium Hypochlorite Solutions



As a result of the pressures brought to bear by Health and Safety requirements, some users of gas have chosen to seek alternative forms of disinfectants for their water and wastewater treatment plants. One of these alternative forms is sodium hypochlorite (**NaOCl**)). This is often purchased commercially at 10 to 15% strength.

The handling and storage of NaOCl presents the plant with a new and sometimes unfamiliar, set of equipment installation configurations and operating conditions.

Product Stability The oxidizing nature of this substance means that it should be handled with extreme care. As NaOCl is relatively unstable, it degrades over time.

There are Three Ways in Which NaOCl Solutions Degrade

- Chlorate-forming reaction due to age, temperature, light and minor reduction in pH.
- Oxygen-producing reaction that occurs when metals, such as iron, copper or nickel, or metal oxides are brought into contact with the solution.
- Chlorine-producing reaction when solution pH falls below 6.

There are Many Factors that Affect the Stability of a NaOCl Solution

- Initial solution strength.
- pH solution.
- Temperature of the solution.
- Exposure of the solution to sunlight.

Exposure

There is no threshold value for to sodium hypochlorite exposure. Various health effects occur after exposure to sodium hypochlorite. People are exposed to sodium hypochlorite by inhalation of aerosols. This causes coughing and a sore throat. After swallowing sodium hypochlorite the effects are stomach ache, a burning sensation, coughing, diarrhea, a sore throat and vomiting. Sodium hypochlorite on skin or eyes causes redness and pain. After prolonged exposure, the skin can become sensitive. Sodium hypochlorite is poisonous for water organisms. It is mutagenic and very toxic when it comes in contact with ammonium salts.

Routes of Exposure
Inhalation
Hypochlorite solutions can liberate toxic gases such as chlorine. Chlorine's odor or irritant properties generally provide adequate warning of hazardous concentrations. However, prolonged, low-level exposures, such as those that occur in the workplace, can lead to olfactory fatigue and tolerance of chlorine's irritant effects. Chlorine is heavier than air and may cause asphyxiation in poorly ventilated, enclosed, or low-lying areas.

Children exposed to the same levels of gases as adults may receive a larger dose because they have greater lung surface area/body weight ratios and higher minute volumes/weight ratios. Children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of chlorine found nearer to the ground.

Skin/Eye Contact
Direct contact with hypochlorite solutions, powder, or concentrated vapor causes severe chemical burns, leading to cell death and ulceration. Because of their relatively larger surface area/weight ratio, children are more vulnerable to toxicants affecting the skin.

Ingestion
Ingestion of hypochlorite solutions causes vomiting and corrosive injury to the gastrointestinal tract. Household bleaches (3 to 6% sodium hypochlorite) usually cause esophageal irritation, but rarely cause strictures or serious injury such as perforation. Commercial bleaches may contain higher concentrations of sodium hypochlorite and are more likely to cause serious injury. Metabolic acidosis is rare, but has been reported following the ingestion of household bleach. Pulmonary complications resulting from aspiration may also be seen after ingestion.

Sources/Uses
Sodium and calcium hypochlorite are manufactured by the chlorination of sodium hydroxide or lime. Sodium and calcium hypochlorite are used primarily as oxidizing and bleaching agents or disinfectants. They are components of commercial bleaches, cleaning solutions, and disinfectants for drinking water and waste water purification systems and swimming pools.

Sodium Hypochlorite as a Disinfectant has the Following Advantages:
It can be easily stored and transported when it is produced on-site. Dosage is simple; transport and storage of sodium hypochlorite are safe. Sodium hypochlorite is as effective as chlorine gas for disinfection. Sodium hypochlorite produces residual disinfectant.

Disadvantages
Sodium hypochlorite is a dangerous and corrosive substance. While working with sodium hypochlorite, safety measures have to be taken to protect workers and the environment.

Sodium hypochlorite should not come in contact with air, because that will cause it to disintegrate. Both sodium hypochlorite and chlorine do not deactivate Giardia Lambia and Cryptosporidium. The regulation for sodium hypochlorite is the same as the regulation considering chlorine.

Household bleaches usually contain sodium hypochlorite in a 3% to 6% solution. Some sodium hydroxide (lye) is added to keep the pH high to avoid decomposition. If the solution is made more acidic, sodium hypochlorite will dissociate, producing chlorine gas and oxygen. It is made by bubbling chlorine gas through a solution of sodium hydroxide. In the environment, it breaks down into water, oxygen, and table salt.

Conditions that tend to increase gassing in Sodium Hypochlorite Solutions are:
* Elevated temperatures
* High concentration solution
* Exposure to sunlight or UV rays
* Reduction in pressure
* Cavitation
* Poor piping conditions
* Contact with metallic impurities
* Contact with organic impurities
* Age of solution
* Quality of solution

Reciprocating Piston Metering Pumps
When handling sodium hypochlorite and acids, be certain to wear gloves and a face shield for protection. Sodium hypochlorite is introduced to treated water by a chemical feeder (pump.) Chemical feeders tend to clog often, so it's important to clean the feeder regularly. Sodium Hypochlorite is subject to degradation within the piping and pump systems as it releases oxygen gas and results in crystallization of the residual. If the oxygen gas or vapor is allowed to build up within the piping and reagent head in sufficient volume, a typical reciprocating piston metering pump, used for accurately feeding chlorine to the process, will not function properly as gas in the pump head is compressed, minimizing the discharge check valve to open upon discharge stroke of the pump. Consequently, this effect could require that the pump be re-primed for operation. Reciprocating piston metering pumps or diaphragm metering pumps have been historically preferred in the dispensing of Sodium Hypochlorite because of their superior ability to accurately dose chemicals into a process stream with great precision and repeatability at a constant pressure. Additionally, the diaphragm metering pump is sealless and leak proof by design with negligible maintenance and simple commissioning.

Traditionally, the diaphragm metering pump industry has promoted the use of degas valves on the discharge port of the pump which diverts gas back to the suction supply source of the bleach. This method has been widely accepted and successful in many applications. However, the small diameter ports in the valve system tend to plug and require continuous flushing or cleaning through human intervention since the system is open to atmosphere on the discharge side of the orifice. Additionally, an external bypass piping system and degas valve assembly require additional costs and maintenance while presenting more opportunities for undesired chlorine leak paths.



A ten minute emergency air escape pack. We suggest that any one that utilizes chlorine gas purchase several of these type devices and station these around the facility and in your work vehicle.

Troubleshooting Hypochlorination Problems

Problem
1. Chemical feed pump won't run.
2. Low chlorine residual at POE.
2. Low chlorine residual at POE.
3. Chemical feed pump won't prime.
4. Loss of prime

Possible Causes
1A. No power.
1B. Electrical problem with signal from well pump or flow sensor.
1C. Motor failure.
2A. Improper procedure for running chlorine residual test or expired chemical reagents.
2B. Pump not feeding an adequate quantity of chlorine.
2C. Change in raw water quality.
2D. Pump **air bound**.
2E. Chlorine supply tank empty.
2F. Reduced effectiveness of chlorine solution.
2G. Damaged suction or discharge lines. (cracks or crimps)
2H. Connection at point of **injection** clogged or leaking.
3A. Speed and stroke setting inadequate.
3B. Suction lift too high due to feed pump relocation.
3C. Discharge pressure too high.
3D. Suction fitting clogged.
3E. Trapped air in suction line.
3F. Suction line not submerged in solution.
4A. Solution tank empty.
4B. Air leaks in suction fittings.
4C. Foot valve not in vertical position.
4D. Air trapped in suction tubing.

Possible Solutions
1A. Check to see if plug is securely in place.
Insure that there is power to the outlet and control systems.
1B. Check pump motor starter. Bypass flow sensor to determine if pump will operate manually.
1C. Check manufacturer's information.
2A Check expiration date on **chemical reagents**. Check test procedure as described in test kit manual. Speed or stroke setting too low.
2B. Damaged **diaphragm** or suction leak.
2C. Test raw water for constituents that may cause increased chlorine demand. (i.e. iron, manganese, etc.)
2D. Check foot valve.
2E. Fill supply tank.
2F. Check date that chlorine was received. Sodium hypochlorite solution may lose effectiveness after 30 days. If that is the case, the feed rate must be increased to obtain the desired residual.
2G. Clean or repair lines with problems.

2H. Flush line and connection with mild acid such as **Acetic** or **Muriatic**. Replace any damaged parts that may be leaking.
3A. Check manufacturers' recommendations for proper settings to prime pump.
3B. Check maximum suction lift for pump and relocate as necessary.
3C. Check well pump discharge pressure.
Check pressure rating on chemical feed pump.
3D. Clean or replace screen.
3E. Insure all fittings are tight.
3F. Add chlorine solution to supply tank.
4A. Fill tank.
4B. Check for cracked fittings.
4C. Adjust foot valve to proper position.
4D. Check connections and fittings.



Small Chlorine solution tank.

Shock Chlorination — Well Maintenance

Shock chlorination is a relatively inexpensive and straightforward procedure used to control bacteria in water wells. Many types of bacteria can contaminate wells, but the most common are iron and sulfate-reducing bacteria.

Although not a cause of health problems in humans, bacteria growth will coat the inside of the well casing, water piping and pumping equipment, creating problems such as:

- Reduced well yield
- Restricted water flow in distribution lines
- Staining of plumbing fixtures and laundry
- Plugging of water treatment equipment
- ***"Rotten egg"*** odor.

Bacteria may be introduced during drilling of a well or when pumps are removed for repair and laid on the ground. However, iron and sulfate-reducing bacteria (as well as other bacteria) can exist naturally in groundwater.

A well creates a direct path for oxygen to travel into the ground where it would not normally exist. When a well is pumped, the water flowing in will also bring in nutrients that enhance bacterial growth.

Note: All iron staining problems are not necessarily caused by iron bacteria. The iron naturally present in the water can be the cause.

Ideal Conditions for Iron Bacteria
Water wells provide ideal conditions for iron bacteria. To thrive, iron bacteria require 0.5-4 mg/L of dissolved oxygen, as little as 0.01 mg/L dissolved iron and a temperature range of 5 to 15°C. Some iron bacteria use dissolved iron in the water as a food source.



Signs of Iron and Sulfate-Reducing Bacteria
There are a number of signs that indicate the presence of iron and sulfate-reducing bacteria. They include:

- Slime growth
- Rotten egg odor
- Increased staining.

Slime Growth
The easiest way to check a well and water system for iron bacteria is to examine the inside surface of the toilet flush tank. If you see a greasy slime or growth, iron bacteria are probably present. Iron bacteria leave this slimy by-product on almost every surface the water is in contact with.

Rotten Egg Odor
Sulfate-reducing bacteria can cause a rotten egg odor in water. Iron bacteria aggravate the problem by creating an environment that encourages the growth of sulfate-reducing bacteria in the well. Sulfate-reducing bacteria prefer to live underneath the slime layer that the iron bacteria form.

Some of these bacteria produce hydrogen sulfide as a by-product, resulting in a "***rotten egg***" or sulfur odor in the water. Others produce small amounts of sulfuric acid which can corrode the well casing and pumping equipment.

Increased Staining Problems
Iron bacteria can concentrate iron in water sources with low iron content. It can create a staining problem where one never existed before or make an iron staining problem worse as time goes by.

Use the following checklist to determine if you have an iron or sulfate-reducing bacteria problem. The first three are very specific problems related to these bacteria. The last two problems can be signs of other problems as well.

Checklist to Determine an Iron or Sulfate-Reducing Bacteria Problem
- Greasy slime on inside surface of toilet flush tank
- Increased red staining of plumbing fixtures and laundry
- Sulfur odor
- Reduced well yield
- Restricted water flow

Mixing a Chlorine Solution
Add a half gallon of bleach to a clean pail with about 3 gallons of water. This is generally sufficient to disinfect a 4 inch diameter well 100 feet deep or less. For wells greater than 100 feet deep or with a larger casing diameter, increase the amount of bleach proportionately.

If you have a dug well with a diameter greater than 18 inches, use 2 to 4 gallons of bleach added directly to the well. Please note that many dug wells are difficult or impossible to disinfect due to their unsanitary construction.

Shock Chlorination — Well Maintenance

Shock Chlorination Method
Shock chlorination is used to control iron and sulfate-reducing bacteria and to eliminate fecal coliform bacteria in a water system. To be effective, shock chlorination must disinfect the following:
- The entire well depth
- The formation around the bottom of the well
- The pressure system
- Some water treatment equipment
- The distribution system.

To accomplish this, a large volume of super chlorinated water is siphoned down the well to displace all the water in the well and some of the water in the formation around the well.

Effectiveness of Shock Chlorination
With shock chlorination, the entire system (from the water-bearing formation, through the well-bore and the distribution system) is exposed to water which has a concentration of chlorine strong enough to kill iron and sulfate reducing bacteria. Bacteria collect in the pore spaces of the formation and on the casing or screened surface of the well.

To be effective, you must use enough chlorine to disinfect the entire cased section of the well and adjacent water-bearing formation.

The procedure described below does not completely eliminate iron bacteria from the water system, but it will hold it in check.

To control the iron bacteria, you may have to repeat the procedure each spring and fall as a regular maintenance procedure. If your well has never been shock chlorinated or has not been done for some time, it may be necessary to use a stronger chlorine solution, applied two or three times, before you notice a significant improvement in the water.

You might also consider hiring a drilling contractor to thoroughly clean and flush the well before chlorinating in order to remove any buildup on the casing.

In more severe cases, the pump may have to be removed and chemical solutions added to the well and vigorous agitation carried out using special equipment. This is to dislodge and remove the bacterial slime. This should be done by a drilling contractor.

Shock Chlorination Procedure for Small Drilled Wells

A modified procedure is also provided for large diameter wells.

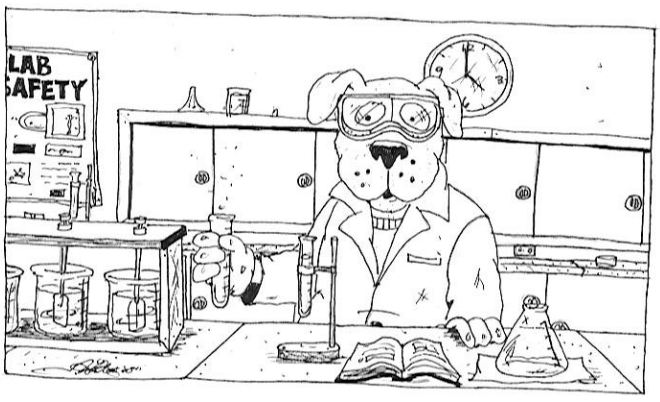
Caution: If your well is low yielding or tends to pump any silt or sand, you must be very careful using the following procedure because over pumping may damage the well.

When pumping out the chlorinated solution, monitor the water discharge for sediment.

Follow these steps to shock chlorinate your well.

Store sufficient water to meet farm and family needs for 8 to 48 hours.

Pump the recommended amount of water (see Amount of Chlorine Required to Obtain a Chlorine Concentration of 1000 PPM) into clean storage. A clean galvanized stock tank or pickup truck box lined with a 4 mil thick plastic sheet is suitable. The recommended amount of water to use is twice the volume of water present in the well casing. To measure how much water is in the casing, subtract the non-pumping water level from the total depth of the well.



12% industrial sodium hypochlorite and 70% high test hypochlorite are available from:
• Water treatment suppliers
• Drilling contractor
• Swimming pool maintenance suppliers
• Dairy equipment suppliers
• Some hardware stores.

Amount of Chlorine required to obtain a Chlorine Concentration of 1000 PPM. Since a dry chemical is being used, it should be mixed with water to form a chlorine solution before placing it in the well.

Calculate the amount of chlorine that is required. Mix the chlorine with the previously measured water to obtain a 1000 ppm chlorine solution.

Calculating Amount of Chlorine Example
If your casing is 6 in. and you are using 12% industrial sodium hypochlorite, you will require .091 L per ft. of water in the casing. If you have 100 ft. of water in the casing, you will use 0.091 L x 100 ft. = 9.1 L of 12% chlorine.
Calculate the amount of chlorine you will need for your well.

Casing diameter_____ Chlorine strength_____

L needed per 1 ft. of water_____ x _____ ft. of water in casing = _____ L of chlorine.

Caution: Chlorine is corrosive and can even be deadly.
If your well is located in a pit, you must make sure there is proper ventilation during the chlorination procedure. Well pits are no longer legal to construct. Use a drilling contractor who has the proper equipment and experience to do the job safely.

Shock Chlorination — Well Maintenance
Siphon this solution into the well.
Open each hydrant and faucet in the distribution system (including all appliances that use water such as dishwasher, washing machine, furnace humidifier) until the water coming out has a chlorine odor. This will ensure all the plumbing fixtures are chlorinated.

Allow the hot water tank to fill completely. Consult your water treatment equipment supplier to find out if any part of your water treatment system should be bypassed to prevent damage.

Leave the chlorine solution in the well and distribution system for 8 to 48 hours. The longer the contact time, the better the results.

- Open an outside tap and allow the water to run until the chlorine odor is greatly reduced. Make sure to direct the water away from sensitive plants or landscaping.
- Flush the chlorine solution from the hot water heater and household distribution system. The small amount of chlorine in the distribution system will not harm the septic tank.

Backwash and regenerate any water treatment equipment.

If you have an old well that has not been routinely chlorinated, consider hiring a drilling contractor to thoroughly clean the well prior to chlorinating. Any floating debris should be removed from the well and the casing should be scrubbed or hosed to disturb the sludge buildup.

Modified Procedure for Large Diameter Wells
Due to the large volume of water in many bored wells the above procedure can be impractical. A more practical way to shock chlorinate a bored well is to mix the recommended amount of chlorine right in the well. The chlorinated water is used to force some of the chlorine solution into the formation around the well. Follow these steps to shock chlorinate a large diameter bored well.

Pump 200 gal. (1000 L) of water into a clean storage tank at the well head.

Mix 20 L of 5 1/4% domestic chlorine bleach (or 8 L of 12% bleach or 1.4 kg of 70% calcium hypochlorite) into the 200 gal. of stored water.

Calculate the amount of chlorine you require per foot of water in the casing and add directly into the well. (Note that the 70% hypochlorite powder should be dissolved in water to form a solution before placing in the well.)

Circulate chlorine added to the water in the well by hooking a garden hose up to an outside faucet and placing the other end back down the well. This circulates the chlorinated water through the pressure system and back down the well. Continue for at least 15 minutes.

Siphon the 200 gal. bleach and water solution prepared in Steps 1 and 2 into the well. Complete the procedure as described in Steps 5 to 9 for drilled wells.

Don't mix acids with chlorine. This is dangerous.

Calculating Water and Chlorine Requirements for Shock Chlorination
Complete the following table using your own figures to determine how much water and chlorine you need to shock chlorinate your well.

Casing Volume of 5 1/4% 12% Industrial **1**70%
Diameter Water Needed Domestic Sodium High Test
Chlorine Bleach Hypochlorite
Imperial gal. needed per Dry weight
1 ft. of water L per 1 ft. (30 cm) L per 1 ft. (30 cm) per 1 ft. (30 cm)
(in) (mm) in the casing of water of water of water
4 (100) _____ft. x 1.1 gal. =_____ _____ft. x 0.095 L =_____ _____ft. x 0.042 L =_____ _____ft. x 7.2 g =_____
6 (150) _____ft. x 2.4 gal. =_____ _____ft. x 0.21 L =_____ _____ft. x 0.091 L =_____ _____ft. x 15.6 g =_____
8 (200) _____ft. x 4.2 gal. =_____ _____ft. x 0.36 L =_____ _____ft. x 0.16 L =_____ _____ft. x 27.3 g =_____
24 (600)**2** extra 200 gal. _____ft. x 1.7 L =_____ _____ft. x 0.74 L =_____ _____ft. x 127 g =_____
36 (900)**2** extra 200 gal. _____ft. x 3.8 L =_____ _____ft. x 1.7 L =_____ _____ft. x 286 g =_____

What Should I do if My Well Water is Contaminated with Bacteria?

First, don't panic'! Bacterial contamination is very common. Studies have found that over 40 percent of private water supplies are contaminated with coliform bacteria. Spring water supplies are the most frequently contaminated, with over 70 percent containing coliform bacteria.

Improving protection of a well or spring from the inflow of surface water is an important option to consider if the supply is contaminated with bacteria. It is important to remember that the groundwater is not necessarily contaminated in these cases; rather the well is acting to funnel contaminants down into the groundwater. Although well pits were the common method of construction several years ago, they are no longer considered sanitary construction.

A properly protected well is evidenced by the well casing extending above the surface of the ground and the ground sloping away from the well to prevent water from collecting around the casing. A properly protected spring is developed underground and the water channeled to a sealed spring box. At no time should the water be exposed to the ground surface.

Keeping the plumbing system clean is an important part of maintaining a sanitary water supply. Anytime work is performed on the plumbing or pump, the entire water system should be disinfected with chlorine. Simply pulling the pump out of the well, setting it on the grass to work on it, and returning it to the well is enough to contaminate the well with bacteria.

The procedure for cleaning and sanitizing a well or spring with chlorine is called **shock chlorination**. Concentrations of chlorine ranging from 50 to 200 mg/1 are used in the shock chlorination process. This is 100 to 400 times the amount of chlorine found in "***city water***." The highly chlorinated water is held in the pipes for 12 to 24 hours before it is flushed out and the system is ready to be used again.

Periodic shock chlorination also may be effective in reducing an **iron bacteria** problem. The amount of chlorine needed to shock chlorinate a water system is determined by the amount of water standing in the well.

Table 3 lists the amount of chlorine laundry bleach or powdered high-test hypochlorite (**HTH**) that is needed for wells. If in doubt, it is better to use more chlorine than less.

Calcium Hypochlorite Section ($CaCl_2O_2$)



Physical Properties - Calcium Hypochlorite
Description: White powder, pellets or flat plates
Warning properties: Chlorine odor; inadequate warning of hazardous concentrations
Molecular weight: 142.98 daltons
Boiling point (760 mm Hg): Decomposes at 100ºC (HSDB 2001)
Freezing point: Not applicable
Specific gravity: 2.35 (water = 1)
Water solubility: 21.4% at 76ºF (25ºC)
Flammability: Not flammable

There are two forms of calcium hypochlorite: powder and tablets. Tablets range in size from 5 mg about the size of an Aspirin to 3 inch tablets. Synonyms of calcium hypochlorite include Losantin, hypochlorous acid, calcium salt, BK powder, Hy-Chlor, chlorinated lime, lime chloride, chloride of lime, calcium oxychloride, HTH, mildew remover X-14, perchloron, and pittchlor.

Calcium hypochlorite is generally available as a white powder, pellets, or flat plates; sodium hypochlorite is usually a greenish yellow, aqueous solution. Although not flammable, they may react explosively.

Calcium hypochlorite decomposes in water to release chlorine and oxygen; sodium hypochlorite solutions can react with acids or ammonia to release chlorine or chloramine. Odor may not provide an adequate warning of hazardous concentrations.

Toxic
Both hypochlorites are toxic by the oral and dermal routes and can react to release chlorine or chloramine which can be inhaled. The toxic effects of sodium and calcium hypochlorite are primarily due to the corrosive properties of the hypochlorite moiety. Systemic toxicity is rare, but metabolic acidosis may occur after ingestion.

Description
Solid chlorine stands alone as the safest form of chlorine disinfection. Requiring only minimal safety equipment for handling, users can breathe easy knowing our tablets are safe for both people and the environment. The elimination of costly scrubbers, containment, or hazard response capability, guarantees lower initial costs and reduced operating expense. Calcium hypochlorite is generally available as a white powder, pellets, or flat plates. It decomposes readily in water or when heated, releasing oxygen and chlorine. It has a strong chlorine odor, but odor may not provide an adequate warning of hazardous concentrations. Calcium hypochlorite is not flammable, but it acts as an oxidizer with combustible material and may react explosively with ammonia, amines, or organic sulfides. Calcium hypochlorite should be stored in a dry, well-ventilated area at a temperature below 120ºF (50ºC) separated from acids, ammonia, amines, and other chlorinating or oxidizing agents.

Chlorine Tablet Feeder
These feed systems are low maintenance and an extremely effective means to treat water or wastewater. Dry tablet feeder may or may not have mechanical components and most require no electricity. The dry tablet feeding system is a good alternative to liquid bleach and potential gas hazards. With no chlorine gas cylinders to handle, chlorine releases are non existent. Process safety Management and Risk Management Program compliance worries disappear.

Chlorine Tablet Feeder Capacities: range - 1,500 to 200,000 (GPD)

Chlorine tablets are stable for 3 years or more.

If a tablet produces 1000 PPM in a liter of water when first off the press, the tablet will produce 1000 PPM plus. This guarantees the activity will be at least 100% 3 years later and probably for much longer than that. In fact, tablets have been stored for 6 years at 6% C and 42% C and still contained the specified levels of available chlorine. Sodium hypochlorite liquid, on the other hand, is inherently unstable and degrades with age until all the active strength disappears. This degradation accelerates in conditions of high temperature or strong sunlight.





These two different tablet chlorinator feeding systems are installed as a sidestream (see the clear plastic line) to the mainstream water flow or directly in the well casing. Using a flow meter or timed device, a chlorine tablet is dropped or delivered inside the well casing or to another location in the distribution system. Sometimes, the chlorinated balance is piped to an integrated solution tank. Then the resulting concentrated chlorine solution is pumped into a pressurized line or holding tank. By mixing chlorinated water from the solution tank with unchlorinated water from the main stream, a controllable level of available chlorine is achieved.



Accuracy
Because of their stability, chlorine tablets are an accurate dose, always yielding the stated level of available chlorine in water or very slightly over, never under. Liquid chlorine strengths vary so widely and are mostly unknown (the container usually says "less than 5%") that it is impossible to make up accurate in-use solutions without access to laboratory equipment.

Storage and Distribution
In recent years, concern regarding the safety hazards associated with liquid chlorine has grown to such an extent that several major cities now restrict transportation of chlorine within their boundaries. Tablets, on the other hand, are easy and convenient to store and transport. One pallet containing 600 jars each of 200 tablets is equivalent to 120,000 x 1 liter in use bleach solutions of 1,000 PPM active chlorine concentration.

Liquid chlorine is bulky, heavy and prone to leakage and spillage. Chlorine tablets are compact, economical and safe to ship and can even be sent by airfreight.

Effectiveness
Both chlorine tablets and liquid Sodium hypochlorite produce Hypochlorous Acid (HOCl) and Hypochlorite ion (OCl-) in solution. It has been postulated by Ortenzio and Stuart in 1959 and again by Trueman in 1971 that Hypochlorous Acid is the predominantly active species whilst Hypochlorite ion has little activity due to its negative charge impeding penetration of the cell wall and membrane. The ratio of Hypochlorous Acid to Hypochlorite ion increases with acidity. Chlorine tablets have a pH of 6.7 and liquid hypochlorite a pH of between 9 and 12. Ergo; tablets have a greater disinfection capacity and are less prone to inactivation due to soiling.

Safety
Chlorine tablets in dry form will not leak or splash and do not damage clothing. Liquid chlorine can affect eyes, skin and mucous membranes; it is easily splashed and rots clothing.

Corrosion
Chlorine tablets are much less corrosive than liquid chlorine, which is highly corrosive to most metals

Comparison
The final very important comparison to be made between Sodium hypochlorite (NaOCL) and Sodium dichloroisocyanurate (NaDCC) is their neutralization by organic matter. They are both prone to this but by using horse serum, it has been shown (Coates 1988) that the degree of neutralization is directly proportional to the concentration of serum present. However, the degree of neutralization of Naocl disinfectant is much greater than that of NaDCC disinfectant and the disparity increases with the concentration of serum. Hence, where there is a high concentration of organic material present, NaDCC will be very much more effective than NaOCL.

The degree of inactivation of NaOCL and NaDCC solutions by different concentrations of horse serum demonstrates that NaDCC solutions are less prone to inactivation by serum than are NaOCL solutions. For example, in 30% serum it required only 4000 PPM av. CI of NaDCC as opposed to 17000 PPM av CI of NaOCL to exhibit similar bactericidal activity.

System Sizing
To determine the correct system for your application, some specific information is required:

What form of chlorination is used now? ___ Gas ___ Liquid Bleach ___ Granular

Source water temperature _____ ___ Other (specify: _____)

Is chlorination performed ___ intermittently or ___ continuously?

What is the current consumption? _____ LBS or GALS/DAY

What is the system pressure to be treated?_____ PSI

What is the system flow rate to be treated?_____ GPM

What is the final desired chlorine concentration?_____ PPM

Dose Rate Math

_____ System Flow Rate (GPM)

x _____ Tablets Demand (PPM)

x 0.0005 Conversion Factor

Total _____ = LBS/HR Tablets Required

System Selection
Compare the LBS/HR of chlorine requirement above with the following system capacity and delivery characteristics. An additional factor that needs to be considered is how frequently the unit must be refilled in light of the volume of tablets that the feeder holds.

Health Effects
* Hypochlorite powder, solutions, and vapor are irritating and corrosive to the eyes, skin, and respiratory tract. Ingestion and skin contact produces injury to any exposed tissues. Exposure to gases released from hypochlorite may cause burning of the eyes, nose, and throat; cough as well as constriction and edema of the airway and lungs can occur.
* Hypochlorite produces tissue injury by liquefaction necrosis. Systemic toxicity is rare, but metabolic acidosis may occur after ingestion.

Acute Exposure
The toxic effects of sodium and calcium hypochlorite are primarily due to the corrosive properties of the hypochlorite moiety. Hypochlorite causes tissue damage by liquefaction necrosis. Fats and proteins are saponified, resulting in deep tissue destruction. Further injury is caused by thrombosis of blood vessels. Injury increases with hypochlorite concentration and pH. Symptoms may be apparent immediately or delayed for a few hours. Calcium hypochlorite decomposes in water releasing chlorine gas.

Sodium Hypochlorite Solutions
Sodium hypochlorite solutions liberate the toxic gases chlorine or chloramine if mixed with acid or ammonia (this can occur when bleach is mixed with another cleaning product). Thus, exposure to hypochlorite may involve exposure to these gases.

Children do not always respond to chemicals in the same way that adults do. Different protocols for managing their care may be needed.

Gastrointestinal
Pharyngeal pain is the most common symptom after ingestion of hypochlorite, but in some cases (particularly in children), significant esophagogastric injury may not have oral involvement. Additional symptoms include dysphagia, stridor, drooling, odynophagia, and vomiting. Pain in the chest or abdomen generally indicates more severe tissue damage. Respiratory distress and shock may be present if severe tissue damage has already occurred. In children, refusal to take food or drink liquid may represent odynophagia.

Ingestion of hypochlorite solutions or powder can also cause severe corrosive injury to the mouth, throat, esophagus, and stomach, with bleeding, perforation, scarring, or stricture formation as potential sequelae.

Dermal
Hypochlorite irritates the skin and can cause burning pain, inflammation, and blisters. Damage may be more severe than is apparent on initial observation and can continue to develop over time. Because of their relatively larger surface area/body weight ratio, children are more vulnerable to toxins affecting the skin.

Ocular
Contact with low concentrations of household bleach causes mild and transitory irritation if the eyes are rinsed, but effects are more severe and recovery is delayed if the eyes are not rinsed. Exposure to solid hypochlorite or concentrated solutions can produce severe eye injuries with necrosis and chemosis of the cornea, clouding of the cornea, iritis, cataract formation, or severe retinitis.

Respiratory
Ingestion of hypochlorite solutions may lead to pulmonary complications when the liquid is aspirated. Inhalation of gases released from hypochlorite solutions may cause eye and nasal irritation, sore throat, and coughing at low concentrations. Inhalation of higher concentrations can lead to respiratory distress with airway constriction and accumulation of fluid in the lungs (pulmonary edema). Patients may exhibit immediate onset of rapid breathing, cyanosis, wheezing, rales, or hemoptysis. Pulmonary injury may occur after a latent period of 5 minutes to 15 hours and can lead to reactive airways dysfunction syndrome (RADS), a chemical irritant-induced type of asthma.

Children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways. Children may also be more vulnerable to gas exposure because of increased minute ventilation per kg and failure to evacuate an area promptly when exposed.

Metabolic
Metabolic acidosis has been reported in some cases after ingestion of household bleach.

Potential Sequelae
Exposure to toxic gases generated from hypochlorite solutions can lead to reactive airways dysfunction syndrome (RADS), a chemical irritant-induced type of asthma. Chronic complications following ingestion of hypochlorite include esophageal obstruction, pyloric stenosis, squamous cell carcinoma of the esophagus, and vocal cord paralysis with consequent airway obstruction.

Chronic Exposure
Chronic dermal exposure to hypochlorite can cause dermal irritation.

Carcinogenicity
The International Agency for Research on Cancer has determined that hypochlorite salts are not classifiable as to their carcinogenicity to humans.

Reproductive and Developmental Effects
No information was located regarding reproductive or developmental effects of calcium or sodium hypochlorite in experimental animals or humans. Calcium and sodium hypochlorite are not included in Reproductive and Developmental Toxicants, a 1991 report published by the U.S. General Accounting Office (GAO) that lists 30 chemicals of concern because of widely acknowledged reproductive and developmental consequences.

Sources/Uses
Sodium and calcium hypochlorite are manufactured by the chlorination of sodium hydroxide or lime. Sodium and calcium hypochlorite are used primarily as oxidizing and bleaching agents or disinfectants. They are components of commercial bleaches, cleaning solutions, and disinfectants for drinking water and waste water purification systems and swimming pools.



Chlorine-Based Disinfectants Chloramines

This process involves the addition of ammonia and chlorine compounds to a water filtration plant. When properly controlled, the mixture forms chloramines. They are commonly used to maintain a residual in the distribution system following treatment with a stronger disinfectant, such as free chlorine.

Chloramine Advantages

- Persistent residual.
- Taste and odor minimization.
- Lower levels of trihalomethane (THM) and haloacetic acid (HAA) formation.
- Effective disinfection of biofilms in the distribution system.

Chloramine Disadvantages

- Produces disinfection by-products (DBPs), including nitrogen-based compounds and chloral hydrate, which may be regulated as a DBP in the future. There is limited information on the toxicity of chloramine DBPs. In an analysis of the health effects of alternatives, Bull states that "there is little information on which to base an estimate of the health hazard that chloramine poses."
- Presents problems to individuals on dialysis machines. Chloramine residuals in tap water can pass through membranes in dialysis machines and directly induce oxidant damage to red blood cells.
- Causes eye irritation. Exposure to high levels of chloramine may result in eye irritation.
- Requires increased dosage and contact time (higher CT values, *i.e.,* concentration X time).
- Has questionable value as viral and parasitic biocide.
- Can promote growth of algae in reservoirs and an increase in distribution system bacteria due to residual ammonia.
- Can produce HAAs.
- Provides weaker oxidation and disinfection capabilities than free chlorine.

Chloramine Section

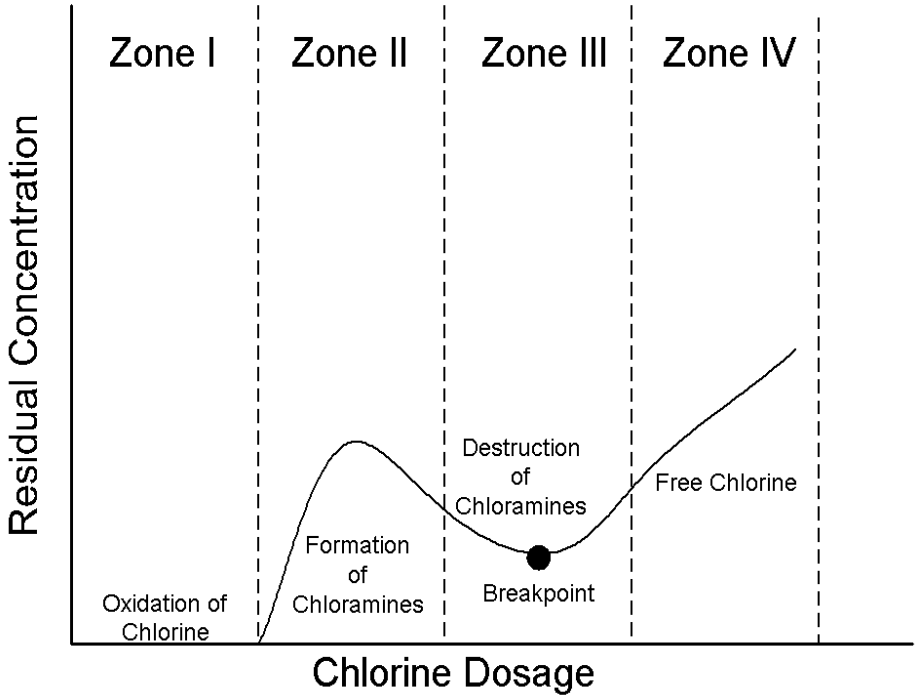
Monochloramine and dichloramine are formed in the pH range of 4.5 to 8.5, however, monochloramine is most common when the pH is above 8. When the pH of the wastewater is below 4.5, the most common form of chloramine is trichloramine which produces a very foul odor. The equations for the formation of the different chloramines are as follows: (Reynolds & Richards, 1996)

Monochloramine: $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$
Dichloramine: $NH_2Cl + 2HOCl \rightarrow NHCl2 + 2H_2O$
Trichloramine: $NHCl2 + 3HOCl \rightarrow NHCl_3 + 3H_2O$

Chloramines are an effective disinfectant against bacteria but not against viruses. As a result, it is necessary to add more chlorine to the wastewater to prevent the formation of chloramines and form other stronger forms of disinfectants.
d) The final step is that additional free chlorine reacts with the chloramine to produce hydrogen ion, water, and nitrogen gas which will come out of solution. In the case of the monochloramine, the following reaction occurs:
$2NH_2Cl + HOCl \rightarrow N_2 + 6HCl + H_2O$

Thus, added free chlorine reduces the concentration of chloramines in the disinfection process. Instead the chlorine that is added is allowed to form the stronger disinfectant, hypochlorus acid.

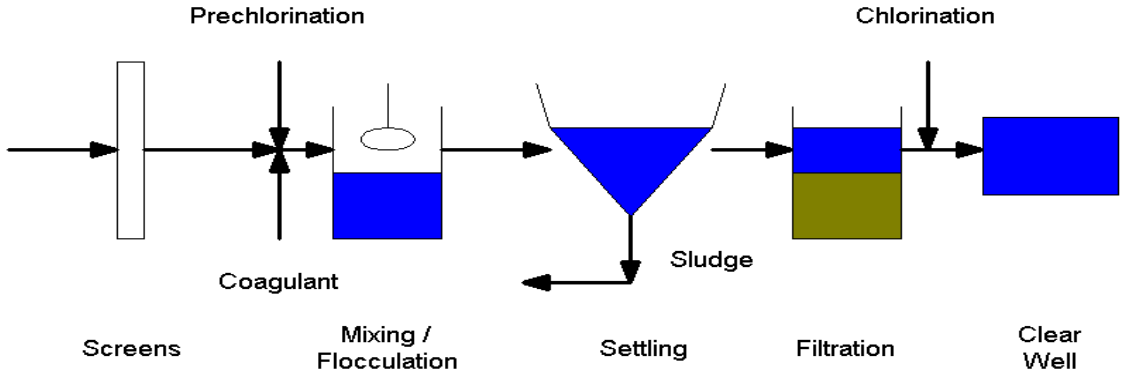
Perhaps the most important stage of the water or wastewater treatment process is the disinfection stage. This stage is most critical because it has the greatest effect on public health as well as the health of the world's aquatic systems. It is important to realize that wastewater treatment is not a cut and dry process but requires in depth knowledge about the type of wastewater being treated and its characteristics to obtain optimum results.



The graph shown on the last page depicts the chlorine residual as a function of increasing chlorine dosage with descriptions of each zone given below (Drawing by Erik Johnston, adapted from Reynolds and Richards, 1996).
· Zone I: Chlorine is reduced to chlorides.
· Zone II: Chloramines are formed.
· Zone III: Chloramines are broken down and converted to nitrogen gas which leaves the system (Breakpoint).
· Zone IV: Free residual.
Therefore, it is very important to understand the amount and type of chlorine that must be added to overcome the difficulties in the strength of the disinfectant which results from the water or wastewater's characteristics.

Water Treatment

The following is a schematic of a water treatment plant (Drawing by Matt Curtis).



In water treatment, pre-chlorination is utilized mainly in situations where the inflow is taken from a surface water source such as a river, lake, or reservoir. Chlorine is usually added in the rapid mixing chamber and effectively prevents the majority of algal growth. Algae is a problem in water treatment plants because it builds up on the filter media and increases the head which means that the filters need to be backwashed more frequently. In addition, the algal growth on the filter media causes taste and odor problems in the treated water.

Post Chlorination

Post chlorination is almost always done in water treatment, but can be replaced with chlorine dioxide or chloramines. In this stage chlorine is fed to the drinking water stream which is then sent to the chlorine contact basin to allow the chlorine a long enough detention time to kill all viruses, bacteria, and protozoa that were not removed and rendered inactive in the prior stages of treatment.

Drinking water requires a large addition of chlorine because there must be a residual amount of chlorine in the water that will carry through the system until it reaches the tap of the user. After post chlorination, the water is retained in a clear well prior to distribution.

Chlorine Production Section

Faraday's laws states that one coulomb of electricity deposits 0.00111801 grams (g) of silver, or 1 ampere of electric current deposits 0.00111801 g of silver in one second.

Faraday's second law states that the quantity of electricity that liberates one gram equivalent weight (e.w.) of an element is the same for all elements. Since the equivalent weight of silver is 107.880, it takes one faraday, or 96,493 coulombs (107.88 e.w. / 0.00111801 g), to liberate 1 gram equivalent weight of any element.

The 1986 recommended value for one faraday is 96,485.309 coulomb. Being consistent with Faraday's laws, one faraday (96,485.3 coulomb) of electricity liberates 1.008 gram of hydrogen and 35.457 grams of chlorine in the chlor-alkali process. Since there are 86,400 seconds in each day (60 sec/min x 60 min/hour x 24 hour/day) and 454 grams in each pound of element, 14.3 amperes of current are required to liberate one pound of chlorine gas during a 24 hour period:

454 g/lb x 96,485.3 coulomb = 14.3 amperes
35.457 g Cl 86,400 sec/day

Coulomb (C)

The SI unit of electric charge. One coulomb is the amount of charge accumulated in one second by a current of one ampere. Electricity is actually a flow of charged particles, such as electrons, protons, or ions. The charge on one of these particles is a whole-number multiple of the charge *e* on a single electron, and one coulomb represents a charge of approximately 6.241 506 x 10^{18} *e*. The coulomb is named for a French physicist, Charles-Augustin de Coulomb (1736-1806), who was the first to measure accurately the forces exerted between electric charges.

Considering typical current efficiencies of 95 percent, the actual electrochemical reaction requires roughly 15 amperes of direct current to produce one pound of chlorine gas during a 24-hour period: 14.3 amperes / 0.95 (efficiency factor) = 15 amperes

The chlorine gas is vacuum swept from the chlorine cell by a Venturi ejector. The chlorine mixes with the ejector water to form hypochlorous acid and/or hypochlorite ion depending on the water pH.

Industrial Uses

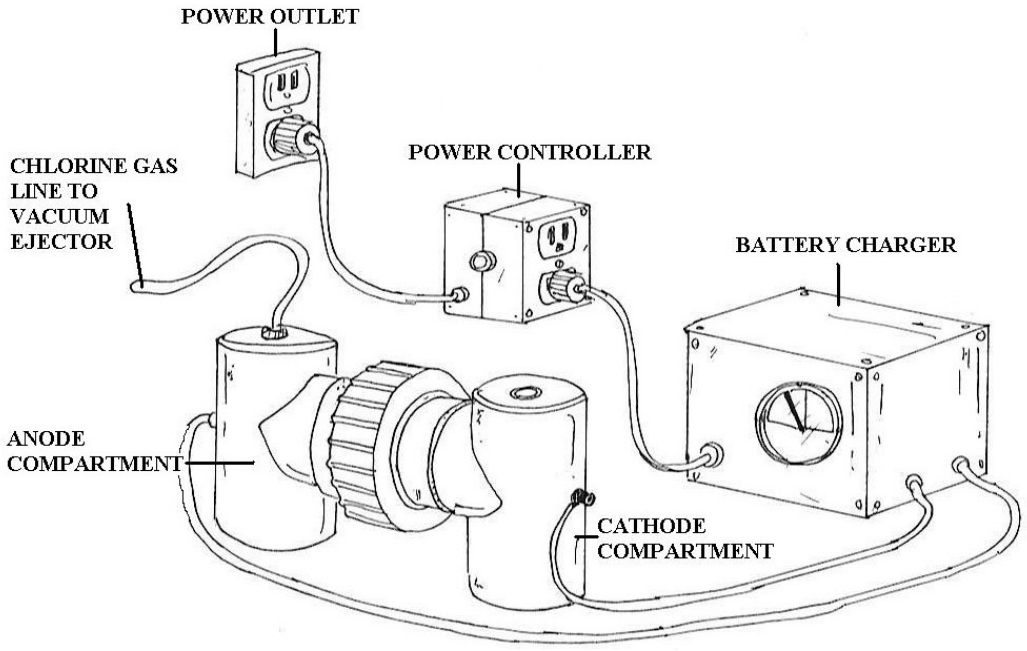
In addition to water treatment chemicals, chlorine is used to make plastics such as PVC (polyvinyl chloride) and polyurethanes, pulp and paper treatment chemicals, solvents and a large number of chemicals.

The most common industrial use of chlorine is the manufacture of a versatile plastic known as polyvinyl chloride, or PVC. PVC is a polymer, meaning on a microscopic level many small units of the same types of atoms are bound together to form long chains, similar to the linking of multiple paper clips. One use of PVC is as lightweight, durable pipes for safe drinking water delivery. PVC pipes resist the pitting and corrosion common in metal pipes which often develop a slimy build-up of disease-causing microbes known as "biofilm." Biofilms can be destroyed by elevated levels of chlorine disinfectant. With so many varied and valuable uses, chlorine chemistry is truly an indispensable asset to modern life.

Chlorine Generator Process
The chlorine generator is as simple as a battery. There are no moving parts; however, the chlorine generator does require operation and maintenance. On this page you will discover:

- Process Components
- Process Installation
- System Operation
- Dose Control
- Salt Addition
- Sodium Hydroxide Dilution
- System Maintenance

Process Components
The chlorine generator requires a cell, DC power supply (battery charger, battery, solar power), power controller, energy source (power outlet, generator), and a pressurized water supply to operate the Venturi ejector.



Cell: The cell includes the anode and cathode compartments that are hydraulically isolated by an ion selective membrane located between the two cell compartments. The anode compartment contains the anode (electrode), salt, saltwater electrolyte, and chlorine. Chlorine gas generated from the anode compartment is swept under vacuum by the Venturi ejector into the water supply. The cathode compartment contains the cathode (electrode), sodium hydroxide (caustic soda) electrolyte, and hydrogen.

The hydrogen produced from the cathode compartment is vented to the outside atmosphere. The two cell compartments are joined together by a union pipe fitting that also holds the ion selective membrane between the union flanges. Please note that the use of a union pipe fitting in the cell configuration is patented and subject to royalty fees.

DC Power Supply
The DC power supply can be any DC battery charger of adequate size to handle the needed chlorine demand. See our power supply sizing page for determining the size of power supply needed.

Power Controller
The power controller is simply a common dimmer switch (used to dim lights) that the power supply is plugged into to adjust the voltage input to the power supply. Like dimming your lights, the power controller will "dim" your chlorine production to the desired chlorine level.

Energy Source
The energy source can basically be a 120 VAC power outlet you plug the Power Controller into.

Pressurized Water Supply
The water passes through a Venturi creating a vacuum that is applied to the anode compartment of the cell. The Venturi ejector also includes a flow switch connected to a relay that operates the Power Controller. This safety feature ensures that flow is going through the Venturi ejector before chlorine is generated. The discharge from the vacuum ejectors is highly chlorinated water in the form of hypochlorous acid and/or hypochlorite ion.

Process Installation
Provided the plumbing for the system is complete (existing vacuum ejector, or simply using a garden hose connected to the ejector); it should not take longer than 30 minutes to an hour to have your chlorine generator completely operational. The installation includes the following steps:

- Remove components from the box, check contents for any missing or broken parts
- Soak the membrane in warm water
- Install the membrane on the cell flange
- Add salt and water to the anode compartment
- Add water and dry sodium hydroxide (i.e. Draino) to the cathode compartment
- Connect water supply to Venturi ejector
- Connect the vacuum tubing from the anode compartment to the Venturi ejector
- Clamp red (positive) power clamp from power supply to anode
- Clamp black (negative) power clamp from power supply to cathode
- Turn on power supply switch
- Plug power supply into power controller
- Plug power controller into power circuit
- Operate Venturi ejector and energize power circuit, adjust power controller to desired chlorine level.

System Operation

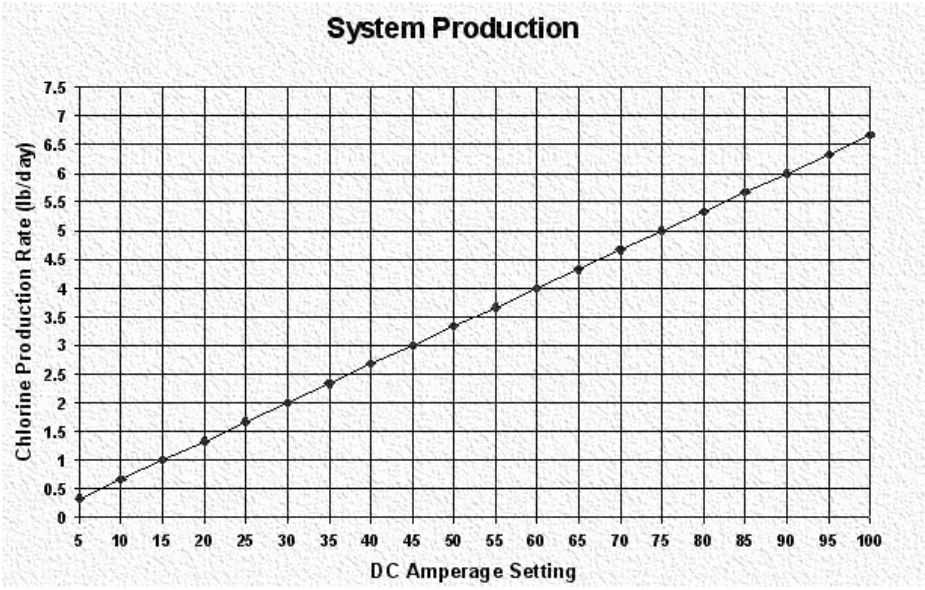
The system operation includes the control of the system, addition of salt and water to the anode compartment, periodic dilution of the sodium hydroxide in the cathode compartment, and occasional cleaning of the cell membrane. There are several ways the chlorine generator can be operated. The simplest way is to plug the power controller into a power outlet that is only energized at times when the generator is needed for chlorine production. This on/off operation procedure can be accomplished by installing a power control relay on the power outlet circuit. Nearly all municipal well installations include this type of circuit typically used for a hypochlorination pump.

The power controller includes a flow switch that ensures operation of the chlorine generator only when there is flow through the Venturi ejector. Having the pool filter and water supply fill line on the vacuum ejector will allow the chlorine generator to operate at any moment when water is moving into the pool.

At a booster pump station having multiple pumps, a chlorine generator for each pump circuit will supply the step chlorine dosage needed depending on the number of pumps operating. This operational procedure eliminates the need for an electronic logic controlled loop and/or pacing valve systems.

The chlorine generator can be controlled in an automatic mode associated with a chlorine demand change. The automatic mode requires an electronic input signal (4-20 mA) associated with the demand change that controls an optional EP1 Series - SCR Power.

Dose Control
Chlorine output is adjusted by the power input of the process. Every 15 amps of direct current (DC) provide a chlorine production rate of 1 pound per day (24 hours). The graph below illustrates the equivalent chlorine production at the desired amperage setting.



Note: With time, the membrane accumulates calcium and other mineral deposits that increase the resistance between the electrodes. The increased resistance causes a reduced amperage output and a corresponding reduced chlorine output. The system needs periodic membrane cleaning to recover the desired amperage output. A water softener system can be added to the system water supply to reduce the amount of calcium, thus increasing the service life of the membrane.

Connecting your power supply (battery charger) into the power controller will allow you to manually adjust the voltage supply to your battery charger, thus controlling the DC amperage output to the cell. The power controller provided with each cell includes a 600 watt dimmer switch to manually adjust the input voltage to the battery charger. Adjustment of the dimmer switch will increase or decrease the voltage output of your battery charger to the desired amperage setting. For example, a chlorine output of 0.5 lbs per day is desired for a 50 gpm well. Based on amperage conversions, approximately 8 amperes of DC power is needed for the well. The operator would adjust the dimmer switch to achieve a power output of 8 DC amperes for the cell.

Salt Addition

For every 50 lb bag of water softener salt, approximately 30 lbs of chlorine is made. The amount of salt that can be added to the cell depends on the shape of the salt pellets; however, a typical amount of salt added in each cycle is roughly 12 lbs. Twelve pounds of salt in the anode compartment will generate 7 lbs of chlorine considering that not all the salt is used in each cycle. The frequency of salt addition depends on the operating cycle. For example:

A 150 gpm municipal well operating a total of 6 hours per day (54,000 gpd) using a 1.5 lb/day (22 amperes) chlorine dosage rate (0.5 mg/l chlorine residual w/ a 0.35 mg/l chlorine demand) will need to have salt added every 18 days (7 lbs/cell / [1.5 lb/day * 6 hr / 24 hr]).

Salt replenishment in the anode compartment requires the drainage of the brine, flushing the anode compartment with water, and addition of new salt and water to the cell. Adding of salt to the cell without flushing and cleaning is not recommended for several reasons. First, the anode compartment contains residual chlorine gas that will be displaced when salt is added. The amount of chlorine gas in this space is small (0.02 lbs); however, this amount of chlorine gas is irritating, especially if in a confined space. Second, the brine contains concentrated mineral impurities that will foul the membrane at a more rapid rate if it is not removed.

Replenishing the Salt Consists of:

- Turn off the power supply to the cell.
- Add roughly 1 cup of sodium hydroxide to the anode compartment. The sodium hydroxide will neutralize the residual chlorine in the brine and make a salt saturated hypochlorite solution.
- Drain the brine solution to waste and flush out the cell removing all the residual matter in the bottom of the cell.
- Add new salt and water to the anode compartment (it is desirable to use softened water to reduce the mineral fouling of the membrane).
- Place system back into service.

Sodium Hydroxide Dilution

Safety: Please note that sodium hydroxide is corrosive and irritating to the skin. If sodium hydroxide touches the skin, wash with water immediately to prevent chemical burn. Wear protective clothing such as rubber gloves and goggles when handling sodium hydroxide.

For every 50 lb bag of water softener salt, approximately 36 gallons of 18 percent sodium hydroxide solution is made. The actual amount of sodium hydroxide produced is dependent upon the level and frequency of dilution. Assuming a 7 lb chlorine cycle per cell, the amount of sodium hydroxide produced from the cell is approximately 8.5 gallons. Using the same 18-day operational cycle as discussed above, approximately one-half gallon of sodium hydroxide solution is produced every day of operation.

Dilution of sodium hydroxide in the cathode compartment requires the removal of approximately one-half gallon of sodium hydroxide and the addition of dilution water to 4-inches from the top of the cathode compartment (Note: more sodium hydroxide is produced than water added for dilution). It is desirable to use softened water for the dilution to reduce the mineral fouling of the membrane.

Maintenance of the sodium hydroxide solution within the optimum range (10-18 percent) provides extended life of the membrane. Daily testing of the sodium hydroxide solution with a typical battery hydrometer will verify the need to dilute the sodium hydroxide. The following table illustrates the specific gravity and concentration of sodium hydroxide at a temperature of 60 degrees F (15.5 degrees C):

% NaOH	Specific Gravity
2	1.023
4	1.045
6	1.067
10	1.090
12	1.112
14	1.134
16	1.156
18	1.178
20	1.201
22	1.223

Dilution of the Sodium Hydroxide Consists of:

- Turn off the power supply to the cell.
- Removal of 1/2 gallon of solution (or more if operating the cell at higher rates or longer periods of time), and dispose/store as desired (this could be disposal down a sanitary drain if solution is not needed; however, sodium hydroxide is needed for pH adjustment in the pool, CIP cleaning for diaries, lift station cleaning in sewer systems, and/or pH adjustment of water for lead and copper corrosion control in municipal drinking water).
- Add dilution water (preferably softened water) to a level of 4-inches from the top of the cathode compartment.

- place system back into service.
- check small amount of solution daily with a hydrometer.

System Maintenance
The chlorine generator has no moving parts and requires minimal maintenance. The system maintenance involves the periodic cleaning of the membrane. The salt and water added to the chlorine generator contain calcium and other minerals that accumulate on the surface of the membrane. These mineral deposits increase the electrical resistance across the membrane eventually reducing the amperage to the cell, thus reducing the chlorine production. Using the same 18-day operational cycle as discussed above, you may achieve two to four months of cell operation before needing to clean the cell membrane (depends on the dilution water quality) Operating the cell at 1 lb/day for 24 hours/day may require membrane cleaning every month (again, depending on the dilution water quality).

Cleaning the Membrane Involves the Following:
- Turn off the power supply to the cell.
- Remove brine and salt from anode compartment as described above.
- Draining and storage of sodium hydroxide from the cathode compartment.
- Flushing the interior of both compartments with water to remove all loose deposits.
- Encrusted mineral deposits on the membrane can be removed by one of two methods.
    - The membrane removal and replacement method requires the removal of the membrane from the union fitting and replacement with a cleaned membrane. The membrane removed from the cell is then cleaned in a weak hydrochloric acid solution (muriatic or pool acid) to dissolve the mineral deposits. After cleaning, observe the condition of the membrane and discard if pin-holes are observed in the membrane. Otherwise, store membrane in a water solution for the next cleaning cycle.
    - The insitu cleaning method involves the addition of water to top of the horizontal pipe connecting the anode and cathode compartments. Addition of 1 cup of muriatic acid to each cell compartment and agitate cell. After five minutes, drain the cell and flush with water.
- Restore sodium hydroxide to the cathode chamber and salt and water to the anode chamber.
- Reconnect power to cell and resume operation.

Replacement Items
Items that wear and need eventual replacement include the vacuum tubing, rubber gasket/o-ring, membrane, and the anode. The anode has an expected life of five years based on a chlorine rate of 1 lb/day under moderate usage. The membrane has an anticipated life of one year depending on the frequency and dilution of the sodium hydroxide (see above). The rubber gasket in the cathode compartment may also need to be replaced every few years as needed.

The vacuum tubing should be checked annually and replace when cracks are observed. Use chlorine compatible tubing such as polyethylene tubing when replacing.

Conclusions

- Chlorine is one of nature's most common chemical elements.
- Electricity applied to salt solutions enables the chlor-alkali industry to harness chlorine captured in salt deposits of ancient oceans.
- Chlorine's chemical properties make it an extremely effective disinfectant and essential component in the chemical manufacture of literally thousands of vital products used every day.
- Pairs of substances that chlorine will react explosively or form explosive compounds with are acetylene and ether, turpentine and ammonia and hydrogen and finely divided metals.
- Monochloramine, dichloramine, and trichloramine are known as combined available chlorine.
- The chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator.
- Chlorine is added to the effluent before the contact chamber for complete mixing. What is the reason for not adding it directly to the chamber? The chamber has very little mixing due to low velocities.
- The two main chemical species formed by chlorine in water and the name that are they are known collectively are HOCl and OCl-; free available chlorine.
- When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equation that best describes this reaction is $C_{l2} + H_2O$ --> H+ + Cl- + HOCl.
- Yoke-type connectors should be used on a chlorine cylinder's valve; always assume the threads on the valve may be worn.
- Excessive chlorine can kill the aerobic organisms in the secondary treatment plant. Take precaution when applying chlorine in the sewer line near a wastewater treatment plant to control hydrogen sulfide production and anaerobic bacteria.
- When replacing the connection from a chlorine cylinder to a chlorinator always use a new, approved gasket on the connector and follow the manufacturer’s instructions.
- Safety precautions when using chlorine gas: In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate.
- Several symptoms of chlorine exposure: Burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting; headaches and dizziness; fatal pulmonary edema, pneumonia, and skin blisters.
- Approved method for storing a chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve and firmly secure each cylinder.
- Emergency procedures in the case of a large uncontrolled chlorine leak: Notify local emergency response team, warn and evacuate people in adjacent areas, be sure that no one enters the leak area without adequate self-contained breathing equipment.

Chlorination Equipment Requirement Section

For all water treatment facilities, chlorine gas under pressure shall not be permitted outside the chlorine room. A chlorine room is where chlorine gas cylinders and/or ton containers are stored. Vacuum regulators shall also be located inside the chlorine room. The chlorinator, which is the mechanical gas proportioning equipment, may or may not be located inside the chlorine room.

For new and upgraded facilities, from the chlorine room, chlorine gas vacuum lines should be run as close to the point of solution application as possible. Injectors should be located to minimize the length of pressurized chlorine solution lines. A gas pressure relief system shall be included in the gas vacuum line between the vacuum regulator(s) and the chlorinator(s) to ensure that pressurized chlorine gas does not enter the gas vacuum lines leaving the chlorine room.

The gas pressure relief system shall vent pressurized gas to the atmosphere at a location that is not hazardous to plant personnel; vent line should be run in such a manner that moisture collecting traps are avoided. The vacuum regulating valve(s) shall have positive shutdown in the event of a break in the downstream vacuum lines.

As an alternative to chlorine gas, it is permissible to use hypochlorite with positive displacement pumping. Anti-siphon valves shall be incorporated in the pump heads or in the discharge piping.

Capacity
The chlorinator shall have the capacity to dose enough chlorine to overcome the demand and maintain the required concentration of the "***free***" or "***combined***" chlorine.

Methods of Control
A chlorine feed system shall be automatic proportional controlled, automatic residual controlled, or compound loop controlled. In the automatic proportional controlled system, the equipment adjusts the chlorine feed rate automatically in accordance with the flow changes to provide a constant pre-established dosage for all rates of flow. In the automatic residual controlled system, the chlorine feeder is used in conjunction with a chlorine residual analyzer which controls the feed rate of the chlorine feeders to maintain a particular residual in the treated water.

In the compound loop control system, the feed rate of the chlorinator is controlled by a flow proportional signal and a residual analyzer signal to maintain particular chlorine residual in the water.

Manual chlorine feed systems may be installed for groundwater systems with constant flow rates.

Standby Provision
As a safeguard against malfunction and/or shut-down, standby chlorination equipment having the capacity to replace the largest unit shall be provided. For uninterrupted chlorination, gas chlorinators shall be equipped with an automatic changeover system. In addition, spare parts shall be available for all chlorinators.

Weigh Scales
Scales for weighing cylinders shall be provided at all plants using chlorine gas to permit an accurate reading of total daily weight of chlorine used. At large plants, scales of the recording and indicating type are recommended. As a minimum, a platform scale shall be provided. Scales shall be of corrosion-resistant material.



Securing Cylinders
All chlorine cylinders shall be securely positioned to safeguard against movement. Tag the cylinder "***empty***" and store upright and chained.

Ton containers may not be stacked.

Chlorine Leak Detection
Automatic chlorine leak detection and related alarm equipment shall be installed at all water treatment plants using chlorine gas. Leak detection shall be provided for the chlorine rooms. Chlorine leak detection equipment should be connected to a remote audible and visual alarm system and checked on a regular basis to verify proper operation.



Leak detection equipment shall not automatically activate the chlorine room ventilation system in such a manner as to discharge chlorine gas. During an emergency, if the chlorine room is unoccupied, the chlorine gas leakage shall be contained within the chlorine room itself in order to facilitate a proper method of clean-up.

Consideration should also be given to the provision of caustic soda solution reaction tanks for absorbing the contents of leaking one-ton cylinders where such cylinders are in use.

Chlorine leak detection equipment may not be required for very small chlorine rooms with an exterior door (e.g., floor area less than $3m^2$).

You can use a spray solution of ammonia or a rag soaked with ammonia to detect a small Cl_2 leak. If there is a leak, the ammonia will create a white colored smoke, Ammonium chloride.

Safety Equipment
The facility shall be provided with personnel safety equipment including the following:
Respiratory equipment; safety shower, eyewash, gloves, eye protection; protective clothing; cylinder and/or ton repair kits.

Respiratory equipment shall be provided which has been approved under the Occupational Health and Safety Act, General Safety Regulation - Selection of Respiratory Protective Equipment. Equipment shall be in close proximity to the access door(s) of the chlorine room.

Chlorine Room Design Requirements
Where gas chlorination is practiced, the gas cylinders and/or the ton containers up to the vacuum regulators shall be housed in a gas-tight, well illuminated, corrosion resistant and mechanically ventilated enclosure. The chlorinator may or may not be located inside the chlorine room. The chlorine room shall be located at the ground floor level.

Ventilation
Gas chlorine rooms shall have entirely separate exhaust ventilation systems capable of delivering one (1) complete air change per minute during periods of chlorine room occupancy only. The air outlet from the room shall be 150 mm above the floor and the point of discharge located to preclude contamination of air inlets to buildings or areas used by people. The vents to the outside shall have insect screens.

Air inlets should be louvered near the ceiling, the air being of such temperature as to not adversely affect the chlorination equipment. Separate switches for fans and lights shall be outside the room at all entrance or viewing points, and a clear wire-reinforced glass window shall be installed in such a manner as to allow the operator to inspect from the outside of the room.

Heating
Chlorine rooms shall have separate heating systems, if a forced air system is used to heat the building. The hot water heating system for the building will negate the need for a separate heating system for the chlorine room. The heat should be controlled at approximately 15^{o}C.

Cylinders or containers shall be protected to ensure that the chlorine maintains its gaseous state when entering the chlorinator.

Access
All access to the chlorine room shall only be from the exterior of the building. Visual inspection of the chlorination equipment from inside may be provided by the installation of glass window(s) in the walls of the chlorine room. Windows should be at least 0.20 m2 in area, and be made of clear wire reinforced glass.

There should also be a ***'panic bar'*** on the inside of the chlorine room door for emergency exit.

Storage of Chlorine Cylinders
If necessary, a separate storage room may be provided to simply store the chlorine gas cylinders, with no connection to the line. The chlorine cylinder storage room shall have access either to the chlorine room or from the plant exterior, and arranged to prevent the uncontrolled release of spilled gas.

The chlorine gas storage room shall have provision for ventilation at thirty air changes per hour. Viewing glass windows and a panic button on the inside of the door should also be provided.

In very large facilities, entry into the chlorine rooms may be through a vestibule from outside.

Scrubbers
For facilities located within residential or densely populated areas, consideration shall be given to provide scrubbers for the chlorine room.



Chlorine Demand
Chlorine combines with a wide variety of materials. These side reactions complicate the use of chlorine for disinfecting purposes. Their demand for chlorine must be satisfied before chlorine becomes available to accomplish disinfection. The amount of chlorine required to react on various water impurities before a residual is obtained. Also, it means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

Chlorine Questions and Answer Review
Downstream from the point of post chlorination, what should the concentration of a free chlorine residual be in a clear well or distribution reservoir? 0.5 mg/L.

True or False. Even brief exposure to 1,000 ppm of Cl2 can be fatal. True

How does one determine the ambient temperature in a chlorine room? Use a regular thermometer because ambient temperature is simply the air temperature of the room.

How is the effectiveness of disinfection determined? From the results of coliform testing.

How often should chlorine storage ventilation equipment be checked? Daily.

Chlorine Health Hazard Section

Signs and Symptoms of Exposure
1. Acute exposure: Acute exposure to low levels of chlorine results in eye, nose, and throat irritation, sneezing, excessive salivation, general excitement, and restlessness. Higher concentrations causes difficulty in breathing, violent coughing, nausea, vomiting, cyanosis, dizziness, headache, choking, laryngeal edema, acute tracheobronchitis, chemical pneumonia. Contact with the liquid can result in frostbite burns of the skin and eyes [Genium 1992].
2. Chronic exposure: Chronic exposure to low levels of chlorine gas can result in a dermatitis known as chloracne, tooth enamel corrosion, coughing, severe chest pain, sore throat, hemoptysis and increased susceptibility to tuberculosis [Genium 1992].

Inhalation
Immediately remove the exposed person upwind from the contaminated area and contact the poison control center. Inhalation can cause coughing, sneezing, shortness of breath, sensation of tightness in the chest, as well as severe restlessness or anxiety, nausea, and vomiting. The nose and throat may become irritated; a stinging and burning sensation may be experienced. Immediate fatalities can occur as a result of suffocation. Delayed fatalities can occur as a result of pulmonary edema (fluid in the lungs). For this reason, rest and immediate attention after inhalation is important. Persons with known cardiovascular or lung problems should not risk chlorine exposure. If breathing has stopped, give artificial respiration; if breathing is difficult, give oxygen if equipment and trained personnel are available. If exposed person is breathing, place in a comfortable position and keep person warm and at rest until medical assistance becomes available.

Eye/Skin Contact
Liquid and concentrated gas could produce severe burns and injury on contact.
Eye
Pour a gentle stream of warm water through the affected eye for at least 15 minutes. Contact the poison control center, emergency room or physician right away as further treatment will be necessary.
Skin
Run a gentle stream of water over the affected area for 15 minutes. A mild soap may be used if available. Contact the poison control center, emergency room or physician right away as further treatment will be necessary.

Chronic
Repeated exposures can result in a loss of ability to detect the odor of chlorine. Long term exposures may cause damage to teeth and inflammation or ulceration of the nasal passages.

Ingestion
Not applicable for gas. Liquid could produce severe burns and injury on contact.

Pre-hospital Management
* Rescue personnel are at low risk of secondary contamination from victims who have been exposed only to gases released from hypochlorite solutions. However, clothing or skin soaked with industrial-strength bleach or similar solutions may be corrosive to rescuers and may release harmful gases.
* Ingestion of hypochlorite solutions may cause pain in the mouth or throat, dysphagia, stridor, drooling, odynophagia, and vomiting. Hypochlorite irritates the skin and can cause burning pain,

inflammation, and blisters. Acute exposure to gases released from hypochlorite solutions can cause coughing, eye and nose irritation, lacrimation, and a burning sensation in the chest. Airway constriction and noncardiogenic pulmonary edema may also occur.
* There is no specific antidote for hypochlorite poisoning. Treatment is supportive.

Hot Zone
Rescuers should be trained and appropriately attired before entering the Hot Zone. If the proper equipment is not available, or if rescuers have not been trained in its use, assistance should be obtained from a local or regional HAZMAT team or other properly equipped response organization.

Rescuer Protection
Hypochlorite is irritating to the skin and eyes and in some cases may release toxic gases.

Respiratory Protection: Positive-pressure, self-contained breathing apparatus (SCBA) is recommended in response to situations that involve exposure to potentially unsafe levels of chlorine gas.

Skin Protection: Chemical-protective clothing should be worn due to the risk of skin irritation and burns from direct contact with solid hypochlorite or concentrated solutions.

ABC Reminders
Quickly establish a patient airway, ensure adequate respiration and pulse. If trauma is suspected, maintain cervical immobilization manually and apply a cervical collar and a backboard when feasible.

Victim Removal
If victims can walk, lead them out of the Hot Zone to the Decontamination Zone. Victims who are unable to walk may be removed on backboards or gurneys; if these are not available, carefully carry or drag victims to safety.

Consider appropriate management in victims with chemically-induced acute disorders, especially children who may suffer separation anxiety if separated from a parent or other adult.

Decontamination Zone
Victims exposed only to chlorine gas released by hypochlorite who have no skin or eye irritation do not need decontamination. They may be transferred immediately to the Support Zone. All others require decontamination as described below.

Rescuer Protection
If exposure levels are determined to be safe, decontamination may be conducted by personnel wearing a lower level of protection than that worn in the Hot Zone (described above).

ABC Reminders
Quickly establish a patient airway, ensure adequate respiration and pulse. Stabilize the cervical spine with a collar and a backboard if trauma is suspected. Administer supplemental oxygen as required. Assist ventilation with a bag-valve-mask device if necessary.

Basic Decontamination
Rapid decontamination is critical. Victims who are able may assist with their own decontamination. Remove and double-bag contaminated clothing and personal belongings. Flush exposed skin and hair with copious amounts of plain tepid water. Use caution to avoid hypothermia when decontaminating victims, particularly children or the elderly. Use blankets or warmers after decontamination as needed.

Irrigate exposed or irritated eyes with saline, Ringer's lactate, or D5W for at least 20 minutes. Eye irrigation may be carried out simultaneously with other basic care and transport. Remove contact lenses if it can be done without additional trauma to the eye. If a corrosive material is suspected or if pain or injury is evident, continue irrigation while transferring the victim to the support zone.

In Cases of Ingestion, Do Not Induce Emesis or Offer Activated Charcoal.
Victims who are conscious and able to swallow should be given 4 to 8 ounces of water or milk; if the victim is symptomatic, delay decontamination until other emergency measures have been instituted. Dilultants are contraindicated in the presence of shock, upper airway obstruction, or in the presence of perforation.

Consider appropriate management of chemically contaminated children at the exposure site. Provide reassurance to the child during decontamination, especially if separation from a parent occurs.

Transfer to Support Zone
As soon as basic decontamination is complete, move the victim to the Support Zone.

Support Zone
Be certain that victims have been decontaminated properly (see Decontamination Zone above). Victims who have undergone decontamination or have been exposed only to vapor pose no serious risks of secondary contamination to rescuers. In such cases, Support Zone personnel require no specialized protective gear.

ABC Reminders
Quickly establish a patient airway, ensure adequate respiration and pulse. If trauma is suspected, maintain cervical immobilization manually and apply a cervical collar and a backboard when feasible. Administer supplemental oxygen as required and establish intravenous access if necessary. Place on a cardiac monitor, if available.

Additional Decontamination
Continue irrigating exposed skin and eyes, as appropriate.
In cases of ingestion, do not induce emesis or offer activated charcoal.
Victims who are conscious and able to swallow should be given 4 to 8 ounces of water or milk; if the victim is symptomatic, delay decontamination until other emergency measures have been instituted. Dilutants are contraindicated in the presence of shock, upper airway obstruction, or in the presence of perforation.
Advanced Treatment

In cases of respiratory compromise, secure airway and respiration via endotracheal intubation. Avoid blind nasotracheal intubation or use of an esophageal obturator: only use direct visualization to intubate. When the patient's condition precludes endotracheal intubation, perform cricothyrotomy if equipped and trained to do so.

Treat patients who have bronchospasm with an aerosolized bronchodilator such as albuterol. Consider racemic epinephrine aerosol for children who develop stridor. Dose 0.25-0.75 mL of 2.25% racemic epinephrine solution in water, repeat every 20 minutes as needed cautioning for myocardial variability.

Patients who are comatose, hypotensive, or having seizures or who have cardiac arrhythmias should be treated according to advanced life support (ALS) protocols.

Transport to Medical Facility
Only decontaminated patients or those not requiring decontamination should be transported to a medical facility. "Body bags" are not recommended.

Report to the base station and the receiving medical facility the condition of the patient, treatment given, and estimated time of arrival at the medical facility.

If a chemical has been ingested, prepare the ambulance in case the victim vomits toxic material. Have ready several towels and open plastic bags to quickly clean up and isolate vomitus.

Multi-Casualty Triage
Consult with the base station physician or the regional poison control center for advice regarding triage of multiple victims.

Patients who have ingested hypochlorite, or who show evidence of significant exposure to hypochlorite or chlorine (e.g., severe or persistent cough, dyspnea or chemical burns) should be transported to a medical facility for evaluation. Patients who have minor or transient irritation of the eyes or throat may be discharged from the scene after their names, addresses, and telephone numbers are recorded. They should be advised to seek medical care promptly if symptoms develop or recur.

Routes of Exposure
Exposure to chlorine can occur through inhalation, ingestion, and eye or skin contact [Genium 1992].

Summary of Toxicology
1. Effects on Animals: Chlorine is a severe irritant of the eyes, mucous membranes, skin, and lungs in experimental animals. The 1 hour LC(50) is 239 ppm in rats and 137 ppm in mice ()[Sax and Lewis 1989]. Animals surviving sublethal inhalation exposures for 15 to 193 days showed marked emphysema, which was associated with bronchiolitis and pneumonia [Clayton and Clayton 1982]. Chlorine injected into the anterior chamber of rabbits' eyes resulted in severe damage with inflammation, opacification of the cornea, atrophy of the iris, and injury to the lens [Grant 1986].

2. Effects on Humans: Severe acute effects of chlorine exposure in humans have been well documented since World War I when chlorine gas was used as a chemical warfare agent. Other severe exposures have resulted from the accidental rupture of chlorine tanks. These exposures have caused death, lung congestion, and pulmonary edema, pneumonia, pleurisy, and

bronchitis [Hathaway et al. 1991]. The lowest lethal concentration reported is 430 ppm for 30 minutes [Clayton and Clayton 1982].

Exposure to 15 ppm causes throat irritation, exposures to 50 ppm are dangerous, and exposures to 1000 ppm can be fatal, even if exposure is brief [Sax and Lewis 1989; Clayton and Clayton 1982]. Earlier literature reported that exposure to a concentration of about 5 ppm caused respiratory complaints, corrosion of the teeth, inflammation of the mucous membranes of the nose and susceptibility to tuberculosis among chronically-exposed workers.

However, many of these effects are not confirmed in recent studies and are of very dubious significance [ACGIH 1991]. A study of workers exposed to chlorine for an average of 10.9 years was published in 1970. All but six workers had exposures below 1 ppm; 21 had TWAs above 0.52 ppm.

No evidence of permanent lung damage was found, but 9.4 percent had abnormal EKGs compared to 8.2 percent in the control group. The incidence of fatigue was greater among those exposed above 0.5 ppm [ACGIH 1991]. In 1981, a study was published involving 29 subjects exposed to chlorine concentrations up to 2.0 ppm for 4- and 8-hour periods. Exposures of 1.0 ppm for 8 hours produced statistically significant changes in pulmonary function that were not observed at a 0.5 ppm exposure concentration. Six of 14 subjects exposed to 1.0 ppm for 8 hours showed increased mucous secretions from the nose and in the hypopharynx. Responses for sensations of itching or burning of the nose and eyes, and general discomfort were not severe, but were perceptible, especially at the 1.0 ppm exposure level [ACGIH 1991]. A 1983 study of pulmonary function at low concentrations of chlorine exposure also found transient decreases in pulmonary function at the 1.0 ppm exposure level, but not at the 0.5 ppm level [ACGIH 1991]. Acne (chloracne) is not unusual among persons exposed to low concentrations of chlorine for long periods of time. Tooth enamel damage may also occur [Parmeggiani 1983]. There has been one confirmed case of myasthenia gravis associated with chlorine exposure [NLM 1995].

Emergency Medical Procedures: [NIOSH to Supply]

- Rescue: Remove an incapacitated worker from further exposure and implement appropriate emergency procedures (e.g., those listed on the Safety Data Sheet (formerly MSDS) required by OSHA's Hazard Communication Standard [29 CFR 1910.1200]).
- All workers should be familiar with emergency procedures, the location and proper use of emergency equipment, and methods of protecting themselves during rescue operations.

Exposure Sources and Control Methods

The following operations may involve chlorine and lead to worker exposures to this substance:

The Manufacture and Transportation of Chlorine

- Used as a chlorinating and oxidizing agent in organic and inorganic synthesis; in the manufacture of chlorinated solvents, automotive antifreeze and antiknock compounds, polymers (synthetic rubber and plastics), resins, elastomers, pesticides, refrigerants, and in the manufacture of rocket fuel.
- Used as a fluxing, purification, and extraction agent in metallurgy.
- Used as a bacteriostat, disinfectant, odor control, and demulsifier in treatment of drinking water, swimming pools, and in sewage.
- Used in the paper and pulp, and textile industries for bleaching cellulose for artificial fibers; used in the manufacture of chlorinated lime; used in detinning and dezincing iron; used to shrink-proof wool.
- Used in the manufacture of pharmaceuticals, cosmetics, lubricants, flameproofing, adhesives, in special batteries containing lithium or zinc, and in hydraulic fluids; use in the processing of meat, fish, vegetables, and fruit.
- Used as bleaching and cleaning agents, and as a disinfectant in laundries, dishwashers, cleaning powders, cleaning dairy equipment, and bleaching cellulose.

Methods that are effective in controlling worker exposures to chlorine, depending on the feasibility of implementation, are as follows: process enclosure, local exhaust ventilation, general dilution, ventilation and personal protective equipment.

Workers responding to a release or potential release of a hazardous substance must be protected as required by paragraph (q) of OSHA's Hazardous Waste Operations and Emergency Response Standard 29 CFR.

Good Sources of Information about Control Methods are as Follows:

1. ACGIH [1992]. Industrial ventilation--a manual of recommended practice. 21st ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.
2. Burton DJ [1986]. Industrial ventilation--a self-study companion. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.
3. Alden JL, Kane JM [1982]. Design of industrial ventilation systems. New York, NY: Industrial Press, Inc.
4. Wadden RA, Scheff PA [1987]. Engineering design for control of workplace hazards. New York, NY: McGraw-Hill.
5. Plog BA [1988]. Fundamentals of industrial hygiene. Chicago, IL: National Safety Council.



Chlorine Storage

Chlorine should be stored in a cool, dry, well-ventilated area in tightly sealed containers that are labeled in accordance with OSHA's Hazard Communication Standard [29 CFR 1910.1200].

Containers of chlorine should be protected from exposure to weather, extreme temperatures changes, and physical damage, and they should be stored separately from flammable gases and vapors, combustible substances (such as gasoline and petroleum products, hydrocarbons, turpentine, alcohols, acetylene, hydrogen, ammonia, and sulfur), reducing agents, finely divided metals, arsenic, bismuth, boron, calcium, activated carbon, carbon disulfide, glycerol, hydrazine, iodine, methane, oxomonosilane, potassium, propylene, silicon, hydrogen sulfide and water, carbon monoxide and sulfur dioxide, moisture, steam, and water.

Workers handling and operating chlorine containers, cylinders, and tank wagons should receive special training in standard safety procedures for handling compressed corrosive gases. All pipes and containment used for chlorine service should be regularly inspected and tested.

Empty containers of chlorine should have secured protective covers on their valves and should be handled appropriately.

Spills and Leaks



In the event of a spill or leak involving chlorine, persons not wearing protective equipment and fully-encapsulating, vapor-protective clothing should be restricted from contaminated areas until cleanup has been completed. The following steps should be undertaken following a spill or leak:

1. Notify safety personnel.
2. Remove all sources of heat and ignition.
3. Keep all combustibles (wood, paper, oil, etc.) away from the leak.
4. Ventilate potentially explosive atmospheres.
5. Evacuate the spill area at least 50 feet in all directions.
6. Find and stop the leak if this can be done without risk; if not, move the leaking container to an isolated area until gas has dispersed. The cylinder may be allowed to empty through a reducing agent such as sodium bisulfide and sodium bicarbonate.
7. Use water spray to reduce vapors; do not put water directly on the leak or spill area.



Chemical Spill Procedure Example

TOXIC CHEMICAL RELEASE: CHLORINE GAS, AMMONIA, AND LIQUID CHLORINE OR OTHER SUBSTANCE POSING IMMEDIATE HEALTH

DANGER: Evacuate the area. Close all fire doors. Contact the fire department or appropriate emergency response crew immediately. If the substance is liquid and a drain is in the area of the spill, contact the sewer department.

If it is safe for you to clean up the spill:
READ SAFETY DATA SHEET (FORMERLY MSDS) (SDS) FOR SPILLED CHEMICAL.

Read the section STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED.

Read the **WASTE DISPOSAL METHOD** listed.

LOCATE CHEMICAL SPILLS KIT:



Apply gloves and protective eyewear.

Use chemical pads located in the kit to soak up the spill.

Place contaminated pads and gloves in the disposal bag and seal.

IF THE CHEMICAL IS A HAZARDOUS CHEMICAL WASTE, write the name of the chemical on the chemical label, attach the label to the disposal bag and notify a licensed Hazardous Waste Hauler for pick up. All other chemicals can be placed in regular trash.

Follow the methods listed on the SDS for cleaning the contaminated area.

Replace items used from the Chemical Spill Kit.

Evacuation and Emergency Procedures

Leak Procedures

Minor Leak
Note: A minor leak is a small leak which can be discharged to the environment without danger or when the source of the leak can be readily controlled.

If you determine from outside the chlorine feed room that there is a minor leak, do the following:
1. Notify your supervisor.
2.Have your safety partner don SCBA and be watching you from outside the chlorine room.
3. Equip yourself with a SCBA.
4. Enter chlorine gas room.
Once Inside
5. Turn chlorine cylinder(s) OFF, leave water on.
6. Adjust feed rate to maximum to purge system.
7. Vacate room and remove air pack. Wait for 15 minutes, until chlorine pressure drops to zero or vacuum goes to maximum.
8. Do the Pre-Entry Check, put SCBA back on.
9. Crack open cylinder(s) and shut off right away.
10. Use ammonium hydroxide solution to find the leak.



11. Mark the leak.
12. Purge the system of gas as indicated on page 18, Section C (3), with the water still on.
13. Repair gently, using correct tools.
14. Start-up and re-check for leaks.
15. If no more leaks, place system back into service.
NOTE: If unable to repair the source of the leak, call it a Major Leak, and follow the appropriate emergency steps.
16. **Clean up:**
• remove air pack and recharge;
• air or launder clothes; and
• take a shower.
17. **Document the event completely**. Report the events which may have serious health or safety implications to the State or Federal Occupational Safety and Health Administration and/or Environmental Protection Agency (for Possible Air Violations and Toxic Releases). Also contact the local Fire Department and your Risk Management personnel.

Major Leak

If you determine from outside the chlorine gas feed or storage room that there is a major leak, you could have a real problem not only for your fellow workers but also for nearby residents and for the plant equipment! Workers can protect themselves with SCBA. Residents may have to be evacuated.

We recommend the following steps, if you discover a Major Leak at your facility.
1. Protect yourself at all times during the emergency, and make sure you will not be overcome by the leaking gas. Stay out of the chlorine gas room. Keep the SCBA ready. Chlorine gas escaping through the ventilation outlet may be collecting outside the chlorine gas room, so be careful outside as well.
2. Isolate the area.
3. Notify your supervisor.
4. Implement the Emergency Response Contingency Plan that has been established for your facility, in consultation with the Safety/Health Committee and/or Risk Management Department.
NOTE: The following steps should be customized as necessary.
5. Notify your Chlorep/Supplier, fire department, police, Spill Report Center, according to your facility's policy.
6. Follow directions given by Chlorep/Supplier.
7. Document the events. Take photographs. Measure the Chlorine in the air.
8. Notify the State or Occupational Safety and Health Administration and/or Environmental Protection Agency (for Possible Air Violations and Toxic Releases). Also contact the local Fire Department and your Risk Management personnel.



Emergency Response Contingency Plans

General Planning Considerations
This can be utilized as the alternative assignment.
1. The plan should be clear, concise and easy to use.
2. Include diagrams of the surrounding land use and occupancy (e.g. schools, residences, hospitals, businesses, etc.), with the approximate distances.
3. Include a diagram of the chlorine room layout. It should show equipment location, floor drainage direction, and show the north direction with an arrow. If a floor drain is installed, include drainage system details.
4. Prepare a complete telephone list of the current employees, persons, organizations or other necessary contacts, including 24-hour emergency contact telephone numbers. The list should be revised, updated and distributed periodically by an assigned person.
5. List the personal protective equipment available on-site and from the local fire department. Include phone number(s).
6. List the emergency equipment and supplies available 24 hours a day on-site and from local suppliers. Include phone number(s).
7. Refer to the Occupational Safety and Health Administration and/or Environmental Protection Agency for any assistance and advice.
8. Routinely set up an emergency chlorine leak safety exercise. Practice makes perfect.
When developing your detailed Emergency Response Contingency Plan, consider the following questions:
• Who may be affected by a potential incident? This is governed by site location, adjacent population, terrain, the amount of chemical stored at your facility and its potential for release.
• Is an emergency phone list of trained personnel available and updated periodically?
• When should outside emergency response agencies (chlorine supplier, CHLOREP team, government agency, etc.) be called? By whom?
• When should the news media be notified? By whom? What information should a statement include?
• Who should be contacted first, second, etc.? An emergency notification list should be developed and periodically updated. It should prioritize who is called, including your own utility personnel.
• Is there an evacuation procedure for employees at the facility? Was this reviewed in the last year?
• How do you notify the public (in close proximity to the facility) when to evacuate?
• Should barricades be set up to keep unauthorized personnel from the scene?
• Are proper chlorine leak detectors installed and functioning? Are the alarm systems routinely checked? Are audible and/or visual alarms observable from any approach to the contaminated area?
• Is the local fire department familiar with the facility and your chlorine emergency procedure?
• Does the treatment facility heating and air-conditioning system have to be shut down in a chlorine emergency at the facility?
• Should doors and windows in close proximity to the affected area be closed?
• Are the duty operator(s) familiar with emergency procedures?
• Are proper warning signs in place?
• Is a manual with chlorine emergency guidelines readily available?
• Is there proper documentation of training, equipment inspection, and incidents?
• Is emergency training adequate? Is the training instructor knowledgeable?
• Are personnel trained in the use of self-contained air packs and leak-repair kits? Is this equipment routinely inspected?

• Are medical examinations given to personnel who are trained in the use of self-contained air packs?
• Are chlorine Safety Data Sheet (formerly MSDS) (SDS) and first-aid procedures readily available?
• Stopping a leak-who, when and how? Personnel must be trained to determine when it is best to stop the leak or when to spend their efforts in other areas such as evacuation, you often cannot do both. Who is going to help you stop the leak? Are they nearby or must they travel a great distance?
• Who determines when it is safe to return to evacuated neighborhoods?
• How can your facility be put back into operation? What are the most likely sources of help to put your facility back into operation? How does the emergency plan fit into your operational plan for equipment breakdowns?
• Should the facility designer be notified?
• What else should I plan for?

Emergency Phone Contacts

(To Be Posted Next To Chlorine Room Switches and Phone)

Name
Telephone

Supervisor:
Supervisor:
Operator:
Operator:
Operator:

Emergency Measures Organization (EMO) or Risk Management Team/Response Team
EMO Coordinator:

Fire Department:

Local Ambulance:

Local Hospital:

Fire Department

CHLORINE EMERGENCY RESPONSE: 24 hour collect call
Chlorine Supplier:
Chlorine Supplier:
CHLOREP TEAM:

CHLORINE INFORMATION:

Special Requirements

The U.S. Environmental Protection Agency (**EPA**) requirements for emergency planning, reportable quantities of hazardous releases, community right-to-know, and hazardous waste management may change over time. Users are therefore advised to determine periodically whether new information is available.

Emergency Planning Requirements
Employers owning or operating a facility at which there are 100 pounds or more of chlorine must comply with the EPA's emergency planning requirements [40 CFR Part 355.30].

Reportable Quantity Requirements for Hazardous Releases
A hazardous substance release is defined by the EPA as any spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing into the environment, including the abandonment or discarding of contaminated containers) of hazardous substances. In the event of a release that is above the reportable quantity for that chemical, employers are required to notify the proper Federal, State, and local authorities [40 CFR

The Reportable Quantity of Chlorine is 10 Pounds.
If an amount equal to or greater than this quantity is released within a 24-hour period in a manner that will expose persons outside the facility, employers are required to do the following:

Notify the National Response Center immediately at (800) or at (202) 426-2675 in Washington, D.C. [40 CFR 302.6]. Notify the emergency response commission of the State likely to be affected by the release [40 CFR 355.40]. Notify the community emergency coordinator of the local emergency planning committee (or relevant local emergency response personnel) of any area likely to be affected by the release [40 CFR 355.40].

Community Right-to-Know Requirements
Employers who own or operate facilities in SIC codes 20 to 39 that employ 10 or more workers and that manufacture 25,000 pounds or more of chlorine per calendar year or otherwise use 10,000 pounds or more of chlorine per calendar year are required by EPA [40 CFR Part 372.30] to submit a Toxic Chemical Release Inventory form (Form R) to the EPA reporting the amount of chlorine emitted or released from their facility annually.



Hazardous Waste Management Requirements
EPA considers a waste to be hazardous if it exhibits any of the following characteristics: ignitability, corrosivity, reactivity, or toxicity as defined in 40 CFR 261.21-261.24. Under the Resource Conservation and Recovery Act (**RCRA**) [40 USC 6901 et seq.], the EPA has specifically listed many chemical wastes as hazardous.

Although chlorine is not specifically listed as a hazardous waste under RCRA, the EPA requires employers to treat waste as hazardous if it exhibits any of the characteristics discussed above.

Providing detailed information about the removal and disposal of specific chemicals is beyond the scope of this guideline. The U.S. Department of Transportation, the EPA, and State and local regulations should be followed to ensure that removal, transport, and disposal of this substance are conducted in accordance with existing regulations.

To be certain that chemical waste disposal meets the EPA regulatory requirements, employers should address any questions to the RCRA hotline at (703) 412-9810 (in the Washington, D.C. area) or toll-free at (800) 424-9346 (outside Washington, D.C.). In addition, relevant State and local authorities should be contacted for information on any requirements they may have for the waste removal and disposal of this substance.



Example of a Chlorine Storage Room.

Chlorine Dioxide Section

Skin contact	Solutions are highly irritant
Skin Absorption	Gas may be absorbed, causing tissue and blood cell damage.
Eye Contact	Severe Irritant. Exposure may cause visual disturbance, i.e., seeing haloes around lights.
Inhalation	A severe respiratory irritant. May cause bronchiospasm and pulmonary edema, which may be delayed in onset. May also cause severe headache. All symptoms may be delayed and long-lasting. Long term exposure may cause bronchitis. An LC_{50} value of 500 ppm/ $15m^3$ (rat) is quoted in the literature.
Ingestion	Not applicable except for solutions, in which case the symptoms would be expected to parallel those for inhalation.
Exposure Limits	ACGIH 1992-93: TWA 0.1 ppm, STEL 0.3 ppm. Most legal limits are similar.
Irritancy	Severe
Sensitization	Information not available.
Carcinogenicity	Not listed by IARC or ACGIH.
Teratogenicity & Mutagenicity	No information is available.
Reproductive Toxicology	No information is available.
Toxicological Synergism	May have synergistic effects in conjunction with chlorine, other chlorine oxides, and chlorine fluorine compounds

The threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists is 0.1 ppm. Two cases of poisoning (one fatal) resulted from exposure to less than 19 ppm while the victims were inside an empty bleach tank. Concentrations of 150 ppm were fatal to guinea pigs in 44 minutes. Characteristic acute effects from over exposure are coughing, eyes and nose watering and the development of a sore throat. Burns resulting from chlorine are severe since the decomposition produces Cl2.

More about Chlorine Dioxide

Chlorine dioxide is generated on-site at water treatment facilities. The popularity of chlorine dioxide as a water disinfectant increased in the 1970s when it was discovered that it did not promote THM formation. Chlorine dioxide (ClO_2), long used in the paper industry, has been an acceptable and effective alternative to chlorination in cooling systems. Chlorine dioxide is a yellow-green gas with an irritating odor not unlike chlorine. It cannot be compressed and shipped in a container, so it must be generated on site.

There are three proven methods of efficiently generating chlorine dioxide. The most common is the chlorine/chlorite or "one pump" method. ClO_2 generation uses sodium chlorite ($NaClO_2$) and chlorine gas. Chlorine gas is educted into a motive water stream in a ClO_2 generator forming HOCl and HCl. Sodium chlorite is pumped into the stream and allowed to react in a generating column to produce ClO_2.

A second, common method of generation uses NaOCl and HCl in place of chlorine gas. Also referred to as the "three pump" method of generation, this method is valuable to a facility that wants to eliminate gaseous chlorine.

A third, more recent method of generation uses sodium chlorate ($NaClO_3$) and sulfuric acid. This differs from the other two methods in that ClO_2 is generated in a vacuum and is then educted into the motive water stream.

Chlorine dioxide holds many advantages over chlorine in cooling water systems. ClO_2 is considerably more selective than chlorine in the presence of various compounds, which allows it to be more effective in contaminated systems. Table 2 lists a series of compounds for which chlorine would show a greater affinity than ClO_2. Under certain conditions ClO_2 may, in fact, be two-and-one-half times more reactive than chlorine. Under efficient ClO_2 generation, THMs are not formed and THM precursors are reduced. In one application, THM formation was reduced from 34 m g/l to 1 m g/l using ClO_2.

Chlorine dioxide does not hydrolyze in water as does chlorine and there is no dissociation of ClO_2. It remains fully active in a pH range far broader than chlorine or sodium hypochlorite. Since ClO_2 remains a gas in water, it does not have the corrosive tendencies of chlorine gas. Its selectivity generally allows for lower dosages compared to chlorine, limiting the amount of aggressive Cl^- available to attack passivated metal surfaces. Finally, ClO_2 is much less aggressive to traditional corrosion inhibitors.

Hypochlorous acid, whether formed from the dissolution of chlorine gas or sodium hypochlorite in water, has satisfactorily controlled microorganisms in cooling water systems. However, dissolution does yield a mineral acid or caustic soda which may adversely affect system pH, inhibitor passivation layers or metal surfaces.

Hypochlorous acid is heavily pH-dependent, because as system pH increases, there is a correspondingly rapid decrease in the concentration of the biocidally active species. It is also a non-specific oxidant which readily reacts with various organic and inorganic compounds that may be present in a cooling water system. Some of these reactions tend to yield undesirable by-products which are regulated or may be regulated in the future.

The effects of pH on hypochlorous acid and its reactivity with a variety of compounds both combine to vastly diminish its effectiveness in contaminated, high-pH cooling water systems. Conversely, chlorine dioxide remains completely pH-independent in the range where recirculating and once-through cooling systems are typically operated.

Since ClO_2 is a dissolved gas in water, there is no mineral acid or caustic soda formation as happens when using HOCl. Chlorine dioxide tends to be much less, if not totally non-reactive, with many organic and inorganic compounds.

Chlorine Dioxide Advantages

- Acts as an excellent virucide.
- Does not react with ammonia nitrogen to form chlorinated amines.
- Does not react with oxidizable material to form THMs; destroys up to 30% of THM precursors.
- Destroys phenols that cause taste and odor problems in potable water supplies.
- Forms fewer chlorinated DBPs such as THMs, HAAs and TOX.
- Disinfects and oxidizes effectively, including good disinfection of both *Giardia* and *Cryptosporidium*.
- Works at low dosage in post-disinfection step with no need of booster stations.
- Improves removal of iron and manganese by rapid oxidation and settling of oxidized compounds.
- Does not react with bromide to form bromate or brominated by-products.
- Has enhanced turbidity removal under certain conditions.

Chlorine Dioxide Disadvantages

- Reacts with natural organic matter and forms inorganic by-products. Chlorite ion, and to a lesser extent chlorate ion, are formed when chlorine dioxide is used.
- Requires on-site generation equipment and handling of chemicals.
- Occasionally poses unique odor and taste problems.

First Aid and Treatment

a) Remove the victim from the contaminated area at once. Loosen all constrictive clothing around the neck.

b) If breathing has stopped, apply artificial respiration.

c) Oxygen should be administered by an (external) Emergency Response Team in case of severe exposure.

d) Call a physician as soon as possible and keep patient warm and quiet.

e) If conscious, discourage coughing; essence of peppermint is sometimes given.

Reactive Chemical Hazards

- During preparation of gaseous ClO_2 decomposition can occur beyond 100 mm Hg partial pressure or above 100°C.

- Chlorine dioxide is incompatible with ammonia, mercury vapors, methane, phosphine and hydrogen sulfide.

- Chlorine dioxide gas is a highly unstable substance. Long stagnation of the vapors will result in an explosive decomposition. Vapors are reactive with most organics.

Phosphine

Phosphine is the common name for phosphorus hydride (PH^3), also known by the IUPAC name phosphane. It is a colorless, flammable gas with a boiling point of 88 °C at standard pressure. Pure phosphine is odorless, but "technical grade" phosphine has a highly unpleasant odor like garlic or rotting fish, due to the presence of substituted phosphine and diphosphine (P^2H^4).

Phosphine is highly toxic; it can easily kill in relatively low concentrations. Because of this, the gas is used for pest control by fumigation. For farm use, it is often sold in the form of aluminum phosphide pellets, which yield phosphine on contact with atmospheric water. These pellets also contain other chemicals which evolve ammonia which helps to reduce the potential for spontaneous ignition or explosion of the phosphine gas. They also contain other agents (e.g. methanethiol) to give the gas a detectable garlic smell to help warn against its presence in the atmosphere. Phosphine is also used as a dopant in the semiconductor industry.



Chlorine Dioxide Questions

1) Q: Is chlorine dioxide more expensive than chlorine?
A: Yes. Generally, chlorine dioxide is used only when chlorine is not capable of doing the job at hand, or when the use of chlorine creates unacceptable levels of by-products, such as THM or HAA.

2) Q: When applying chlorine dioxide for drinking water treatment, how do I ensure that I don't exceed the chlorite MCL?
A: To make sure that chlorite levels in drinking water treated with chlorine dioxide don't exceed the MCL, you should apply pure chlorine dioxide at a dose of no more than about 1.5ppm, depending on the particular water matrix being treated. (Generally, 50-70% of the applied chlorine dioxide "winds up" as chlorite.)

3) Q: What is the chlorite MCL for drinking water?
A: The chlorite MCL allowed in drinking water is 1.0 mg/l.

4) Q: What is the chlorate MCL for drinking water?
A: There is no MCL for chlorate for drinking water, due to insufficient toxicology data. However, the State of California has set an "advisory level" for chlorate of 0.8mg/l.

5) Q: What is the practical dosage limit for chlorine dioxide for drinking water treatment?
A: The practical dosage limit for chlorine dioxide in drinking is driven by compliance with the chlorite MCL, and is about 1.5mg/l unless chlorite is removed prior to the water leaving the plant.

6) Q: Will switching from chlorine gas to hypochlorite (bleach) help control disinfection byproducts (THM, HAA)?
A: Switching from chlorine gas to hypochlorite addresses risks associated with potential chlorine gas release; the chemistry of chlorine and hypochlorite in water are substantially the same, so far as DBP formation is concerned.

7) Q: Can chlorine dioxide kill Cryptosporidium?
A: Chlorine dioxide can kill Cryptosporidium far better than chlorine. However, a relatively high CxT (concentration x time) product is still required to get substantial Cryptosporidium kill with chlorine dioxide, especially in cold water. Only water treatment utilities with very long intake structures and low-demand raw water can expect to achieve significant Cryptosporidium reduction (1-2 logs) with chlorine dioxide alone.

8) Q: Can chlorine dioxide remove off-tastes and odors from drinking water?
A: Chlorine dioxide is effective at the removal of many off-tastes and odors associated with drinking water. However, chlorine dioxide is substantially ineffective in controlling certain "earthy-musty" odor causing compounds, such as MIB and Geosmin.

9) Q: Does chlorine dioxide form bromate ion when used to treat bromide-containing water?
A: Chlorine dioxide does not oxidize bromide ion to form bromate ion, unless the reaction is photolyzed. That is, some bromate ion may be formed in the presence of light.

10) Q: Does chlorine dioxide react with organics to produce trihalomethanes (THM) or haloacetic acids (HAA)?
A: In contrast to chlorine, chlorine dioxide does not react with organics (to any appreciable extent) to produce THM or HAA.

11) Q: **What is the typical chlorine dioxide dosage for drinking water treatment?**
A: The chlorine dioxide dosage for drinking water treatment depends on the particular application, as well as on the water matrix being treated. For example, a typical dosage for manganese removal might be 0.25-0.50ppm; for primary disinfection, a dose of 0.75 to 1.25ppm is more likely.



Chlorine Dioxide Testing Methods

Most tests for chlorine dioxide rely upon its oxidizing properties. Consequently, numerous test kits are readily available that can be adapted to measure chlorine dioxide. In addition, new methods that are specific for chlorine dioxide are being developed. The following are the common analytical methods for chlorine dioxide:

	DPD glycine	Chlorophenol Red	Direct Absorbance	Iodometric Titration	Amperometric Titration
Method Type:	Colorimetric	Colorimetric	Colorimetric	Titrimetric	Titrimetric
How It Works	Glycine removes Cl2; ClO_2 forms a pink color, whose intensity is proportional to the ClO_2 concentration.	ClO_2 bleaches chlorophenol red indicator. The degree of bleaching is proportional to the concentration of ClO_2.	The direct measurement of ClO_2 is determined between 350 and 450 nM.	Two aliquots are taken one is sparged with N_2 to remove ClO_2. KI is added to the other sample at pH7 and titrated to a colorless endpoint. The pH is lower to 2, the color allowed to reform and the titration continued. These titrations are repeated on the sparged sample.	
Range	0.5 to 5.0 ppm.	0.1 to 1.0 ppm	100 to 1000 ppm	> 1 ppm	< 1ppm
Interferences	Oxidizers	None	Color, turbidity	Oxidizers	
Complexity	Simple	Moderate	Simple	Moderate	High
Equipment Required	Spectrophotometer or Colorimeter			Titration equipment	Amperometric Titrator
EPA Status	Approved	Not approved	Not approved	Not approved	Approved
Recommendation	Marginal	Yes	Marginal	Yes	Marginal

Additional Drinking Water Methods (Non EPA) for Chemical Parameters

Method	Method Focus	Title	Order Number	Source
4500-Cl^- B	Chloride by Silver Nitrate Titration	Standard Methods for the Examination of Water and Wastewater, 18th & 19th Ed.	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl^- D	Chloride by Potentiometric Method	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl D	Chlorine Residual by Amperometric Titration (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl E	Chlorine Residual by Low Level Amperometric Titration (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl F	Chlorine Residual by DPD Ferrous Titration (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl G	Chlorine Residual by DPD Colorimetric Method (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl H	Chlorine Residual by Syringaldazine (FACTS) Method (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-Cl I	Chlorine Residual by Iodometric Electrode Technique (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-ClO_2 C	Chlorine Dioxide by the Amperometric Method I	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-ClO_2 D	Chlorine Dioxide by the DPD Method (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**
4500-ClO_2 E	Chlorine Dioxide by the Amperometric Method II (Stage 1 DBP use SM 19th Ed. only)	Standard Methods for the Examination of Water and Wastewater, 18th, 19th & 20th Editions	Included in Standard Methods	American Water Works Assn. **(AWWA)**

Alternative Disinfectants Chapter 5
Ultraviolet Disinfection

This process involves exposing water to ultraviolet (UV) radiation, which inactivates various microorganisms. The technique has enjoyed increased application in wastewater treatment but very limited application in potable water treatment.

The enormous temperatures on the sun create ultraviolet (**UV**) rays in great amounts, and this radiation is so powerful that all life on earth would be destroyed if these rays were not scattered by the atmosphere and filtered out by the layers of ozone gas that float some 20 miles above the earth.



This radiation can be artificially produced by sending strong electric currents through various substances. A sun lamp, for example, sends out UV rays that, when properly controlled, result in a suntan. Of course, too much will cause sunburn.

The UV lamp that can be used for the disinfection of water depends upon the low-pressure mercury vapor lamp to produce the ultraviolet energy. A mercury vapor lamp is one in which an electric arc is passed through an inert gas. This in turn will vaporize the mercury contained in the lamp; and it is a result of this vaporization that UV rays are produced.



The lamp itself does not come in to contact with water, the lamp is placed inside a quartz tube, and the water is in contact with the outside of the quartz tube. Quartz is used in this case since practically none of the UV rays are absorbed by the quartz, allowing all of the rays to reach the water. Ordinary glass cannot be used since it will absorb the UV rays, leaving little for disinfection.

The UV sterilizer will consist of a various number of lamps and tubes, depending upon the quantity of water to be treated. As water enters the sterilizer, it is given a tangential flow pattern so that the water spins over and around the quartz sleeves. In this way the microorganisms spend maximum time and contact with the outside of the quartz tube and the source of the UV rays. The basic design flow of water of certain UV units is in the order of 2.0 gpm for each inch of the lamp. Further, the units are designed so that the contact or retention time of the water in the unit is not less than 15 seconds.

UV disinfection transfers electromagnetic energy from a mercury arc lamp to a pathogen's DNA material, thus affecting its ability to replicate itself. UV's effectiveness depends on the characteristics of the wastewater, the intensity of the UV radiation being emitted, the length of time that the wastewater comes in contact with the UV radiation, and the arrangement of the UV reactor.

UV has the advantage of being effective at inactivating viruses and, because it's a physical process rather than a chemical process, there are no residual constituents remaining in the treated wastewater after exposure to UV. Also, the contact time for the wastewater with the UV source is the shortest of any of the disinfectant strategies, lasting no longer than 20 to 30 seconds. Disadvantages include the effects of turbidity in the water reducing the infiltration and therefore the effectiveness of UV and the need to provide an effective cleaning and replacement program for the UV components.

"If you just need pure disinfection you probably would tend towards UV rather than ozone". "The cost of the UV just for disinfection is usually less than ozone, and the amount of equipment needed is less. But every water treatment has to be looked at individually in order to get what you want. Sometimes you cannot use UV because the waste treatment is too turbid; if you can filter it to get the turbidity levels down, maybe you'll use UV."

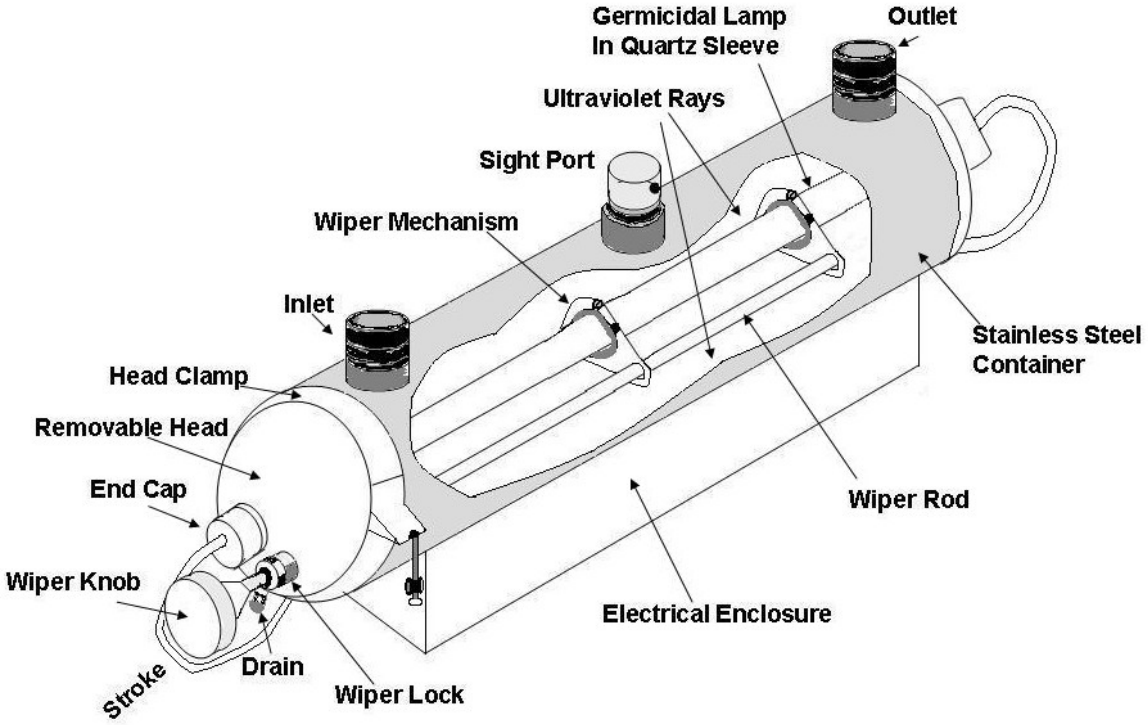
Primarily, there are two designs for UV systems. One system involves a non-contact design in which the UV-light system is suspended away from contact with the water. The second system is the contact reactor-type design in which the lamps are encased in a quartz sleeve that is submerged in the water. This contact-type system is further separated by whether it is an open- or closed-channel system. The open-channel system submerges the lamps in either a horizontal or vertical arrangement. A closed-channel system is within a sealed chamber that can be used in a pressurized system. The open-channel system is mostly used in wastewater treatment.

Ensuring that the UV maintains good contact with the water requires control of the water level within the channel to ensure that the UV is making total contact at the designed depths. Also, because of the heat generated by the electric components of the system, adequate ventilation and cooling must be applied to the UV arrays to reduce heat build-up, otherwise the ballasts could fail. UV lamps have a rated life of up to 14,000 hours, and should be routinely replaced at 12,000 hours or roughly every 1.5 years of continuous operation. The electrical consumption of this system, combined with the cost of routine replacement of ballasts and shields, should be considered against other systems.

Photoelectric Cell

Most manufacturers claim that the UV lamps have a life of about 7,500 hours, which is about 1 year's time. The lamp must be replaced when it loses about 40% to 50% of its UV output; in any installation this is determined by means of a photoelectric cell and a meter that shows the output of the lamp. Each lamp is outfitted with its own photoelectric cell, and with its own alarm that will be activated when the penetration drops to a preset level.

Ultraviolet radiation is an excellent disinfectant that is highly effective against viruses, molds, and yeasts; and it is safe to use. It adds no chemicals to the water, it leaves no residual, and it does not form THMs. It is used to remove traces of ozone and chloramines from the finished water. Alone, UV radiation will not remove precursors, but in combination with ozone, it is said to be effective in the removal of THM precursors and THMs.



The germicidal effect of UV is thought to be associated with its absorption by various organic components essential to the cell's functioning. For effective use of ultraviolet, the water to be disinfected must be clean, and free of any suspended solids. The water must also be colorless and must be free of any colloids, iron, manganese, taste, and odor. These are conditions that must be met.

Also, although a water may appear to be clear, such substances as excesses of chlorides, bicarbonates, and sulfates affect absorption of the ultraviolet rays. These parameters will probably require at least filtration of one type or another. The UV manufacturer will of course stipulate which pretreatment may be necessary.

Removal of Disinfection By-Products		
Disinfectant	***Disinfectant By-product***	***Disinfectant By-product Removal***
Chlorine (HOCl)	Trialomethane (THM) Chloramine Chlorophenol	Granular Activated Carbon (**GAC**), resins, controlled coagulation, aeration. GAC-UV GAC
Chloramine (NH_xCl_y)	Probably no THM Others?	GAC UV?
Chlorine dioxide (ClO_2)	Chlorites Chlorates	Use of Fe2+ in coagulation, RO, ion-exchange
Permanganate ($KMnO_4$)	No THMs	
Ozone (O_3)	Aldehydes, Carboxylics, Phthalates	GAC
Ultraviolet (UV)	None known	GAC

The table indicates that most of the disinfectants will leave a by-product that is or would possibly be inimical to health. This may aid with a decision as to whether or not precursors should be removed before these disinfectants are added to water.

If it is decided that removal of precursors is needed, research to date indicates that this removal can be attained through the application of controlled chlorination plus coagulation and filtration, aeration, reverse osmosis, nanofiltration, GAC or combinations of others processes.

Ultraviolet Radiation *Advantages and Disadvantages*

Ultraviolet Radiation Advantages

- No chemical storage, handling or feed equipment required.
- No identified disinfection by-products.

Ultraviolet Radiation Disadvantages

- No residual action.
- High maintenance requirements.
- High initial capital costs.
- High operating (energy) costs.

Disinfecting action can be compromised by variables such as water clarity, hardness (scaling on the UV tubes), fouling (biological materials) of UV lamps, wavelength of the UV radiation or power failure.

Ozone Section

Ozone has been used for several decades in Europe and is now starting to be found in the U.S. for taste and odor control, color removal and disinfection.

Strongest Oxidizing Agent
Ozone (O_3) is probably the strongest oxidizing agent available for water treatment. Although it is widely used throughout the world, is has not found much application in the United States. Ozone is obtained by passing a flow of air or oxygen between two electrodes that are subjected to an alternating current in the order of 10,000 to 20,000 volts.

$$3O_2 + \text{electrical discharge} \rightarrow 2O_3$$

Liquid ozone is very unstable and can readily explode. As a result, it is not shipped and must be manufactured on-site. Ozone is a light blue gas at room temperature.

It has a self-policing pungent odor similar to that sometimes noticed during and after heavy electrical storms. In use, ozone breaks down into oxygen and nascent oxygen.

$$O_3 \rightarrow O_2 + O$$

It is the nascent oxygen that produces the high oxidation and disinfections, and even sterilization. Each water has its own ozone demand, in the order of 0.5 ppm to 5.0 ppm. Contact time, temperature, and pH of the water are factors to be determined.

Ozone acts as a complete disinfectant. It is an excellent aid to the flocculation and coagulation process, and will remove practically all color, taste, odor, iron, and manganese. It does not form chloramines or THMs, and while it may destroy some THMs, it may produce others when followed by chlorination.

Ozone is not practical for complete removal of chlorine or chloramines, or of THM and other inorganics. Further, because of the possibility of formation of other carcinogens (such as aldehydes or phthalates) it falls into the same category as other disinfectants in that it can produce DBPs.



Oxygen tank is necessary to generate O_3

Ozone Advantages

- Acts as an excellent virucide.
- Disinfects and oxidizes very effectively.
- Produces no chlorinated THMs, HAAs or other chlorinated by-products.
- Enhances turbidity removal under certain conditions.
- Inactivates both *Cryptosporidium* and *Giardia*, as well as other known pathogens.
- Controls taste and odor.

Ozone Disadvantages

- Produces disinfection by-products, including:
  ~Aldehydes
  ~Ketones
  ~Carboxylic acids
  ~Brominated THMs (including bromoform)
  ~Brominated acetic acids
  ~Bromate (in the presence of bromide)
  ~Quinones
  ~Peroxides
- Fosters THM formation when some ozonation by-products combine with secondary disinfection processes. A biologically active filter will likely be necessary to remove these newly formed precursors.
- Does not provide a persistent residual.
- Raises regulatory concerns. Future DBP regulations may require plants using ozone to install costly precursor removal systems (such as granular activated carbon filtration systems).
- Requires capital investment. Ozone must be produced on-site by costly generation that requires a high level of maintenance and substantial operator training.
- Promotes microbial growth. Ozone readily reacts with more complex organic matter and can break this down to smaller compounds that serve to increase nutrients in water supplies, thus enhancing microbial regrowth in water distribution systems.

Alternate Disinfectants Section Summary

Chloramines
Chloramine is a very weak disinfectant for Giardia and virus reduction. It is recommended that it be used in conjunction with a stronger disinfectant. It is best utilized as a stable distribution system disinfectant. In the production of chloramines the ammonia residuals in the finished water, when fed in excess of stoichiometric amount needed, should be limited to inhibit growth of nitrifying bacteria.

Chlorine Dioxide
Chlorine dioxide may be used for either taste and odor control or as a pre-disinfectant. Total residual oxidants (including chlorine dioxide and chlorite, but excluding chlorate) shall not exceed 0.30 mg/L during normal operation or 0.50 mg/L (including chlorine dioxide, chlorite and chlorate) during periods of extreme variations in the raw water supply.

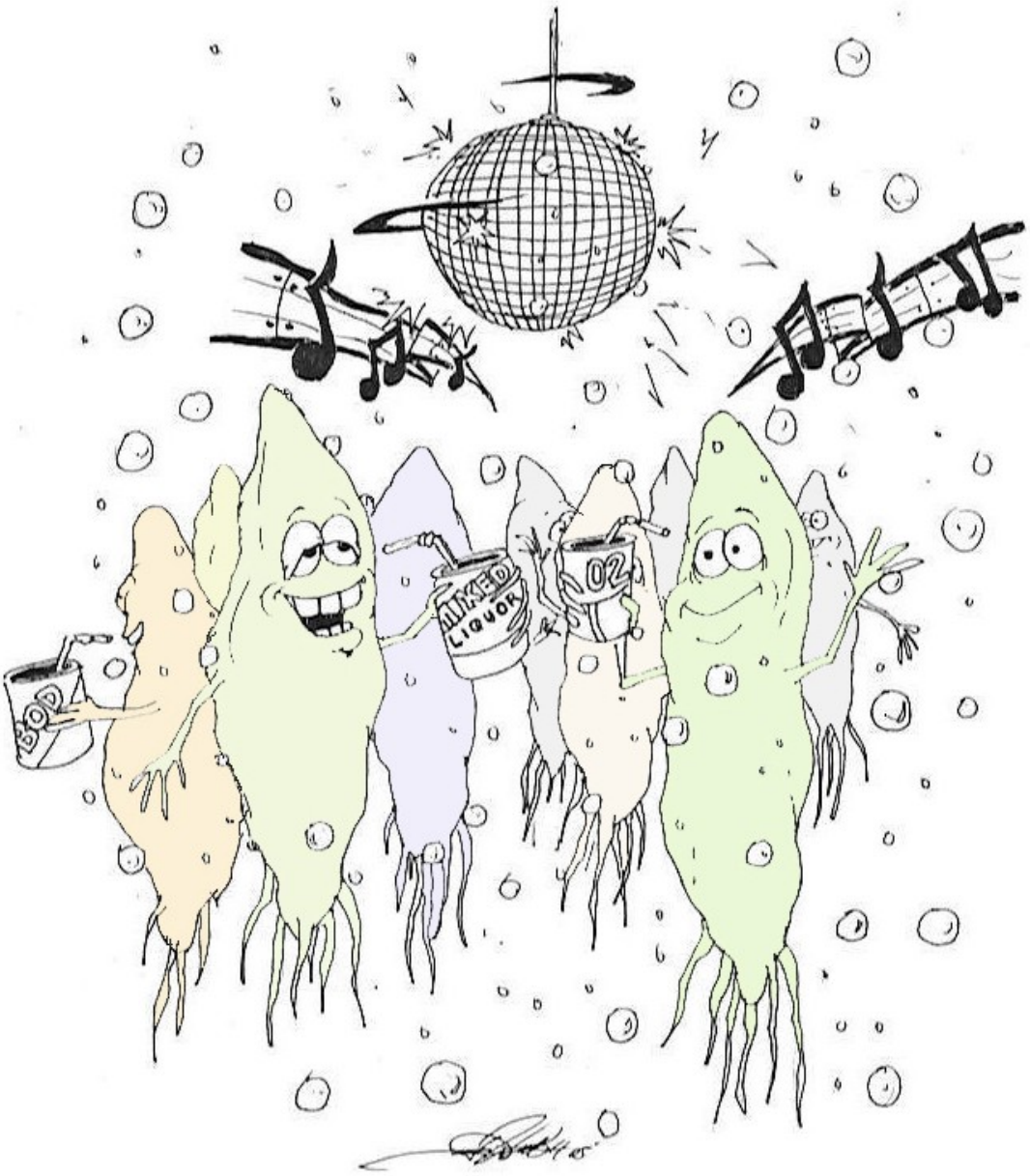
Chlorine dioxide provides good Giardia and virus protection but its use is limited by the restriction on the maximum residual of 0.5 mg/L ClO^2/chlorite/chlorate allowed in finished water. This limits usable residuals of chlorine dioxide at the end of a process unit to less than 0.5 mg/L. Where chlorine dioxide is approved for use as an oxidant, the preferred method of generation is to entrain chlorine gas into a packed reaction chamber with a 25% aqueous solution of sodium chlorite ($NaClO^2$).

Warning: Dry sodium chlorite is explosive and can cause fires in feed equipment if leaking solutions or spills are allowed to dry out.

Ozone
Ozone is a very effective disinfectant for both Giardia and viruses. Ozone CT (Contact time) values must be determined for the ozone basin alone; an accurate T10 value must be obtained for the contact chamber, residual levels measured through the chamber and an average ozone residual calculated. Ozone does not provide a system residual and should be used as a primary disinfectant only in conjunction with free and/or combined chlorine.

Ozone does not produce chlorinated byproducts (such as trihalomethanes) but it may cause an increase in such byproduct formation if it is fed ahead of free chlorine; ozone may also produce its own oxygenated byproducts such as aldehydes, ketones, or carboxylic acids. Any installed ozonation system must include adequate ozone leak detection alarm systems, and an ozone off-gas destruction system. Ozone may also be used as an oxidant for removal of taste and odor, or may be applied as a pre-disinfectant.

UV
The germicidal effect of UV is thought to be associated with its absorption by various organic components essential to the cell's functioning. For effective use of ultraviolet, the water to be disinfected must be clean and free of any suspended solids. The water must also be colorless and must be free of any colloids, iron, manganese, taste, and odor. These are conditions that must be met.


MIXED
LIQUOR
02

Final Review Section

Waterborne Pathogens

Incredibly in 2012, there are around 1.1 billion people globally do not have access to improved water supply sources whereas 2.4 billion people do not have access to any type of improved sanitation facility. About 2 million people die every year due to diarrheal diseases; most of them are children less than 5 years of age. The most affected are the populations in developing countries, living in extreme conditions of poverty, normally peri-urban dwellers or rural inhabitants.

In many cases, source water from a lake, river, reservoir or ground water aquifer needs to be disinfected to inactivate (or kill) microbial pathogens. Microbial pathogens include a few types of bacteria, viruses, protozoa, and other organisms. Some pathogens are often found in water, frequently as a result of:

- Fecal matter from sewage discharges
- Leaking septic tanks
- Runoff from animal feedlots into bodies of water

To protect drinking water from these pathogens, water suppliers often add a disinfectant to drinking water such as chlorine. However, disinfectant practices can be problematic because:

- Certain microbial pathogens, such as *Cryptosporidium*, are highly resistant to traditional disinfection practices.
- Disinfectants themselves can react with naturally-occurring materials in the water to form byproducts, such as trihalomethanes and haloacetic acids, which may pose health risks.

A major challenge for water suppliers is how to balance the risks from microbial pathogens and disinfection byproducts. It is important to provide protection from microbial pathogens while simultaneously minimizing health risks to the population from disinfection byproducts. There are several existing and future rules that are designed to achieve these goals.

Among the main problems which are responsible for this situation are: lack of priority given to the sector, lack of financial resources, lack of sustainability of water supply and sanitation services, poor hygiene behaviors, and inadequate sanitation in public places including hospitals, health centers and schools. Providing access to sufficient quantities of safe water, the provision of facilities for a sanitary disposal of excreta, and introducing sound hygiene behaviors are of capital importance to reduce the burden of disease caused by these risk factors. Water, sanitation and hygiene have important impacts on both health and disease.

Water-related diseases include:

- those due to micro-organisms and chemicals in water people drink;
- diseases like schistosomiasis which have part of their lifecycle in water;
- diseases like malaria with water-related vectors;
- drowning and some injuries;
- and others such as legionellosis carried by aerosols containing certain micro-organisms.

Diseases	Responsible pathogen	Route of exposure	Mode of transmission
Cholera	Vibrio cholerae bacteria	gastro-intestinal	often waterborne
Botulism	Clostridium botulinum bacteria	gastro-intestinal	food/water borne; can grow in food
Typhoid	Salmonella typhi bacteria	gastro-intestinal	water/food borne
Hepatitis A	Hepatitis A virus	gastro-intestinal	water/food borne
Dysentery	Shigella dysenteriae bacteria or Entamoeba histolytica amoeba	gastro-intestinal	food/water
Cryptosporidiosis	Cryptosporidium parvum protozoa	gastro-intestinal	waterborne; resists chlorine
Polio	polioviruses	gastro-intestinal	exposure to untreated sewage; may also be waterborne
Giardia	Giardia lamblia protozoa	gastro-intestinal	waterborne

Microorganisms are Present Everywhere in Our Environment
Invisible to the naked eye, vast numbers of these microbes can be found in soil, air, food and water. Although humans are essentially free of microorganisms before birth, constant circumstances of exposure (e.g., breathing, eating, and drinking) quickly allow the establishment of harmless microbial flora in our bodies.

Microbial pathogens (microorganisms capable of causing disease), however, can and often do harm those who become infected. Moreover, diseases that healthy individuals "weather" well may prove fatal to individuals with compromised immune systems. In some cases, an infection can persist to create a "carrier state" where a disease causing agent is harbored by the body (and spread) without any apparent symptoms.

Waterborne diseases are typically considered to be those diseases resulting from ingestion of contaminated water. Additional pathways of infection being studied by EPA include inhalation of water vapors as well as body contact during bathing (opportunistic pathogens) in the hospital environment.

Since voluntary water ingestion (drinking water) and bathing are universal practices and accidental ingestion during recreational activities (e.g., swimming, water skiing, wading) is common, inadequate protection of water integrity could lead to widespread outbreaks (the Centers for Disease Control defines an outbreak to be two or more cases of illness that can be traced to a common source). Because symptoms can be mild and short-lived, it is estimated that only a fraction of waterborne outbreaks is recognized, reported and investigated. Of these, the pathogenic agent is identified only half of the time. Additionally, experts believe that some food-related disease outbreaks may originate with an initial infection (e.g., of a restaurant worker) caused by contaminated drinking water.

Bacteria, viruses and protozoa are the microorganism groups containing pathogens of primary concern in the study of waterborne diseases. To eliminate these pathogens from our water, especially from our drinking water, seems theoretically straightforward. Simply mix in a disinfectant, allow adequate contact time to assure inactivation (rendering the microbes unable to produce disease), and pump the water into the distribution lines.

In reality, many conditions render the above scenario unworkable. The physical characteristics of the water, primarily represented by dissolved and suspended solids content, can affect the disinfection process. The chemical content, both naturally occurring and anthropogenic (i.e., generated by humans), can also interfere with the chemical reactions desired during treatment and disinfection. Finally, pathogens associated (i.e., imbedded in or clumped) with higher organisms (e.g., algae, rotifers, worms) may be protected from the action of disinfectants. To overcome these obstacles to disinfection, successful treatment of drinking and waste water generally includes a series of steps. In the case of drinking water disinfection, once the impurities have been removed, enough disinfectant is added to inactivate pathogens.

What Progress Has Been Made?

Early in this century, the waterborne diseases of chief concern in the U.S. were typhoid fever and amebiasis. Of the 1,087 deaths associated with waterborne outbreaks between 1920 and 1991, 943 were attributed to typhoid fever while 102 were caused by amebiasis. Overall, 83% of the deaths occurred prior to 1936 and less than 1% occurred after 1970. Additionally, the number of outbreaks in community water systems since 1945 is about half as great as the number documented during the first half of this century. The reduction in fatalities and number of outbreaks indicates that progress has been made in the prevention of certain waterborne diseases. Much of the progress has been the result of increased implementation of important treatment practices (e.g., filtration, disinfection, sewage treatment).

Although progress has been significant, the nation's water continues to be a source of disease. It must be rigorously monitored for indicators of fecal contamination.

A residual level of disinfectant must be maintained throughout the distribution system to guard against potential problems (e.g., microorganisms entering through breaks in distribution lines or regrowth). Proper distribution system operation and maintenance practices are essential deterrents of pathogen entry, recovery and survival. These practices (according to Geldreich *et al., 1992*) include:

• Systematic flushing of the entire distribution system "to get more movement of the chlorine residual into all parts of the pipe network...to remove static water from slow-flow sections, deadends and stratified water in storage tanks on a periodic basis;"

• Effecting repairs and replacement of distribution line components (e.g., broken mains and service meters) in a sanitary manner (i.e., soil-free replacement parts, disinfection and flushing of repaired lines, valves and fittings);

• Preventing pathogens from being drawn into the distribution system by maintaining continuous positive pressure and preserving barriers between public water supplies and sewage or storm water drainage;

• Varying the sample sites during routine monitoring to produce data more representative of the entire system.

While the importance of source water treatment to ensure safe drinking water seems obvious, the need to devote equal effort to pathogen reduction in wastewater is not always recognized. The release of untreated or inadequately treated wastewater into source waters drawn upon by other communities presents a significant health risk. Source waters heavily loaded with disease causing microorganisms can reduce the effectiveness of "downstream" drinking water treatment processes. Such advances as ultraviolet light disinfection systems, initially investigated as a wastewater disinfection option several years ago, are presently becoming more widely accepted and reliable with recent design enhancements. This technology has been demonstrated to be capable of meeting existing disinfection criteria without the release of dangerous disinfection by-products.

Safe Drinking Water Act (SDWA) Review
Start of the Assignment Question 1

In 1974, Congress passed the Safe Drinking Water Act (SDWA) setting up a regulatory program among local, state, and federal agencies to help ensure the provision of safe drinking water in the U.S. The states are expected to administer and enforce these regulations for public water systems (systems that either have 15 or more service connections or regularly serve an average of 25 or more people daily for at least 60 days each year). Public water systems must provide water treatment, ensure proper drinking water quality through monitoring, and provide public notification of contamination problems.

Relating to prevention of waterborne disease, the SDWA required EPA to:
1) set numerical standards, referred to as Maximum Contaminant Levels (MCLs — the highest allowable contaminant concentrations in drinking water) or treatment technique requirements for contaminants in public water supplies;
2) issue regulations requiring monitoring of all regulated and certain unregulated contaminants, depending on the number of people served by the system, the source of the water supply, and the contaminants likely to be found;
3) set criteria under which systems are obligated to filter water from surface water sources; it must also develop procedures for states to determine which systems have to filter;
4) develop disinfection rules for all public water supplies; and
5) require all states to develop Wellhead Protection Programs designed to protect from sources of contamination areas around wells that supply public drinking water systems.

Through the Surface Water Treatment Rule (SWTR), EPA has set treatment requirements to control microbiological contaminants in public water systems using surface water sources (and ground-water sources under the direct influence of surface water). These requirements include the following:
1) treatment must remove or inactivate at least 99.9% of *Giardia lamblia* cysts and 99.99% of viruses;
2) all systems must disinfect, and are required to filter if certain source water quality criteria and site-specific criteria are not met;
3) the regulations set criteria for determining if treatment, including turbidity (suspended particulate matter) removal and disinfection requirements, is adequate for filtered systems; and
4) all systems must be operated by qualified operators as determined by the states.

Current EPA Research –Barriers to Contamination
Although water treatment and disinfection techniques are quite effective at microbe reduction, finished drinking water is not sterile. Survival and regrowth of microorganisms in drinking water distribution systems can lead to the deterioration of water quality and even noncompliance of a supply. Regrowth has largely been associated with heterotrophic bacteria (i.e., those bacteria – including pathogens – that require preformed organic compounds as carbon and energy sources). Bacterial growth occurs on the walls of the distribution system (referred to as “biofilms”) and in the water either as free living cells or cells attached to suspended solids. A multi-faceted phenomenon, bacterial regrowth is influenced primarily by temperature, residence time in mains and storage units, the efficacy of disinfection, and nutrients.

Assimilable Organic Carbon (AOC)
Assimilable organic carbon (AOC) is the portion of the total organic carbon (TOC) dissolved in water that is easily used by microorganisms as a carbon source (i.e., nutrients). Researchers are currently investigating treatment processes to control AOC.
One promising process is biologically active filtration wherein bacterial communities are intentionally established in the filters to use up, or biodegrade, the AOC as it passes through. This treatment process must be employed before final disinfection so that bacteria escaping from the filter can be properly controlled.

Most water utilities do not disinfect with chlorine until late in the treatment train. This limits the formation of disinfection by-products (i.e., those compounds like chloroform produced when chlorine reacts with naturally occurring organic carbon).

To accomplish disinfection earlier in treatment, some water utilities employ ozonation. While ozone is a very strong disinfectant, it also converts a portion of the TOC into AOC. Researchers are examining the advantages (e.g., disinfection of bacteria, viruses and protozoan cysts, control of color, control of taste and odor, enhancement of coagulation, and partial oxidation of the naturally occurring organic carbon that reacts with chlorine) and disadvantages of ozone (e.g., enhancement of AOC, conversion of bromide to bromate, and formation of its own disinfection byproducts like formaldehyde).

EPANET
The project entitled “EPANET” involves the development and testing of a water quality model for drinking water distribution systems. The EPANET model is a computer program that performs extended period simulation of hydraulic and water quality behavior within water distribution networks. It tracks the flow of water in each pipe, the pressure at each pipe junction, the height of water in each tank, and the concentration of a contaminant throughout the network during a multiple time period simulation. Water age and source tracing can also be simulated.

EPANET can be useful for analyzing the loss of disinfectant residual, designing water quality sampling programs, performing drinking water exposure risk assessments, and calibrating network hydraulic models. It can provide insight into how changes in water source utilization, pumping water storage levels, use of satellite treatment and targeted pipe cleaning and replacement would affect drinking water quality. In support of small community and non-community (less than 3,300 people) drinking water treatment systems, researchers are designing, modifying and testing “Hybrid Drinking Water Treatment Package Plants.”

These package plants are factory-built, skid-mounted, and ready to be operated in the field with minimal site preparation. They exhibit lower capital cost than custom designed facilities built onsite and can incorporate any drinking water treatment process. Promising technologies being considered for incorporation include membranes, advanced oxidation, bag filters, and photocatalytic oxidation. By merging, modifying, and adapting conventional treatment trains with innovative treatment technologies, a broader variety of contaminants (including pathogens) can be removed and SDWA compliance can be facilitated. Concern has recently mounted over the ability of certain pathogenic protozoan (*Cryptosporidium)* cysts to survive treatment processes and enter the distribution system.

Microorganisms Associated with Waterborne Disease

The following groups of microorganisms have been linked with the occurrence of waterborne disease. As each pathogen is isolated and identified as a threat to water quality, researchers try to discover the most effective combination of barriers and disinfection methods to minimize risk of human exposure.

Bacteria. Bacteria are the most widely distributed life forms. Pathogenic bacteria range in length from approximately 0.4 to 14 mm (a mm or "micrometer" equals one one-thousandth of a millimeter) and 0.2 to 1.2 mm in width. Key bacterial pathogens responsible for waterborne disease include Legionella, Salmonella typhi, Shigella, and Vibrio cholerae.

Viruses. Viruses are inactive when outside of a living host cell. Viruses linked to waterborne disease have protein coats that provide protection from environmental hazards and range in size from 0.02 to 0.09 mm. Unlike bacteria and protozoa, they contain only one type of nucleic acid (RNA or DNA). Key pathogens include hepatitis A and Norwalk virus.

Protozoa. Protozoa, common in bodies of water, are much larger than bacteria and viruses. To survive harsh environmental conditions, some species can secrete a protective covering and form a resting stage called a "cyst." Encystment can protect protozoa from drinking water disinfection efforts and facilitate the spread of disease. Key protozoa being studied as agents of waterborne disease include Giardia and Cryptosporidium.

Protozoan Diseases
Two protozoans in the news today are Giardia and Cryptosporidium. Their consumption can lead to severe problems of the digestive system, which can be life-threatening to the very young, very old, or those with damaged immune systems.

Bacteriological Monitoring Section Review

Current EPA Research —Bacteria

The new National Primary Drinking Water Regulations require that all drinking water samples testing positive for total coliforms be further tested for the presence of either fecal coliforms or *E. coli*. There is a method currently available that allows the simultaneous detection of total coliforms and *E. coli* in a broth medium in 24 hours; however, there is no equivalent method for use with membrane filters. Development of such a method will allow those who prefer to obtain counts of these organisms in their distribution systems to use a membrane filter method and to have results within the 24-hour time frame. Through the project "Development of a Membrane Filter Medium for the Simultaneous Detection of Total Coliforms and *E. coli*," a membrane filter medium on which both total coliforms and *E. coli* can be distinguished from non-coliforms will be developed and patented.

E. coli are fecal organisms that when present in drinking water are indicative of fecal pollution. Logistical concerns in sample handling and holding require evaluation of conditions for optimizing sample stability and longevity. No current regulations exist for handling samples for analysis of *E. coli*. Through the project entitled "Optimal Sample Holding Conditions for Analysis of Fecal *E.coli* in Drinking Water," sample temperature and holding time will be determined for *E. coli* or fecal coliform analysis methods (i.e., Colilert and M-FC agar).

Relative recovery of methods and storage conditions will be assessed for optimal *E. coli* recovery. The requirement (through the SDWA amendments) to test all coliform-positive drinking water samples for either fecal coliforms or *E. coli* is new. Data from available methods for detecting chlorine damaged *E. coli* in drinking water are limited. The objective of the project entitled "Detection of Low Numbers of Chlorine-Stressed *E. coli* in Drinking Water" is to evaluate and compare the abilities of a commercial method (Colilert) and a standard coliform method (ECMUG) to recover low numbers of chlorine-stressed *E. coli* from potable water. Pure cultures of *E. coli* will be washed, nutrient-stressed in finished drinking water, and treated with chlorine. The chlorine-stressed *E. coli* will then be enumerated, diluted to levels that would be found in marginally unsafe drinking water and assayed in multiple tubes by the three methods.

These experiments will be repeated using naturally occurring *E. coli* from diluted human fecal specimens, contaminated source waters and effluents. The infectious bacterial agent identified from the stools of cholera victims is *Vibrio cholerae*. The epidemic in Latin America has prompted a renewed interest in control measures for this disease. Through the project entitled "Inactivation of *Vibrio cholerae* Biotype El Tor and Biotype Classical by Chlorination," it has been determined that the strain responsible for the epidemic in Peru is capable of reverting to a variant which is more resistant to chlorination than the typical smooth variety of *Vibrio cholerae*.

Cells of the variant appear to be imbedded in a gelatinous mucoid material, facilitating the formation of aggregates, which renders them more resistant to disinfection. Although the variant is more resistant, studies have indicated that all strains are readily inactivated through adequate chlorination.

The *Legionella pneumophila* bacterial strains that cause community- and hospital-acquired pneumonia are usually spread via finished drinking water. Certain free living amoebae (protozoa) support the multiplication of *L. pneumophila* in drinking water systems.

These amoebae may also be responsible for enhancing the virulence (capacity of a microorganism to cause disease) of the *Legionellae* and for protecting them from adverse environmental factors such as high temperature and chlorine disinfection. The project entitled "Multiplication of *Legionellae* in Amoebae and Assessment of Virulence" will determine the effect of intracellular growth of *Legionella* in amoebae on virulence and as protection against chlorine and high temperature. To accomplish this, a method will be established to study the ability of various types of amoebae to provide a protective niche for the multiplication of *Legionellae* under adverse environmental conditions.

Combinations of *Legionella* isolates and specific amoebae that result in high yields of *Legionella* after intracellular growth will be used to study the effects of intracellular growth on virulence. Preliminary studies on the ability of amoebae to supply iron to *Legionellae* growing intracellularly showed no obvious associations between growth and iron concentration. EPA is required by the SDWA to establish appropriate controls and regulations for potable water.

EPA's Office of Research and Development's (ORD) project entitled "Develop Methods for Identifying Potential Bacterial Pathogens in Drinking Water" will develop a data base on potential health hazards (i.e., pathogenicity) associated with bacteria commonly found in water distribution systems. To accomplish this, three rodent species will be compromised using nitrous oxides or immunosuppressive agents, and the animals subsequently will be challenged via the gastrointestinal route.

Although virulence is usually measured *in vivo* (animal research), the need for extensive animal testing can be significantly reduced by the development of a battery of *in vitro* (cell culture) tests for traits known to be virulence-related. This battery can be used to predict the potential an organism has for causing disease in exposed populations. Through the project entitled "Develop *In Vitro* Methods for Identifying Potential Bacterial Pathogens in Drinking Water," model systems will be developed that can be used to determine the potential pathogenicity of bacteria found in potable water distribution systems. Additionally, gene probe and other assays to identify known opportunistic pathogens will be developed and evaluated. Bacteria common to drinking water distribution systems colonize point-of-entry, granular activated carbon (GAC) filters where they are able to grow to very high densities.

Subsequent to reaching the high densities the bacteria begin sloughing off the GAC filters. The number of bacteria in the filter effluent (i.e., water flowing out of the filter) is significantly higher than in the influent water. This amplification of bacteria in drinking water is of concern to EPA because GAC filters are being considered as a substitute for central potable (i.e., fit for drinking drinking) water treatment in small communities where the treatment system has been overwhelmed by organic substances that may be harmful to human health.

EPA's Office of Ground Water and Drinking Water (OGWDW), however, does not want to recommend the use of these filters if the possibility exists that their use poses an acute disease risk due to bacteria that grow on the filters. The health significance of the bacteria known to adsorb and grow on GAC filters used in the home will be evaluated. The OGWDW will use this information to develop appropriate controls and regulations for this type of drinking water treatment as required by the SDWA.

Stage 2 DBP Rule Federal Register Notices

The Stage 2 DBP rule is one part of the Microbial and Disinfection Byproducts Rules (MDBPs), which are a set of interrelated regulations that address risks from microbial pathogens and disinfectants/disinfection byproducts. The Stage 2 DBP rule focuses on public health protection by limiting exposure to DBPs, specifically total trihalomethanes (TTHM) and five haloacetic acids (HAA5), which can form in water through disinfectants used to control microbial pathogens. This rule will apply to all community water systems and nontransient non-community water systems that add a primary or residual disinfectant other than ultraviolet (UV) light or deliver water that has been disinfected by a primary or residual disinfectant other than UV.

In the past 30 years, the Safe Drinking Water Act (SDWA) has been highly effective in protecting public health and has also evolved to respond to new and emerging threats to safe drinking water. Disinfection of drinking water is one of the major public health advances in the 20th century. One hundred years ago, typhoid and cholera epidemics were common through American cities; disinfection was a major factor in reducing these epidemics.

However, the disinfectants themselves can react with naturally-occurring materials in the water to form byproducts, which may pose health risks. In addition, in the past 10 years, we have learned that there are specific microbial pathogens, such as *Cryptosporidium*, which can cause illness, and are highly resistant to traditional disinfection practices.

Amendments to the SDWA in 1996 require EPA to develop rules to balance the risks between microbial pathogens and disinfection byproducts (DBPs). The Stage 1 Disinfectants and Disinfection Byproducts Rule and Interim Enhanced Surface Water Treatment Rule, promulgated in December 1998, were the first phase in a rulemaking strategy required by Congress as part of the 1996 Amendments to the Safe Drinking Water Act.

The Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) builds upon the Stage 1 DBPR to address higher risk public water systems for protection measures beyond those required for existing regulations.

The Stage 2 DBPR and the Long Term 2 Enhanced Surface Water Treatment Rule are the second phase of rules required by Congress. These rules strengthen protection against microbial contaminants, especially *Cryptosporidium*, and at the same time, reduce potential health risks of DBPs.

What is the Stage 2 DBPR?
The Stage 2 Disinfection Byproducts Rule will reduce potential cancer and reproductive and developmental health risks from disinfection byproducts (DBPs) in drinking water, which form when disinfectants are used to control microbial pathogens. Over 260 million individuals are exposed to DBPs.

This final rule strengthens public health protection for customers by tightening compliance monitoring requirements for two groups of DBPs, trihalomethanes (TTHM) and haloacetic acids (HAA5). The rule targets systems with the greatest risk and builds incrementally on existing rules. This regulation will reduce DBP exposure and related potential health risks and provide more equitable public health protection. The Stage 2 DBPR is being promulgated simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule to address concerns about risk tradeoffs between pathogens and DBPs.

What does the rule require?
Under the Stage 2 DBPR, systems will conduct an evaluation of their distribution systems, known as an Initial Distribution System Evaluation (IDSE), to identify the locations with high disinfection byproduct concentrations. These locations will then be used by the systems as the sampling sites for Stage 2 DBPR compliance monitoring.

Compliance with the maximum contaminant levels for two groups of disinfection byproducts (TTHM and HAA5) will be calculated for each monitoring location in the distribution system. This approach, referred to as the locational running annual average (LRAA), differs from current requirements, which determine compliance by calculating the running annual average of samples from all monitoring locations across the system.

The Stage 2 DBPR also requires each system to determine if they have exceeded an operational evaluation level, which is identified using their compliance monitoring results. The operational evaluation level provides an early warning of possible future MCL violations, which allows the system to take proactive steps to remain in compliance. A system that exceeds an operational evaluation level is required to review their operational practices and submit a report to their state that identifies actions that may be taken to mitigate future high DBP levels, particularly those that may jeopardize their compliance with the DBP MCLs.

Who must comply with the rule?
Entities potentially regulated by the Stage 2 DBPR are community and nontransient noncommunity water systems that produce and/or deliver water that is treated with a primary or residual disinfectant other than ultraviolet light.

A community water system (CWS) is a public water system that serves year-round residents of a community, subdivision, or mobile home park that has at least 15 service connections or an average of at least 25 residents.

A nontransient non-community water system (NTNCWS) is a water system that serves at least 25 of the same people more than six months of the year, but not as primary residence, such as schools, businesses, and day care facilities.

What are disinfection byproducts (DBPs)?
Disinfectants are an essential element of drinking water treatment because of the barrier they provide against waterborne disease-causing microorganisms. Disinfection byproducts (DBPs) form when disinfectants used to treat drinking water react with naturally occurring materials in the water (e.g., decomposing plant material).

Total trihalomethanes (TTHM - chloroform, bromoform, bromodichloromethane, and dibromochloromethane) and haloacetic acids (HAA5 - monochloro-, dichloro-, trichloro-, monobromo-, dibromo-) are widely occurring classes of DBPs formed during disinfection with chlorine and chloramine. The amount of trihalomethanes and haloacetic acids in drinking water can change from day to day, depending on the season, water temperature, amount of disinfectant added, the amount of plant material in the water, and a variety of other factors.

Are THMs and HAAs the only disinfection byproducts?
No. The four THMs (TTHM) and five HAAs (HAA5) measured and regulated in the Stage 2 DBPR act as indicators for DBP occurrence. There are many other known DBPs, in addition to the possibility of unidentified DBPs present in disinfected water. THMs and HAAs typically occur at higher levels than other known and unknown DBPs. The presence of TTHM and HAA5 is

representative of the occurrence of many other chlorination DBPs; thus, a reduction in the TTHM and HAA5 generally indicates a reduction of DBPs from chlorination.

What are the costs and benefits of the rule?
Quantified benefits estimates for the Stage 2 DBPR are based on reductions in fatal and non-fatal bladder cancer cases. EPA has projected that the rule will prevent approximately 280 bladder cancer cases per year. Of these cases, 26% are estimated to be fatal. Based on bladder cancer alone, the rule is estimated to provide annualized monetized benefit of $763 million to $1.5 billion.

The rule applies to approximately 75,000 systems; a small subset of these (about 4%) will be required to make treatment changes. The mean cost of the rule is $79 million annually. Annual household cost increases in the subset of plants adding treatment are estimated at an average of $5.53, with 95 percent paying less than $22.40.

What are the compliance deadlines?
Compliance deadlines are based on the sizes of the public water systems (PWSs). Wholesale and consecutive systems of any size must comply with the requirements of the Stage 2 DBPR on the same schedule as required for the largest system in the combined distribution system (defined as the interconnected distribution system consisting of wholesale systems and consecutive systems that receive finished water).

What technical information will be available on the rule?
The following Guidance Documents will be available:

- ✓ Initial Distribution System Evaluation (IDSE) Guidance Manual
- ✓ Operational Evaluation Guidance Manual
- ✓ Consecutive Systems Guidance Manual
- ✓ Small Systems (SBREFA) Guidance Manual
- ✓ Simultaneous Compliance Guidance Manual

Where can I find more information about this notice and the Stage 2 DBPR?
For general information on the rule, please visit the EPA Safewater website at http://www.epa.gov/safewater/disinfection/stage2 or contact the Safe Drinking Water Hotline at 1-800-426-4791. The Safe Drinking Water Hotline is open Monday through Friday, excluding legal holidays, from 10:00 a.m. to 4:00 p.m., Eastern Time. For technical inquiries, email stage2mdbp@epa.gov.

Reviewing Chloramines in Drinking Water

Chloramines are disinfectants used to treat drinking water. Chloramines are most commonly formed when ammonia is added to chlorine to treat drinking water. The typical purpose of chloramines is to provide longer-lasting water treatment as the water moves through pipes to consumers. This type of disinfection is known as secondary disinfection. Chloramines have been used by water utilities for almost 90 years, and their use is closely regulated. More than one in five Americans uses drinking water treated with chloramines. Water that contains chloramines and meets EPA regulatory standards is safe to use for drinking, cooking, bathing and other household uses.

Many utilities use chlorine as their secondary disinfectant; however, in recent years, some of them changed their secondary disinfectant to chloramines to meet disinfection byproduct regulations. In order to address questions that have been raised by consumers about this switch, EPA scientists and experts have answered 29 of the most frequently asked questions about chloramines. We have also worked with a risk communication expert to help us organize complex information and make it easier for us to express current knowledge.

What are Chloramines?

Chloramines are disinfectants used to treat drinking water.

Chloramines are most commonly formed when ammonia is added to chlorine to treat drinking water.

The most typical purpose of chloramines is to protect water quality as it moves through pipes.

Chloramines provide long-lasting protection as they do not break down quickly in water pipes.

The different types of chloramines are monochloramine, dichloramine, trichloramine, and organic chloramines.

When chloramines are used to disinfect drinking water, monochloramine is the most common form.

- ✓ Dichloramine, trichloramine, and organic chloramines are produced when treating drinking water but at much lower levels than monochloramine.
- ✓ Trichloramines are typically associated with disinfected water used in swimming pools.

***The Environmental Protection Agency regulates the safe use of chloramines* in drinking water.**

EPA requires water utilities to meet strict health standards when using chloramines to treat water.

EPA chloramines regulations are based on the average concentration of chloramines found in a water system over time.

EPA regulates certain chemicals formed when chloramines react with natural organic matter4 in water.

Additional Supporting Information:

1. Dichloramine is formed when the chlorine to ammonia-nitrogen weight ratio is greater than 5:1, however, this reaction is very slow. Organic chloramines are formed when chlorine reacts with organic nitrogen compounds. Source: *Optimizing Chloramine Treatment*, 2nd Edition, AwwaRF, 2004
2. Trichloramine formation does not usually occur under normal drinking water treatment conditions. However, if the pH is lowered below 4.4 or the chlorine to ammonia-nitrogen weight ratio becomes greater than 7.6:1, then trichloramine can form. Trichloramine

formation can occur at a pH between 7 and 8 if the chlorine to ammonia-nitrogen weight ratio is increased to 15:1. Source: *Optimizing Chloramine Treatment*, 2nd Edition, AwwaRF, 2004

3. The drinking water standard for chloramines is 4 parts per million (ppm) measured as an annual average. More information on water utility use of chloramines is available at http://www.epa.gov/safewater/disinfection/index.html and in the 1997-1998 Information Collection Rule, a national survey of large drinking water utilities for the Stage 2 Disinfection Byproducts Rule (DBPR). Information on the Stage 2 DBPR is available at http://www.epa.gov/safewater/disinfection/stage2/.

More information on EPA's standard setting process may be found at: http://www.epa.gov/OGWDW/standard/setting.html.

Natural Organic Matter: Complex organic compounds that are formed from decomposing plant, animal and microbial material in soil and water. They can react with disinfectants to form disinfection by products. Total organic carbon (TOC) is often measured as an indicator of natural organic matter.

What Disinfectants are Available for Drinking Water?

Most water utilities use chlorine as a primary disinfectant because of its effectiveness in killing potentially harmful organisms.2 Chlorine is effective in killing bacteria, viruses, and other potentially harmful organisms in water. One disadvantage of chlorine is it can react with natural organic matter3 present in water to form potentially harmful disinfection byproducts. Water utilities sometimes use chlorine several times during treatment because the initial dose loses its effectiveness over time.

Monochloramine is commonly used as a secondary disinfectant to protect the water as it travels from the treatment plant to consumers. Monochloramine is effective in killing bacteria, viruses, and other potentially harmful organisms but takes much longer to act than chlorine. One disadvantage of monochloramine is it can react with natural organic matter present in water to form potentially harmful disinfection byproducts. Monochloramine is more chemically stable than chlorine, which makes it longer lasting and an effective secondary disinfectant.

Water utilities may use ozone, UV light, or chlorine dioxide as primary disinfectants in the treatment plant.

Ozone, UV light, and chlorine dioxide are effective in killing bacteria, viruses, and other potentially harmful organisms in water at the treatment plant. One disadvantage of ozone, UV light, and chlorine dioxide is they do not provide protection as water travels through pipes. Either chlorine or monochloramine should still be used in addition to any primary treatment process to protect the quality of treated water as it travels from the treatment plant to the customer.

How did EPA evaluate the safety of monochloramine for use as a drinking water disinfectant? ***EPA evaluated monochloramine primarily through an analysis of human health and animal data.***

Research reviewed in EPA's safety analysis is contained in EPA's *Drinking Water Criteria Document for Chloramines.* The criteria document for monochloramine provides a complete summary of health and other data considered in establishing a monochloramine standard. EPA periodically updates the monochloramine "criteria document."

EPA's monochloramine standard2 ***is set at a level where no human health effects are expected to occur.***

Data from animal and human studies provide information on the health effects of monochloramine.

EPA reviews and considers new research results as they become available. EPA's standard for monochloramine takes data gaps and uncertainty into account by building safety factors into the regulatory standard.

EPA reviewed historical data in its evaluation of monochloramine.
Monochloramine has been in use as a drinking water disinfectant since the 1930's. Decades of use in the US, Canada, and Great Britain shows that monochloramine is an effective secondary drinking water disinfectant. Denver, Philadelphia, and other large cities have used monochloramine as part of their water treatment process for years.

Additional Supporting Information:
1. The *Drinking Water Criteria Document for Chloramines* can be found at http://www.epa.gov/ncea/pdfs/water/chloramine/dwchloramine.pdf, Publication No.: ECAO-CIN-D002, March, 1994.
2. The Maximum Residual Disinfectant Level (MRDL) for chloramines is 4 parts per million (ppm).
3. See the Contaminant Candidate List online at http://www.epa.gov/OGWDW/ccl/ccl3.html for contaminants that EPA proposes to review. EPA scientists review regulations of disinfectants and disinfection byproducts every six years. For information on EPA's six-year review visit: http://epa.gov/safewater/review.html
4. For additional information regarding how uncertainty factors (also known as safety factors) are applied to risk assessments to provide a wide margin of safety see: http://epa.gov/risk/dose-response.htm.
5. Cleveland, OH, Springfield, IL, and Lansing, MI were among the first cities to use monochloramine in 1929 (see Chapter 1 of *The Quest for Pure Water Vol II*, AWWA, 1981).

Water Disinfection Methods Review

Disinfection is an important step in ensuring that water is safe to drink. Water systems add disinfectants to destroy microorganisms that can cause disease in humans. The Surface Water Treatment Rule requires public water systems to disinfect water obtained from surface water supplies or groundwater sources under the influence of surface water. Primary methods of disinfection are chlorination, chloramines, zone, and ultraviolet light. Other disinfection methods include chlorine dioxide, potassium permanganate, and nanofiltration. Since certain forms of chlorine react with organic material naturally present in many water sources to form harmful chemical by-products, the U.S. Environmental Protection Agency has proposed maximum levels for these contaminants.

Many people in most developing countries suffer from the inadequacy or hazardous condition of public water supplies (WHO 1985). A wide variety of known waterborne diseases, including those associated with children's diarrhea, are rampant (Tartakow and Vorperian 1980; Feachem et al. 1983; WHO 1984, 1987). This prompted the establishment of the International Drinking Water Supply and Sanitation Decade. It aims at providing about 90% of the human population with an adequate, safe community water supply by 1990 (WHO 1985).

In Lebanon, the shortage of community water supplies, their actual or potential pollution from anthropogenic sources, inadequate treatment, and the resultant spread of associated diseases are still unresolved problems (Acra et al. 1985). To curb these issues would require implementing feasible measures for prevention and treatment. These should include sanitation and disinfection of drinking water.

Physical Methods
Formation of mutagenic and carcinogenic agents in water and wastewater effluent treated with chlorine has prompted research to seek alternative disinfecting methods that would minimize environmental and public health impacts. The technology, based on nonchemical methods, is undergoing rapid development. Some techniques are already available commercially. This category is represented by techniques employing such physical principles for disinfection as W radiation, ultrasound, ultrafiltration, reverse osmosis, heating, freezing, and ionizing radiation (Cheremissinoff et al. 1981). Disinfecting small quantities of water by pasteurizing with heat or solar energy is a technology with some potential, but requires further development (Cheremissinoff et al. 1981; Ciochetti and Metcalf 1984). The recently developed method for water disinfection by direct exposure to solar radiation (Acra et al. 1980, 1984) is further described in the following sections.

Chemical methods
Chemical methods depend mostly on selected chemicals with oxidizing and biocidal properties. Their practical applications range from removing undesirable constituents to disinfecting water supplies, wastewater treatment effluent, or industrial waters. The most commonly used chemicals include ozone, chlorine and some of its compounds, potassium permanganate, and hydrogen peroxide.

Ozone has been used for water disinfection for about 80 years in France, Germany, and other European countries. It is now undergoing a critical evaluation as a possible alternative to chlorine when used alone or in conjunction with other disinfection systems (Foster et al. 1980; Kott et al. 1980; Dolora et al. 1981; Venosa 1983; Rakness et al. 1984; Wickramanayake et al. 1984; Den-Blanken 1985). There is some evidence that it forms smaller amounts of hazardous trihalomethanes (THM) when employed to treat polluted waters or wastewater effluent than

either chlorine or chlorine dioxide. However, its potential for producing other equally toxic substances is still not clearly defined (Glaze 1987). Ozonation has become popular in North America partly because of its superiority over chlorination. It enhances the coagulation process despite its inherent weakness in leaving practically no residual in the distribution system.

Interhalogen compounds, formed from two different halogens, resemble their parent substances in properties and germicidal characteristics. The interhalogens BrCl, ICl, and IBr have recently been investigated as possible alternative disinfectants for water and wastewater effluent (Groninger and Mills 1980; Cheremissinoff et al. 1981). Added to water, they rapidly hydrolyze to the corresponding hypohalous acids, which are stronger oxidants and disinfectants than hypochlorous acid. For instance, BrCl is hydrolyzed to HCl and HOBr. However, their improved germicidal activity is counterbalanced by the formation of haloforms. They react with humates in water or wastewater effluent by the haloform reaction (HOBr, for example, reacts with humates yielding bromoform). In this context, hypobromite would be formed in seawater by reaction of the natural bromides with hypochlorites in chlorinated wastewater effluent or cooling waters from power plants (Sugam and Helz 1980; Wong 1982; Bousher et al. 1986). This also applies to natural waters rich in bromides with subsequent formation of bromoform and other trihalomethanes (Amy et al. 1984; Rav-Acha, Choshen et al. 1985; Rav-Acha, Serri et al. 1985; Ishikawa et al. 1986; Guttman-Bass et al. 1987). Consequently, coastal groundwater affected by seawater infiltration should create some concern if used for drinking.

Using hydrogen peroxide for water disinfection began in the 1950s in Eastern Europe (Laubusch 1971). Although it has been well known for its high oxidative and germicidal activity, its application as a water disinfectant has not gained wide acceptance. Its increasing use, however, has been noted (Gaudy and Gaudy 1980). The degradation of organic matter in water treated sequentially with up to 0.5% by weight of hydrogen peroxide and W radiation (>200 nm) has been reported (Malaiyandi et al. 1982). In another form of application, hydrogen peroxide produced no significant oxidation of soluble manganese in water containing organic matter in the pH range of 5.0-8.0 (Knocke et al. 1987). A newly marketed product (Sanosil, Sanosil AG, Feldmeilen, Switzerland) is claimed to be applicable to large-scale water disinfection; its effective bacteriostatic and fungicidal activity has been demonstrated at concentrations of 10-35 mg/L on *Escherichia coli, Klebsiella pneumoniae, Streptococcus aureus, Pseudomonas aeroginosa, Proteus mirabilis, Micobacter* spp., *Clamidia sporogenes*, and *Candida albicans*. The two active biocidal constituents of this product are hydrogen peroxide and colloidal silver.

Chlorination and Dechlorination

The use of chlorine and some of its derivatives will continue as an integral part of the disinfection process in water and wastewater treatment. This also applies to developing countries, where this mode of disinfection is fairly well established (Mara 1978; Droste and McJunkin 1982; Smethurst 1983). Apart from almost a century of chlorination practices (Laubusch 1962a, b; Cheremissinoff et al. 1981), two other favourable determinants are the technical expertise already acquired and the relatively low costs involved. In the wake of the recent discovery of the formation of THM in chlorinated natural waters (Rook 1974), and their potential health hazards (Glaze et al. 1980; Williamson 1981; Carpenter and Beresford 1986), its credibility is diminishing.

Alternative disinfecting agents such as chlorine dioxide (Rav-Acha et al. 1985b), UV light (Severin et al. 1984; Scheible 1987), and UV light in conjunction with hydrogen peroxide (Crandall 1986) are being considered. However, the formation of mutagens and carcinogens in chlorinated waters and wastewaters can be abolished or minimized by modifying the unit processes (Stelter et al. 1984; Fiessinger et al. 1985; Finger et al. 1985; Huang et al. 1985; Kool

et al. 1985; Moyers and Wu 1985; Suh and Abdel-Rahman 1985; Means et al. 1986; Rogers and Lauer 1986; Guttman-Bass et al. 1987; Knocke et al. 1987). The potential health impacts that are yet to be clearly discerned and the toxicity to aquatic life resulting from discharged chlorinated effluent (Brungs 1973; Jolley et al. 1980) do not seem to outweigh the public health benefits derived from chlorination practices (Cortruvo 1985). However, as the controversy continues, epidemiological studies (Craun 1985) and the pertinent drinking water standards and legislation (Toft 1985) are being revised.

Reactions of chlorine in water that form the basis for its application as a disinfectant and oxidant are as follows:

$Cl_2 + H_20 \rightarrow HCl + HOCl$

$HOCl \rightarrow H^+ + OCl^-$

These reactions in water devoid of other inorganic or organic matter that could react with chlorine are pH and temperature dependent. The products, hypochlorous acid (HOCl) and hypochlorite ions (OCl^-, are referred to as free available chlorine (FAC). The biocidal activity is attributed chiefly to HOCl, as it is more effective than the OCl^-. In the presence of natural or added ammonium ions, HOCl reacts to form chloramines, known as combined available chlorine (CAC). As a disinfectant, FAC is more effective. It is essential to chlorinate beyond the subsequent attainment of FAC at the desired level for optimal biocidal effectiveness ("free residual" chlorination).

The influencing factors to be considered in chlorination practices are the following:

- chlorine concentration,
- contact time,
- pH,
- temperature, and
- interfering substances.

The relationship between chlorine concentration (*C*, milligrams per litre) and contact time (*T*, minutes) required for a specific percentage destruction of microorganisms is expressed as a constant ($CT = K$) (Gaudy and Gaudy 1980). The proper use of this *CT* relationship to determine adequate water chlorination requirements has been emphasized as an approach to prevent and control waterborne diseases. Minimum CT values of 15-30 for systems using groundwater as a source and 100-150 for those using surface supplies have been recommended (Lippy 1986). Based on these values, the required FAC concentration can be determined mathematically for a given contact time. Once the chlorine demand (D) for a water supply is determined by testing, then the optimal chlorine dose to attain the desired free chlorine residual (C) can be calculated by addition: chlorine dose = D + C.

One of the factors in the many waterborne disease outbreaks in the United States in the past decades was failure to comply with the *CT* relationship in chlorination practices (Lippy and Waltrip 1984; Bitton et al. 1986; Lippy 1986; Williams and Akin 1986). In addition, the need for the disinfection of wastewater discharged into streams has been emphasized and justified by the 23 different kinds of pathogenic organisms present in wastewater from US communities (Shertzer 1986).

Excess chlorine residuals can be controlled by a dechlorination procedure. Of the various chemicals used for the partial or complete removal of the residual chlorine in water or wastewater, sulfur dioxide gas (SO_2) is the most common (Laubusch 1971; Cheremissinoff et al.

1981; Finger et al. 1985; Huang et al. 1985). Dechlorination is often applied to heavily dosed water supplies as they are aesthetically objectionable to consumers or undesirable for industrial water uses. Chlorinated cooling waters and wastewaters need to be dechlorinated before discharging into water bodies in view of their toxicity to aquatic life. They have also potentially harmful effects because of the formed THM.

Household Methods

There are many situations where individuals or families would need to resort to simple and effective methods for drinking-water disinfection. These include the following:

- catastrophic conditions leading to displacement (earthquakes, floods, hurricanes, wars, or civil disturbances);
- emergencies arising from flourishing waterborne diseases; and
- resident populations and foreigners at risk in endemic areas with unsafe water supplies.

Physical methods (boiling or the use of ceramic filters), chemical methods (chlorine compounds in solution or tablet form, e.g., sodium hypochlorite solutions, calcium hypochlorite tablets, organic chlorine compounds, iodine solution, and organic iodine compounds) and others have been recommended for such cases (Morris et al. 1953; Gershenfeld 1957; Hadfield 1957; Cox 1969; O'Connor and Cooper 1970; WHO 1972, 1973; Rajagopalan and Shiffman 1974; UNHCR 1982).

None of these methods is entirely free from practical problems that could induce users to revert to untreated water. Fuelwood, for instance, for boiling is no longer a tenable practice, particularly in areas where it is absent or being depleted. Besides, the flat taste of boiled water discourages some consumers. The diverse types of ceramic filters have a wide range of pore sizes and present difficulties in selection. They suffer frequent clogging of the ceramic candles and often leak through disguised fine cracks. Proprietary halogen preparations frequently lead to consumer complaints and rejection because of the undesirable tastes and odors imparted to the water. It is especially so if high doses are applied inadvertently or as required in cases of heavily polluted waters. Relief agencies are often trapped in a dilemma by the requirements for importing and distributing, in addition to shortages, cost acceptability, and expiry dates. These issues encourage attempts to resolve them through the development of practical and effective techniques, simple enough to be applied by individuals or households.

Photo-Inactivation

The concept of photodynamic inactivation (PDI) of microorganisms evolved from experiments made in the early l9th century. It was firmly established, however, after the discovery of the inactivation of *Paramecium* spp. by visible light in the presence of an exogenous photosensitizing dye (acridine) (Raab 1900). Two types of photosensitizing compounds are known (Harrison 1967; Chamberlin and Mitchell 1978; Senger 1980):

- exogenous: fluorescent substances or dyes such as eosin, methylene blue, and benzopyrene; and
- endogenous: porphyrins, cytochromes, cytochrome oxidase, aromatic amino acids, flavins, tryptophan, and chlorophylls.

Several microorganisms and aquatic ecosystems have shown sensitivity to solar UVR, including viruses, algae, and fungi (Perdrau and Todd 1933; Hiatt et al. 1960; Crowther and Melnick 1961; Jagger 1967, 1981; Billen and Green 1975; Berry and Noton 1976; Propst and Lubin 1978; Acher and Elgavish 1980; Calkins and Thordardottir 1980; Kapuscinski and Mitchell 1981; Worrest et al. 1981; Jabara 1984; Wei et al. 1985). The rapid destruction of saprophytic strains of Mycoplasma by artificial visible light in the presence of toluidine blue and air has been

reported (Cooney and Krinsky 1972). Coliforms in water and sewage have been completely inactivated by exposure to sunlight for about 1 h in the presence of methylene blue or rose bengal; the added dye is removed by absorption on bentonite (Acher and Juven 1977).

A new technique for the photodynamic disinfection of municipal and industrial wastewaters, which also results in the photodegradation of pesticides and anionic surfactants therein, has been suggested. The technique is based on the use of exogenous dye sensitizers, aeration, and sunlight, with the possibility of reusing the treated effluent for crop irrigation (Acher 1985).

Aquatic Photochemistry

Photochemical reactions induced by natural or artificial light have been known for some time, but much of this field remains obscure. Of particular interest is the photochemistry of the hydrosphere, which is continuously experiencing light-induced chemical reactions in the surface layer (photic zone). Inorganic and organic chemical pollutants in natural surface waters capable of absorbing solar energy with consequent chemical changes, referred to as photoreactive chromophores, can lead to direct photolysis reactions. Some of the better known chromophores include inorganic substances such as nitrites, nitrates, iodates, hydrogen peroxide, and ferrous compounds (Zafiriou et al. 1984).

The fate of disinfectants added to wastewater effluent and cooling waters used in industries that are discharged to surface waters is of importance in aquatic biology. Sunlight plays a prime role in their photodecomposition, as was demonstrated with experiments in which hypochlorite and hypobromite (formed by interaction of chlorine and natural bromides in seawater) were found to be photosensitive, the latter being easier to decompose (Wong 1982). In addition, volatilization into the atmosphere has been proposed as a possible pathway for the dissipation of the haloforms formed in water, with subsequent enhanced dilution and further photochemical degradation (Groninger and Mills 1980). Decay and dissipation models for chlorine residuals in natural waters have been developed. These models predict that the nocturnal discharge of chlorinated effluent would have a much greater impact on aquatic life, given the absence of light-induced decomposition (Lin et al. 1983; Yamomoto et al. 1985).

The photochemical reactions of the hypohalites formed in aqueous solutions of chlorine, bromine, and iodine are somewhat similar, except for the absorption spectra and reaction rates (Allmand et al. 1927; Allmand and Webb 1928; Farkas and Klein 1948). Their photodecomposition is wavelength-dependent, with increased decay rates in the shorter wavebands within the spectral region of 200-440 nm and the possible liberation of the highly reactive singlet oxygen, as has been noted for the surface of fresh and coastal waters (Zafiriou et al. 1984). It can be postulated, then, that aqueous halogen solutions are subject to photodecomposition by the effective radiation in the UV-B, UV-A, and blue light bands of the solar spectrum, and that these reactions could be of practical importance.

pH Scale

Alkalinity is the capacity of water to neutralize acids. This capacity is caused by the water's content of carbonate, bicarbonate, hydroxide and occasionally borate, silicate and phosphate. pH is an expression of the intensity of the basic or acid condition of a liquid. EPA has a suggested range of 6.5 to 8.5 for pH (called a secondary maximum contaminant level or SMCL). Furthermore, alkalinity and pH are different because water does not have to be strongly basic (high pH) to have a high alkalinity.

Conclusion *Answers 51- down*

Emerging waterborne pathogens constitute a major health hazard in both developed and developing nations. A new dimension to the global epidemiology of cholera-an ancient scourge-was provided by the emergence of Vibrio cholerae O139. Also, water-borne enterohaemorrhagic Escherichia coli (E. coli O157:H7), although regarded as a problem of the industrialized west, has recently caused outbreaks in Africa. Outbreaks of chlorine-resistant Cryptosporidium have motivated water authorities to reassess the adequacy of current water-quality regulations. Of late, a host of other organisms, such as hepatitis viruses (including hepatitis E virus), Campylobacter jejuni, microsporidia, cyclospora, Yersinia enterocolitica, calciviruses and environmental bacteria like Mycobacterium spp, aeromonads, Legionella pneumophila and multidrug-resistant Pseudomonas aeruginosa have been associated with water-borne illnesses.

The protection and enhancement of our nation's water quality remains a chief concern of the U.S. Environmental Protection Agency. The Office of Research and Development is committed, through the extensive waterborne disease research efforts earlier described, to ensure that the most effective and efficient methods are developed to identify, detect, and inactivate/remove pathogens that may be present in our drinking water supplies.

Life cycles, mechanisms of infection, protective or dormant states, emergence of disinfection resistant variants, optimal pathogen removal techniques, regrowth in distribution lines…all are areas that must be investigated and understood to afford the water quality safeguards that are so often taken for granted. The successes and failures of these research efforts, relayed to the public and appropriate federal, state, and local agencies, have helped to ensure safe drinking water.

Salmonella Typhi
Salmonella typhi, the basics. It's a bacteria. It causes diarrheal illness, also known as typhoid fever. And humans are the reservoir for this pathogen. Salmonella typhi, prevention. Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Shigella Species
Shigella species, the basics. It's a bacteria. It causes diarrheal illness known as shigellosis. Humans and primates are the reservoir for this pathogen. Shigella species, in the United States two-thirds of the shigellosis in the U.S. is caused by Shigella sonnei, and the remaining one-third is caused by Shigella flexnieri. In developing countries, Shigella dysenteriae is the primary cause of illness associated with this pathogen. Shigella species prevention. Prevention strategies for this pathogen include source protection,
halogenation of water, and boiling water for one minute.

Campylobacter
Campylobacter, the basics. It's a bacteria. It causes diarrheal illness. And Campylobacter is primarily associated with poultry, animals, and humans. Campylobacter prevention. Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Vibrio Cholerae
Vibrio cholerae, the basics. It's a bacteria. It causes diarrheal illness, also known as cholera. It is typically associated with aquatic environments, shell stocks, and human. Vibrio cholerae has also been associated with ship ballast water, and there will be a discussion later on in this presentation of an outbreak associated with ship ballast water. Vibrio cholerae prevention. Prevention strategies for this pathogen include source protection, halogenation of water, and boiling water for one minute.

Legionella
Legionella, the basics. It's a bacteria. It causes a respiratory illness known as legionellosis. There are two illnesses associated with legionellosis: the first, Legionnaire's disease, which causes a severe pneumonia, and the second, Pontiac fever, which is a nonpneumonia illness; it's typically an influenza-like illness, and it's less severe. Legionella is naturally found in water, both natural and artificial water sources. Legionella, prevention. Maintaining hot water systems at or above 50 degrees Centigrade and cold water below 20 degrees Centigrade can prevent or control the proliferation of Legionella in water systems. Hot water in tanks should be maintained between 71 and 77 degrees Centigrade. Proper recreational water system maintenance and disinfection can prevent the proliferation of Legionella in recreational water systems. It is important to prevent water stagnation. This can be accomplished by eliminating dead ends in distribution systems and in recreational water systems. Additionally, preventing biofilm development is important to control this particular pathogen in water systems.

Pseudomonas
Pseudomonas, the basics. It's a bacteria. It is caused by dermal contact with water. It can cause dermatitis, which is an inflammation of the skin, or it can cause otitis, which is an infection of the ear. Pseudomonas is typically associated with soil and water. Pseudomonas prevention. Proper maintenance and disinfection of recreational water systems is important in preventing Pseudomonas.

Hepatitis A
Hepatitis A, the basics. It's a virus. It causes inflammation of the liver. And the reservoir for Hepatitis A virus is humans.

Hepatitis A, Prevention
Prevention strategies for this pathogen include source protection and adequate disinfection. Fecal matter can protect Hepatitis A virus from chlorine. Additionally, Hepatitis A virus is resistant to combined chlorines, so it is important to have an adequate free chlorine residual.

Norovirus
Norovirus, the basics. It's a virus. It causes diarrheal illness. And humans are the reservoir for this virus. Norovirus, prevention. Prevention strategies for this pathogen include source protection.

Cryptosporidium
Cryptosporidium, the basics. It's a parasite. It causes diarrheal illness known as cryptosporidiosis. It is typically associated with animals and humans, and it can be acquired through consuming fecally contaminated food, contact with fecally contaminated soil and water. Cryptosporidium, prevention. Prevention strategies for this pathogen include source protection. A CT value of 9,600 is required when dealing with fecally accidents. CT equals a concentration, in parts per million, while time equals a contact time in minutes. Cryptosporidium can also be prevented or eliminated by boiling water for one minute.

Filtration with an "absolute" pore size of one micron or smaller can eliminate Cryptosporidium. And reverse osmosis is known to be effective as well.

Giardia
Giardia, the basics. It is a parasite. It causes diarrheal illness known as giardiasis. It is typically associated with water. It is the most common pathogen in waterborne outbreaks. It can also be found in soil and food. And humans and animals are the reservoir for this pathogen. Giardia prevention. Prevention strategies for this pathogen include source protection; filtration, coagulation, and halogenation of drinking water.

Schistosomatidae
Schistosomatidae, the basics. It is a parasite. It is acquired through dermal contact, cercarial dermatitis. It is commonly known as swimmer's itch. The reservoir for this pathogen are aquatic snails and birds. Schistosomatidae, prevention. Prevention strategies for this pathogen include eliminating snails with a molluscicide or interrupting the life cycle of the parasite by treating birds with an antihelmetic drug.

E-Coli Section
Escherichia coli. There are several pathogenic strains of Escherichia coli, which are classified under enterovirulent E. coli. They are enterohemorrhagic, enteroinvasive, enterotoxigenic, enteropathogenic, and enteroaggregative. Escherichia coli O157:H7, the basics. It's a bacteria. It causes diarrheal illness, and it's classified as an enterohemorrhagic E. coli. In its most severe form, it can cause hemorrhagic colitis. The reservoir for this bacteria are cattle, deer, goats, and sheep. Humans can also be a reservoir. It is typically associated with contaminated food and water. E. coli O157:H7 prevention. Prevention strategies for this pathogen include source protection, halogenation of water, or boiling water for one minute. Salmonella species, the basics. It's a bacteria. It causes diarrheal illness known as salmonellosis. Humans and animals are the reservoir, and it's typically associated with contaminated food and water. Salmonella species, prevention. Prevention strategies for this pathogen include source protection, halogenation of water, and also boiling water for one minute.

Disinfection Rules

In the past 25 years, the Safe Drinking Water Act (SDWA) has been highly effective in protecting public health and has also evolved to respond to new and emerging threats to safe drinking water. Disinfection of drinking water is one of the major public health advances in the 20th century. One hundred years ago, typhoid and cholera epidemics were common through American cities; disinfection was a major factor in reducing these epidemics.

However, the disinfectants themselves can react with naturally-occurring materials in the water to form unintended byproducts which may pose health risks. In addition, in the past ten years, we have learned that there are specific microbial pathogens, such as *Cryptosporidium*, which can cause illness and is resistant to traditional disinfection practices.

Chlorine is the most widely used water disinfectant due to its effectiveness and cost. Using chlorine as a drinking water disinfectant has prevented millions of water borne diseases, such as typhoid, cholera, dysentery, and diarrhea. Most states require community water systems to use chlorination. However, research shows that chlorine has side effects. It reacts with organic matter present in water and forms a
series of compounds that have been linked to cancer in animals.

These compounds are called disinfection by-products (DBPs). All disinfectants form DBPs in one of two reactions:
(1) chorine and chlorine-based compounds (halogens) react with organics in water causing the chlorine atom to substitute other atoms resulting in halogenated by-products and
(2) oxidation reactions, where chlorine oxidizes compounds present in water. Secondary by-products are also formed when multiple disinfectants are used.

All living organisms have carbon as an essential element in their cells. When trees shed their leaves, they start decomposing and are ultimately broken down by bacteria into carbon-containing compounds. Similarly, dead animals on land and fish and other aquatic life decompose and disintegrate into compounds that contain carbon as an essential element. Hence, all surface water and groundwater contain varying amounts of carbon-containing compounds called organic matter (primarily humic and fulvic acids).

The EPA Surface Water Treatment Rule (SWTR) requires systems using public water supplies from either surface water or groundwater under the direct influence of surface water to disinfect. Also, since some disinfectants produce chemical by-products, the dual objective of disinfection is to provide the required level of organism destruction and remain within the maximum contaminant level (MCL) for the SWTR disinfection set by EPA. At this time, an MCL is set for only Total Trihalomethanes, and proposed for additional disinfection byproducts.

What are the microbial/disinfection byproducts (MDBP) rules and which ones apply to me?
The MDBP requirements have been in place for close to 30 years and include the following federal rules:

- Total Trihalomethanes monitoring and MCL, promulgated Nov 1979
- Surface Water Treatment Rule, promulgated June 1989
- Interim Enhanced Surface Water Treatment Rule and Stage 1 Disinfectants / Disinfection Byproducts Rule, promulgated Dec 1998
- Filter Backwash Rule, promulgated June 2001
- Long Term 1 Enhanced Surface Water Treatment Rule, promulgated Jan 2002

- Long Term 2 Enhanced Surface Water Treatment Rule and Stage 2 Disinfectants / Disinfection Byproducts Rule, promulgated Jan 2006
- Groundwater Rule, promulgated Nov 2006

The Disinfectants and Disinfection Byproducts (DBP) rules apply to all community and non-community water systems using a disinfectant such as chlorine, chloramines, ozone and chlorine dioxide.

Compliance with the Stage 1 DBP requirements began in 2000. The Stage 2 DBP requirements began in 2006 with the Initial Distribution System Evaluation (IDSE). Compliance monitoring for the Stage 2 DBP begins in April 2012. See phased compliance schedule dependent on system population below.

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2) rule applies to all water systems using surface water, groundwater under the influence of a surface water, as well as groundwater/surface water blends. The LT2 requirements began in 2006 with the characterization of raw water Cryptosporidium and E.coli levels. Systems serving <10,000 monitor for E.coli only every two weeks for one year. Compliance with the LT2 requirements begin in April 2013.

The Groundwater Rule (GWR) applies to all public water systems using groundwater. The GWR requirements begin in March 2009 with 6-months investigative monitoring (IM) for source water E.coli, for systems currently applying disinfection only. All other requirements for the GWR began back in Dec 2009.

Amendments to the SDWA in 1996 require EPA to develop rules to balance the risks between microbial pathogens and disinfection byproducts (DBPs). It is important to strengthen protection against microbial contaminants, especially *Cryptosporidium*, and at the same time, reduce potential health risks of DBPs.

The Stage 1 Disinfectants and Disinfection Byproducts Rule and Interim Enhanced Surface Water Treatment Rule, announced in December 1998, are the first of a set of rules under the 1996 SDWA Amendments. This fact sheet focuses on the Stage 1 Disinfectants and Disinfection Byproducts Rule. A separate fact sheet focuses on the Interim Enhanced Surface Water Treatment Rule (EPA 815-F-98-009).

Public Health Concerns

While disinfectants are effective in controlling many microorganisms, they react with natural organic and inorganic matter in source water and distribution systems to form DBPs. Results from toxicology studies have shown several DBPs (e.g., bromodichloromethane, bromoform, chloroform, dichloroacetic acid, and bromate) to be carcinogenic in laboratory animals. Other DBPs (e.g., chlorite, bromodichloromethane, and certain haloacetic acids) have also been shown to cause adverse reproductive or developmental effects in laboratory animals. Several epidemiology studies have suggested a weak association between certain cancers (e.g., bladder) or reproductive and developmental effects, and exposure to chlorinated surface water. More than 200 million people consume water that has been disinfected. Because of the large population exposed, health risks associated with DBPs, even if small, need to be taken seriously.

Who Must Comply With The Rule?

The Stage 1 Disinfectants and Disinfection Byproducts Rule applies to all community and nontransient non-community water systems that treat their water with a chemical disinfectant for either primary or residual treatment.

What Does The Rule Require?
The Stage 1 Disinfectant and Disinfection Byproduct Rule updates and supersedes the 1979 regulations for total trihalomethanes. In addition, it will reduce exposure to three disinfectants and many disinfection byproducts.

The rule establishes maximum residual disinfectant level goals (MRDLGs) and maximum residual disinfectant levels (MRDLs) for three chemical disinfectants - chlorine, chloramine and chlorine dioxide (see Table 1). It also establishes maximum contaminant level goals (MCLGs) and maximum contaminant levels (MCLs) for total trihalomethanes, haloacetic acids, chlorite and bromate (see Table 1).

Table 1
MRDLGs, MRDLs, MCLGs and MCLs for Stage 1 Disinfectants and Disinfection Byproducts Rule

DISINFECTANT RESIDUAL	MRDLG (mg/L)	MRDL (mg/L)	COMPLIANCE BASED ON
Chlorine	**4 (as Cl_2)**	**4.0 (as Cl_2)**	**Annual Average**
Chloramine	**4 (as Cl_2)**	**4.0 (as Cl_2)**	**Annual Average**
Chlorine Dioxide	**0.8 (as ClO_2)**	**0.8 (as ClO_2)**	**Daily Samples**
DISINFECTION BYPRODUCTS	MCLG (mg/L)	MCL (mg/L)	COMPLIANCE BASED ON
Total trihalomethanes (TTHM)[1] **- Chloroform** **- Bromodichloromethane** **- Dibromochloromethane** **- Bromoform**	**N/A** ******* **0** **0.06** **0**	**0.080**	**Annual Average**
Haloacetic acids (five) (HAA5)[2] **- Dichloroacetic acid** **- Trichloroacetic acid**	**N/A** **0** **0.3**	**0.060**	**Annual Average**
Chlorite	**0.8**	**1.0**	**Monthly Average**
Bromate	**0**	**0.010**	**Annual Average**

N/A - Not applicable because there are individual MCLGs for TTHMs or HAAs
1-Total trihalomethanes is the sum of the concentrations of chloroform, bromodichloromethane, dibromochloromethane, and bromoform.
2-Haloacetic acids (five) is the sum of the concentrations of mono-, di-, and trichloroacetic acids and mono- and dibromoacetic acids.
*** EPA removed the zero MCLG for chloroform from its National Primary Drinking Water Regulations, effective May 30, 2000, in accordance with an order of the U.S. Court of Appeals for the District of Columbia Circuit.

Water systems that use surface water or ground water under the direct influence of surface water and use conventional filtration treatment are required to remove specified percentages of

organic materials, measured as total organic carbon (TOC) that may react with disinfectants to form DBPs (See Table 2). Removal will be achieved through a treatment technique (enhanced coagulation or enhanced softening) unless a system meets alternative criteria.

Table 2
Required Removal of Total Organic Carbon by Enhanced Coagulation and Enhanced Softening for Subpart H Systems Using Conventional Treatment[1]

Source Water TOC (mg/L)	Source Water Alkalinity (mg/L as $CaCO_3$)		
	0-60	>60-120	$>120_2$
>2.0-4.0	35.0%	25.0%	15.0%
>4.0-8.0	45.0%	35.0%	25.0%
>8.0	50.0%	40.0%	30.0%

[1]Systems meeting at least one of the alternative compliance criteria in the rule are not required to meet the removals in this table.
[2]Systems practicing softening must meet the TOC removal requirements in the last column to the right.
What Are The Compliance Deadlines?
Large surface water systems are required to comply with the Stage 1 Disinfectants and Disinfection Byproducts Rule and Interim Enhanced Surface Water Treatment Rule by January 2002. Ground water systems and small surface water systems must comply with the Stage 1 Disinfectants and Disinfection Byproducts Rule by January 2004.

What Are The Costs And Benefits Of The Rule?
EPA estimates that implementation of the Stage 1 Disinfectants and Disinfection Byproducts Rule will result in:

- As many as 140 million people receiving increased protection from DBPs.
- 24 percent national average reduction in TTHM levels.
- Reduction in exposure to the major DBPs from use of ozone (bromate) and chlorine dioxide (chlorite).

The total annual cost of the rule is about $700 million. EPA believes that the benefits exceed the costs of the Stage 1 Disinfectants and Disinfection Byproducts Rule. An estimated 116 million households are affected by the Stage 1 Disinfectants and Disinfection Byproducts Rule. EPA estimates that 95 percent of the households will incur additional costs of less than $1 per month on their water bills. An additional four percent will pay between $1 and $10 per month more, and one percent are expected to incur increased water bills of $10 to $33 per month, if they choose to install treatment. However, many of these systems may chose less costly non-treatment options, such as consolidation. The majority of households incurring the highest costs are small systems serving less than 10,000 people that have never been regulated for DBPs.

Conditions for Respirator Use
Good industrial hygiene practice requires that engineering controls be used where feasible to reduce workplace concentrations of hazardous materials to the prescribed exposure limit.

However, some situations may require the use of respirators to control exposure. Respirators must be worn if the ambient concentration of chlorine exceeds prescribed exposure limits. Respirators may be used (1) before engineering controls have been installed, (2) during work operations such as maintenance or repair activities that involve unknown exposures, (3) during operations that require entry into tanks or closed vessels, and (4) during emergencies. Workers should only use respirators that have been approved by NIOSH and the Mine Safety and Health Administration (MSHA).

Respiratory Protection Program
Employers should institute a complete respiratory protection program that, at a minimum, complies with the requirements of OSHA's Respiratory Protection Standard [29 CFR 1910.134]. Such a program must include respirator selection, an evaluation of the worker's ability to perform the work while wearing a respirator, the regular training of personnel, respirator fit testing, periodic workplace monitoring, and regular respirator maintenance, inspection, and cleaning. The implementation of an adequate respiratory protection program (including selection of the correct respirator) requires that a knowledgeable person be in charge of the program and that the program be evaluated regularly.

For additional information on the selection and use of respirators and on the medical screening of respirator users, consult the latest edition of the NIOSH Respirator Decision Logic [NIOSH 1987b] and the NIOSH Guide to Industrial Respiratory Protection [NIOSH 1987a].

Personal Protective Equipment
Workers should use appropriate personal protective clothing and equipment that must be carefully selected, used, and maintained to be effective in preventing skin contact with chlorine. The selection of the appropriate personal protective equipment (**PPE**) (e.g., gloves, sleeves, encapsulating suits) should be based on the extent of the worker's potential exposure to chlorine.

The resistance of various materials to permeation by both chlorine liquid and chlorine gas is shown below:

Material Breakthrough Time (hr) Chlorine Liquid Responder
Chlorine gas butyl rubber neoprene Teflon viton saranex barricade chemrel responder trellchem HPS nitrile rubber H (**PE/EVAL**) polyethylene polyvinyl chloride. Material is estimated (but not tested) to provide at least four hours of protection. Not recommended, degradation may occur.

To evaluate the use of these PPE materials with chlorine, users should consult the best available performance data and manufacturers' recommendations. Significant differences have been demonstrated in the chemical resistance of generically similar PPE materials (e.g., butyl) produced by different manufacturers. In addition, the chemical resistance of a mixture may be significantly different from that of any of its neat components.

Any chemical-resistant clothing that is used should be periodically evaluated to determine its effectiveness in preventing dermal contact. Safety showers and eye wash stations should be located close to operations that involve chlorine.

Splash-proof chemical safety goggles or face shields (20 to 30 cm long, minimum) should be worn during any operation in which a solvent, caustic, or other toxic substance may be splashed into the eyes.



In addition to the possible need for wearing protective outer apparel (e.g., aprons, encapsulating suits), workers should wear work uniforms, coveralls, or similar full-body coverings that are laundered each day. Employers should provide lockers or other closed areas to store work and street clothing separately. Employers should collect work clothing at the end of each work shift and provide for its laundering. Laundry personnel should be informed about the potential hazards of handling contaminated clothing and instructed about measures to minimize their health risk.

Protective clothing should be kept free of oil and grease and should be inspected and maintained regularly to preserve its effectiveness. Protective clothing may interfere with the body's heat dissipation, especially during hot weather or during work in hot or poorly ventilated work environments.



SCBA Fit Test

Respiratory Protection Breakdown

General
In the Respiratory Protection program, hazard assessment and selection of proper respiratory PPE is conducted in the same manner as for other types of PPE. In the control of those occupational diseases caused by breathing air contaminated with harmful dusts, fogs, fumes, mists, gases, smokes, sprays, or vapors, the primary objective shall be to prevent atmospheric contamination.

This shall be accomplished as far as feasible by accepted engineering control measures (for example, enclosure or confinement of the operation, general and local ventilation, and substitution of less toxic materials). When effective engineering controls are not feasible, or while they are being instituted, appropriate respirators shall be used. References: OSHA Standards ***Respiratory Protection*** (**29 CFR 1910.134**)

Why Respirators Are Needed
Respirators protect against the inhalation of dangerous substances (vapors, fumes, dust, gases). They can also provide a separate air supply in a very hazardous situation.
Some of the health hazards that respirators prevent include:
- ***Lung damage***
- ***Respiratory diseases***
- ***Cancer and other illnesses.***



Respiratory Protection Responsibilities
The employer is responsible for:
- Providing training in the use and care of respirators.
- Ensuring that equipment is adequate, sanitary, and reliable.
- Allowing employees to leave area if ill, for breaks, and to obtain parts.
- Fit testing.
- Providing an annual medical evaluation.
- Providing a powered air-purifying respirator (**PAPR**) if an employee cannot wear a tight-fitting respirator.



The employee is responsible for:
- Properly using respirators.
- Maintaining respirator properly.
- Reporting malfunctions.
- Reporting medical changes.

Selection of Proper Respiratory Protection

When choosing the correct respiratory protection for your work environment, it is important to consider:

- Identification of the substance or substances for which respiratory protection is necessary.
- A substance's Safety Data Sheet (formerly MSDS) (**SDS**) (it will state which type of respirator is most effective for the substance).
- Activities of the workers.
- Hazards of each substance and its properties.
- Maximum levels of air contamination expected.
- Probability of oxygen deficiency.
- Period of time workers will need to use the respiratory protection devices.
- Capabilities and physical limitations of the device used.

Types of Respirators The following is a description of different types of respirators.

Commonly Used Respirators (*Air Purifying*)

- **Disposable Dust masks** are worn over the nose and mouth to protect the respiratory system from certain nuisance dusts, mists, etc. They can only provide protection against particular contaminants as specified by the manufacturer (e.g., general dust, fiberglass, etc.). These dust masks cannot be fit tested, and are generally single use. They are not recognized as respiratory protection and may not be worn if a potential for overexposure exists. They are not included in most companies' Respiratory Protection Program.

- **Half-Face Respirators** with interchangeable filter cartridges can protect the respiratory system from hazardous dusts, fumes, mists, etc. They can only provide protection against certain contaminants up to limited concentrations specified by the manufacturer for the particular cartridge type used (e.g., toluene, acetone). These generally operate under negative pressure within the respirator which is created by the wearer's breathing through the filter cartridges. As the protection is only gained if there is a proper seal of the respirator face piece, this type requires fit testing prior to respirator assignment and a fit check prior to each use.

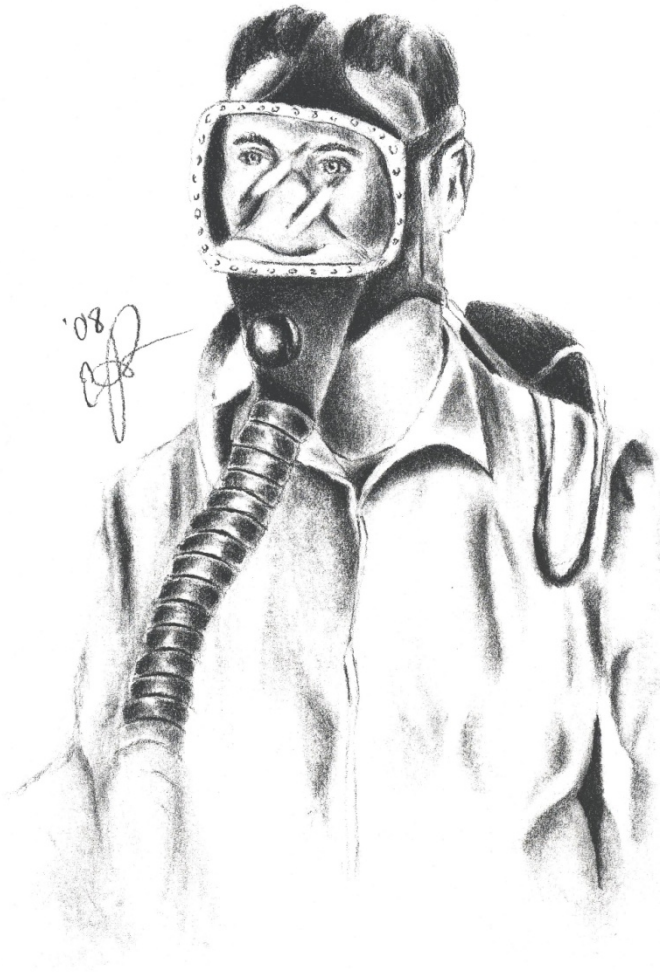
- **Full-Face Respirators** operate under the same principle and requirements as the half-face type, however, they offer a better facepiece fit and also protect the wearer's eyes from particularly irritating gases or vapors.

- **Full-face, helmet or hood type powered air purifying respirators** (**PAPRs**) operate under positive pressure inside the facepiece using a battery operated motor blower assembly to force air through a filter cartridge into the wearer's breathing zone. Use of these respirators is also subject to the manufacturers' guidelines.

Less Commonly Used Types Respirators (Air Supplying)

- **Air-Line Respirators** supply clean air through a small diameter hose from a compressor or compressed air cylinders. The wearer must be attached to the hose at all times, which limits mobility. Use of these respirators is subject to the manufacturers' guidelines.
- **Self-Contained Breathing Apparatus** (**SCBA**) respirators supply clean air from a compressed air tank carried on the back of the wearer. These types of respirators are highly mobile and are used primarily for emergency response or rescue work, since only a limited amount of air can be supplied by a single tank, generally 20-60 minutes. Units must be thoroughly inspected on a monthly basis and written records must be kept of all inspections, operator training, etc. Use of these respirators is subject to the manufacturer's guidelines

Basic Types of Respirators
Air-purifying or filtering respirators. Such respirators are used when there is enough oxygen (at least 19.5 percent) and contaminants are present below IDLH level. The respirator filters out or chemically "**scrubs**" contaminants, usually with a replaceable filter. Use color-coded filter cartridges or canisters for different types of contaminants. It's important to select the right filter for the situation.



Air-supplying respirators. These respirators are required when air-purifying respirators aren't effective. Air-purifying respirators are not sufficient in the following settings:

- When there is not enough oxygen.
- In Confined spaces.
- When contaminants cannot be filtered out.
- When contaminants are at or above IDLH level.

Different kinds of air-supplying respirators include

- Those connected by hose to a stationary air supply (air line)
- Portable tank self-contained breathing apparatus (**SCBA**).

The Importance of Correct Fit

Even a tiny gap between the respirator and the face can allow contaminants to enter. Respirators should be comfortable and properly fitted.

Proper fit includes:
- Secure but not too tight.
- No slipping or pinching.
- Allowance for head movement and speech.

An OSHA-accepted qualitative fit test or quantitative fit test must be performed prior to an employee using any tight-fitting respirator.

Tight-fitting respirators must be seal checked before each use by using positive- or negative-pressure check procedures or the manufacturer's instructions.

Respirator Filters/Cartridges
For protection against gases and vapors, the cartridges used for air-purifying respirators must be either equipped with an end-of-service-life indicator (**ESLI**), certified by NIOSH for the contaminant, or a cartridge change schedule has to be established.

For protection against particulates, there are nine classes of filters (three levels of filter efficiency, each with three categories of resistance to filter efficiency degradation). Levels of filter efficiency are 95 percent, 99 percent, and 99.97 percent. Categories of resistance to filter efficiency degradation are labeled N, R, and P.

Protection Factors
The protection factor of a respirator is an expression of performance based on the ratio of two concentrations: The contaminant concentration outside the respirator to the contaminant concentration inside the respirator.

Each class of respirator is also given an assigned protection factor (**APF**). The APF is a measure of the minimum anticipated level of respiratory protection that a properly functioning respirator or class of respirators would provide to a percentage of properly fitted and trained users. When a contaminant concentration is known, the APF can be used to estimate the concentration inside a particular type of respirator worn by a user.



Who Cannot Wear a Respirator?

Respirator fit is essential. Employees must have a medical checkup to make sure they can wear respirators safely.

Generally, Respirators cannot be Worn when a Person:

- Wears glasses or personal protective equipment that interferes with the seal of the face piece to the face of the user.
- Has facial hair that comes between the sealing surface of the face piece and the face or interferes with valve function.
- Has a breathing problem, such as asthma.
- Has a heart condition.
- Is heat sensitive.

Sometimes a person's facial features will not permit a good fit. Check with the supervisor or medical department if the fit is a problem.

Checking for Damage

Before each use, make sure there are no holes, tears, etc., in the respirator. Rubber parts can wear out and should be checked very carefully every time a respirator is used. Replace worn and damaged parts when necessary. Make sure air and oxygen cylinders are fully charged.

Staying Prepared for Respirator Use

Respirators are bulky and awkward, so getting used to them takes practice. Possible problems with wearing respirators may include heat exhaustion or heat stroke. Be alert for symptoms, use the "**buddy system**," and wear a lifeline or harness when necessary. Drink plenty of fluids and take frequent breaks.

Poor Maneuverability. Practice with respirators in narrow passages, on ladders, etc., if your use of respirators may be in these types of conditions.

Using up the Air Supply. When a SCBA is in use, keep checking the gauges and listening for alarms; be ready to leave the area immediately if there is a problem.

Panic. Remember the importance of staying calm in a hot, stressful, or awkward situation.

Cleaning Respirators

Respirators should be cleaned and disinfected after every use. Check the respirator for damage before putting it away; look for holes, cracks, deterioration, dented cartridges, etc. If any damage is found, it should be reported to a supervisor. Respirators stored for emergency use must be inspected monthly when not in use, as well as after each use.

Respirators should be stored away from light, heat, cold, chemicals, and dust. Store respirators in a "***normal***" (natural, undistorted) position to hold their shape. Do not allow respirators to get crushed, folded, or twisted.

OSHA Regulation Overview

OSHA requires that supervisors consult with employees and encourage their participation in the process safety management plan. In fact, managers must have a written plan of action for employee participation in process safety management. Employee participation is critical because;

- Employees know a lot about the process they work on.
- They play key roles in making sure that process operation is conducted safely.

Operating Procedures

Managers must furnish written operating procedures that clearly explain how to perform each covered process safely. The procedures must be accurate and must be written in language that people can understand. Avoid technical jargon and, if necessary, supply translations.

Operating procedures must include at least the following:

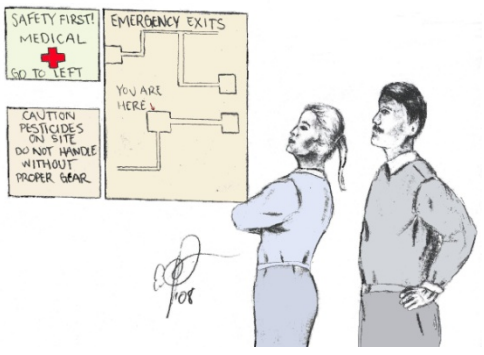
- Operating steps for initial startup, normal and temporary operations, emergency shutdown (including when it's called for and who does it), emergency operations, normal shutdown, and startup after a turnaround or an emergency shutdown.
- Operating limits, including what happens if workers don't conform to operating limits and how to avoid or correct such problems.
- Safety and health considerations, such as chemical or other hazards, precautions to prevent exposure, quality and inventory control for chemicals, and what to do if an employee is exposed to a hazardous substance.
- Safety systems and their functions, including up-to-date operating procedures and safe work practices.

Contractor Employees

Process safety training and safety programs are also required for contractors who work on-site. Managers must check out the safety performance and programs of any contractors being considered for maintenance, repair, turnaround, major renovation, or specialty work on or around a process covered by the regulation.

When a contractor is hired, the manager must provide the contractor with information on the hazards of the process the contractor will work on. To further ensure contractor safety, managers must also:

- Provide the contractor with information on safe work practices for the process they're involved with and tell them what to do in an emergency.
- Keep a log of contractor employees' injuries or illnesses related to their work in process areas.
- Evaluate the contractor's performance to make sure they're living up to their safety obligations under the standard.



The Contractor has Responsibilities, too…

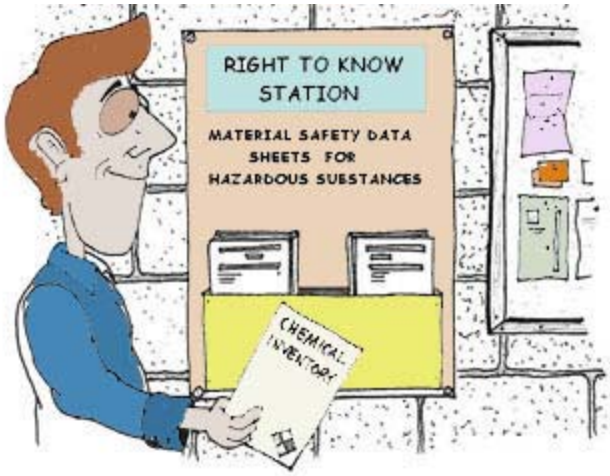
- Document that employees are trained to recognize hazards and to follow safe work practices on the job.
- Make sure that the contractor's employees understand potential job-related hazards, are trained to work safely, and follow the safety rules of the facility in which they're working.

Written Respiratory Protection Program

This paragraph requires the employer to develop and implement a written respiratory protection program with required worksite-specific procedures and elements for required respirator use. The program must be administered by a suitably trained program administrator. In addition, certain program elements may be required for voluntary use to prevent potential hazards associated with the use of the respirator.

The Small Entity Compliance Guide contains criteria for the selection of a program administrator and a sample program that meets the requirements of this paragraph. Copies of the Small Entity Compliance Guide will be available on or about April 8, 1998 from the Occupational Safety and Health Administration's Office of Publications, Room N 3101, 200 Constitution Avenue, NW, Washington, DC, 20210 (202-219-4667).

(c)(1) In any workplace where respirators are necessary to protect the health of the employee or whenever respirators are required by the employer, the employer shall establish and implement a written respiratory protection program with worksite-specific procedures. The program shall be updated as necessary to reflect those changes in workplace conditions that affect respirator use. The employer shall include in the program the following provisions of this section, as applicable:



(c)(1)(i) Procedures for selecting respirators for use in the workplace;

(c)(1)(ii) Medical evaluations of employees required to use respirators;

(c)(1)(iii) Fit testing procedures for tight-fitting respirators;

(c)(1)(iv) Procedures for proper use of respirators in routine and reasonably foreseeable emergency situations;

(c)(1)(v) Procedures and schedules for cleaning, disinfecting, storing, inspecting, repairing, discarding, and otherwise maintaining respirators;

(c)(1)(vi) Procedures to ensure adequate air quality, quantity, and flow of breathing air for atmosphere-supplying respirators;

(c)(1)(vii) Training of employees in the respiratory hazards to which they are potentially exposed during routine and emergency situations;

Example of RP Employee Responsibilities

All Employees shall follow the requirements of the Respiratory Protection Program.

Management

- implement the requirements of this program
- provide a selection of respirators as required
- enforce all provisions of this program
- appoint a *Specific Designated* individual to conduct the respiratory protection program

Administrative Department

- Review sanitation/storage procedures.
- ensure respirators are properly stored, inspected and maintained
- monitor compliance for this program
- provide training for affected Employees
- review compliance and ensure monthly inspection of all respirators
- provide respirator fit testing



Designated Occupational Health Care Provider

- conducts medical aspects of program

Program Administrator

Each Department will designate a program administrator who is qualified by appropriate training or experience that is commensurate with the complexity of the program to administer or oversee the respiratory protection program and conduct the required evaluations of program effectiveness.

Voluntary Use of Respirators is Prohibited

OSHA requires that voluntary use of respirators, when not required by the Employer, must be controlled as strictly as under required circumstances. To prevent violations of the Respiratory Protection Standard, Employees are not allowed voluntary use of their own or Employer supplied respirators of any type.

Exception: Employees whose only use of respirators involves the voluntary use of filtering (non-sealing) face pieces (dust masks).
See appendix D.



Respiratory Protection Program Statement Example

Facility _____
Policy Statement

A respiratory protection program is hereby established so as to coordinate the use and maintenance or respiratory protective equipment as determined necessary to:

1. Reduce Personnel exposure to toxic chemical agents, harmful dusts, mist and fumes and

2. Allow trained personnel to work safely in hazardous environments, such as welding, oxygen deficient atmospheres, toxic atmospheres, etc.

Designation of Program Administrator

Management has designated _____
to be responsible for the respiratory protection program at this facility. He/she has been delegated authority by Management to make decisions and implement changes in the respirator program anywhere in this facility.
The following responsibilities apply:

1. Supervision of respirator selection process and procedures
2. Establishment of respiratory protection training sessions
3. Establishment of a continuing program of cleaning and inspections
4. Establishment of medical screening program
5. Establishment of issuing procedures
6. Establishment of periodic inspections
7. Continuing evaluation of all aspects of the respiratory protection program to assure continued effectiveness
8. Establishment of annual fit tests procedures

Any questions or problems concerning respirators or their use should be directed to the Program Administrator

_____ _____
Facility Manager Date



Inspection of the respirator includes the filter and storage areas.

Program Evaluation

Evaluations of the workplace are necessary to ensure that the written respiratory protection program is being properly implemented; this includes consulting with employees to ensure that they are using the respirators properly.

Evaluations shall be conducted as necessary to ensure that the provisions of the current written program are being effectively implemented and that it continues to be effective.

Program evaluation will include discussions with employees required to use respirators to assess the employees' views on program effectiveness and to identify any problems.

Any problems that are identified during this assessment shall be corrected. Factors to be assessed include, but are not limited to:

- Respirator fit (including the ability to use the respirator without interfering with effective workplace performance);
- Appropriate respirator selection for the hazards to which the employee is exposed;
- Proper respirator use under the workplace conditions the employee encounters; and
- Proper respirator maintenance.



Examples of half-face and full-face respirators with HEPA filters (left and center). The mask on the right is only suitable for non-hazardous, non-respirable nuisance dusts.

Recordkeeping

The Employer will retain written information regarding medical evaluations, fit testing, and the respirator program. This information will facilitate employee involvement in the respirator program, assist the Employer in auditing the adequacy of the program, and provide a record for compliance determinations by OSHA.

Training and Information
Effective training for employees who are required to use respirators is essential. The training must be comprehensive, understandable, and recur annually and more often if necessary. Training will be provided prior to requiring the employee to use a respirator in the workplace.

The training shall ensure that each employee can demonstrate knowledge of at least the following:

- Why the respirator is necessary and how improper fit, usage, or maintenance can compromise the protective effect of the respirator.
- Limitations and capabilities of the respirator.
- How to use the respirator effectively in emergency situations, including situations in which the respirator malfunctions.
- How to inspect, put on and remove, use, and check the seals of the respirator.
- Procedures for maintenance and storage of the respirator.
- How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators.
- The general requirements of this program.



Retraining shall be conducted annually and when:

- changes in the workplace or the type of respirator render previous training obsolete
- inadequacies in the employee's knowledge or use of the respirator indicate that the employee has not retained the requisite understanding or skill
- other situation arises in which retraining appears necessary to ensure safe respirator use

Training is divided into the following sections:
Classroom Instruction
1. Overview of the Employer's Respiratory Protection Program & OSHA Standard
2. Respiratory Protection Safety Procedures
3. Respirator Selection
4. Respirator Operation and Use
5. Why the respirator is necessary
6. How improper fit, usage, or maintenance can compromise the protective effect.
7. Limitations and capabilities of the respirator.
8. How to use the respirator effectively in emergency situations, including respirator malfunctions
9. How to inspect, put on and remove, use, and check the seals of the respirator.
10. Procedures for maintenance and storage of the respirator.
11. How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators.
12. Change out schedule and procedure for air purifying respirators.

Respiratory Protection Program

Training Certificate Example

Name:_____

Department:_____ Date:_____
I have received Training on the Respiratory Protection Program. The Training included the following:

Classroom Training

- ✓ Overview of the Company Respiratory Protection Program
- ✓ Respiratory Protection Safety Procedures
- ✓ Respirator Selection
- ✓ Respirator Operation and Use
- ✓ Why the respirator is necessary
- ✓ How improper fit, usage, or maintenance can compromise the protective effect.
- ✓ Limitations and capabilities of the respirator.
- ✓ How to use the respirator effectively in emergency situations, including respirator malfunctions.
- ✓ How to inspect, put on and remove, use, and check the seals of the respirator.
- ✓ Procedures for maintenance and storage of the respirator.
- ✓ How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators.
- ✓ Respirator filter & cartridge change-out schedule
- ✓ The general requirements of this program

Hands-on Training

- ✓ Respirator Inspection
- ✓ Respirator cleaning and sanitizing
- ✓ Fit Check
- ✓ Record Keeping
- ✓ Respirator Storage
- ✓ Emergencies

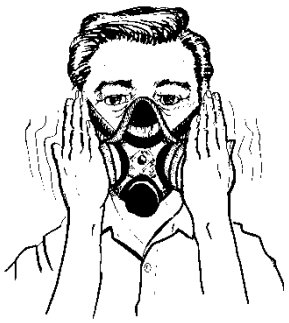
_____

Employees Signature

_____

Trainer's Signature

Fit Testing Hands-on Respirator Training



(see appendix A for more information)

1. **Respirator Inspection**
2. **Respirator cleaning and sanitizing**
3. **Record Keeping**
4. **Respirator Storage**
5. **Respirator Fit Check**
6. **Emergencies**

Basic Respiratory Protection Safety Procedures

1. Only authorized and trained Employees may use Respirators. Those Employees may use only the Respirator that they have been trained on and properly fitted to use.
2. Only Physically Qualified Employees may be trained and authorized to use Respirators. A pre-authorization and annual certification by a qualified physician will be required and maintained. Any changes in an Employees health or physical characteristics will be reported to the Occupational Health Department and will be evaluated by a qualified physician.
3. Only the proper prescribed respirator or SCBA may be used for the job or work environment. Air cleansing respirators may be worn in work environments when oxygen levels are between 19.5 percent to 23.5 percent and when the appropriate air cleansing canister, as determined by the Manufacturer and approved by NIOSH or MESA, for the known hazardous substance is used. SCBAs will be worn in oxygen deficient and oxygen rich environments (below 19.5 percent or above 23.5 percent oxygen).
4. Employees working in environments where a sudden release of a hazardous substance is likely will wear an appropriate respirator for that hazardous substance (example: Employees working in an ammonia compressor room will have an ammonia APR respirator on their person.).
5. Only SCBAs will be used in oxygen deficient environments, environments with an unknown hazardous substance or unknown quantity of a known hazardous substance or any environment that is determined "Immediately Dangerous to Life or Health" (IDLH).
6. Employees with respirators loaned on "permanent check out" will be responsible for the sanitation, proper storage and security. Respirators damaged by normal wear will be repaired or replaced by the Employer when returned.
7. The last Employee using a respirator and/or SCBA that are available for general use will be responsible for proper storage and sanitation. Monthly and after each use, all respirators will be inspected with documentation to assure its availability for use.
8. All respirators will be located in a clean, convenient and sanitary location.
9. In the event that Employees must enter a confined space, work in environments with hazardous substances that would be dangerous to life or health should an RPE fail (a SCBA is required in this environment), and/or conduct a HAZMAT entry, a "**buddy system**" detail will be used with a Safety Watchman with constant voice, visual or signal line communication. Employees will follow the established Emergency Response Program and/or Confined Space Entry Program when applicable.
10. Management will establish and maintain surveillance of jobs and work place conditions and degree of Employee exposure or stress to maintain the proper procedures and to provide the necessary RPE.

11. Management will establish and maintain safe operation procedures for the safe use of RPE with strict enforcement and disciplinary action for failure to follow all general and specific safety rules. Standard Operation Procedures for General RPE use will be maintained as an attachment to the Respiratory Protection Program and Standard Operation Procedures for RPE use under emergency response situations will be maintained as an attachment to the Emergency Response Program.

Selection of Respirators
The Employer is responsible and needs to have evaluated the respiratory hazard(s) in each workplace, identified relevant workplace and user factors and have based respirator selection on these factors. Also included are estimates of employee exposures to respiratory hazard(s) and an identification of the contaminant's chemical state and physical form.

This selection has included appropriate protective respirators for use in IDLH atmospheres, and has limited the selection and use of air-purifying respirators. All selected respirators are NIOSH-certified.

Filter Classifications - These classifications are marked on the filter or filter package
N-Series: Not Oil Resistant
- Approved for non-oil particulate contaminants
- Examples: dust, fumes, mists not containing oil



R-Series: Oil Resistant
- Approved for all particulate contaminants, including those containing oil
- Examples: dusts, mists, fumes
- Time restriction of 8 hours when oils are present

P-Series: Oil Proof
- Approved for all particulate contaminants including those containing oil
- Examples: dust, fumes, mists
- See Manufacturer's time use restrictions on packaging

Respirators for IDLH Atmospheres.
- The following respirators will be used in IDLH atmospheres:
- A full face piece pressure demand SCBA certified by NIOSH for a minimum service life of thirty minutes, or
- A combination full face piece pressure demand supplied-air respirator (**SAR**) with auxiliary self-contained air supply.
- Respirators provided only for escape from IDLH atmospheres shall be NIOSH-certified for escape from the atmosphere in which they will be used.

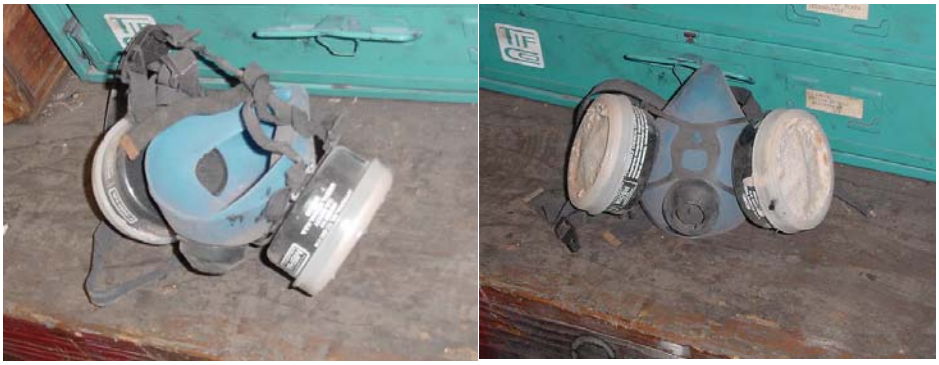
Respirators for Atmospheres that are not IDLH.

The respirators selected shall be adequate to protect the health of the employee and ensure compliance with all other OSHA statutory and regulatory requirements, under routine and reasonably foreseeable emergency situations. The respirator selected shall be appropriate for the chemical state and physical form of the contaminant.

Identification of Filters & Cartridges

All filters and cartridges shall be labeled and color coded with the NIOSH approval label; the label is never to be removed and must remain legible. A change out schedule for filters and canisters has been developed to ensure these elements of the respirators remain effective.

Respirator Filter & Canister Replacement
An important part of the Respiratory Protection Program includes identifying the useful life of canisters and filters used on air-purifying respirators. Each filter and canister shall be equipped with an end-of-service-life indicator (**ESLI**) certified by NIOSH for the contaminant; or If there is no ESLI appropriate for conditions, a change schedule for canisters and cartridges that is based on objective information or data that will ensure that canisters and cartridges are changed before the end of their service life.



Unacceptable maintenance and storage (OSHA Violation).

Filter & Cartridge Change Schedule
Stock of spare filters and cartridges shall be maintained to allow immediate change when required or desired by the employee.

Cartridges shall be changed based on the most limiting factor below:
- Prior to expiration date
- Manufacturer's recommendations for the specific use and environment
- After each use
- When requested by employee
- When contaminate odor is detected
- When restriction to air flow has occurred as evidenced by increase effort by user to breathe normally
- Cartridges shall remain in their original sealed packages until needed for immediate use

Filters shall be changed on the most limiting factor below:
- Prior to expiration date
- Manufacturer's recommendations for the specific use and environment
- When requested by employee
- When contaminate odor is detected
- When restriction to air flow has occurred as evidenced by increase effort by user to breathe normally
- When discoloring of the filter media is evident
- Filters shall remain in their original sealed package until needed for immediate use.

Respirator Selection and Use

HAZARD	RESPIRATOR TYPE
Asbestos	Half-mask, air-purifying respirator with HEPA filters Full-face, air-purifying respirator with HEPA filters Full-face, powered air-purifying respirator with HEPA filters
Epoxy- or Oil-based Paints	Half-face, air-purifying respirators with organic vapor filters Full-face powered air-purifying respirator with organic vapor filters
Lead-based Paint removal	Half-face, air-purifying respirators with HEPA filters Full-face, air-purifying respirators with HEPA filters Full-face, powered air-purifying respirators with HEPA filters
Use of Pesticides, Herbicides, and Rodenticides	Full-face, air-purifying respirator with combination particulate and pesticide cartridges Full-face, powered air-purifying respirator with combination particulate and pesticide cartridges
Use of Formaldehyde	Full-face, air-purifying respirator with organic vapor or specific formaldehyde cartridges Full-face, powered air-purifying respirator with organic vapor or specific formaldehyde cartridges Type C supplied air respirator with pressure- demand mode

RESPIRATORY PROTECTION PROGRAM CHECKLIST			PAGE 1 OF 1 PAGES		
DIVISION:	**SECTION:**	**SUPERVISOR:**	**DATE:**		
		YES	NO	NA	
1	Is respiratory protection (RP) being worn in the section?				
2	Has air sampling been accomplished that mandates using RP?				
3	Where air sampling results greater than Occupational Exposure Limits? (If NO, why are you using a respirator?)				
4	Has a Hazard Assessment been generated concerning the task or process that placed the section on the RP Program?				
5	Have all processes that may warrant the use of RP been evaluated? (If NO, request an assessment from the Department Safety Analyst /Personnel Safety, unless the operation is emergency response).				
6	Have workers received physicals and been found medically qualified to wear RP?				
7	Is there documentation that workers were formally briefed on air sampling results and why RP is required?				
8	Is respiratory protection training and fit-testing documentation available on everyone who wears a respirator?				
9	Are RP wearers being fit-tested at least annually?				
10	Are section employees wearing RP voluntarily when conditions have not mandated their use?				
11	Are employees wearing contacts in hazardous atmospheres or using eye-wear that negates face to face piece seal?				
12	Do RP users have facial hair that negates face to face piece seal?				
13	Has a respirator inventory been compiled that list the type of respirator(s) used in the workplace? (Use Respirator Inventory Worksheet attach to this checklist)				
14	Has the Section Supervisor received formal RP training on OSHA, City Personnel Safety and Respiratory Protection Program requirements and his or her responsibilities?				
15	Does the section have written standard operating instructions governing the selection, fit-testing, use, cleaning, storage and maintenance of respirators?				
16	Is the Fire Department the only source being used to charge SCBA's with compressed air?				
17	Are SCBA's being inspected at least every 30 days?				
18	Does the section have on hand, applicable OSHA, CITY, and Section Respiratory Protection Program guidance documents?				
19	Are periodic audits of the section's RP program conducted with discrepancies tracked until closed out?				
20	Have program deficiencies been elevated to the Director and Department Safety Analyst?				

SURVEYED BY:	REVIEWED BY:



SDS Terminology

The Hazard Communication Standard requires employees to understand chemical hazards, labels, and SDSs and to use them on the job. Before starting jobs involving possible exposure to hazardous substances, employees must read SDSs to know what they're working with and procedures for safe handling.

Hundreds, perhaps thousands, of terms could be included in a listing like the one provided in this session. The list of terms and definitions included in this session is not comprehensive or all-inclusive.

The intent is to provide users with a brief list of some of the terms that may appear in common SDSs. Additional terms specific to the substances your company uses or keeps on-site should be added to the list.

Respiratory Protection Schedule by Job and Working Condition

The Employer needs to maintain a Respiratory Protection Schedule by Job and Working Condition. This schedule is provided to each authorized and trained Employee. ***The Schedule provides the following information:***



1. **Job/Working Conditions**
2. **Work Location**
3. **Hazards Present**
4. **Type of Respirator or SCBA Required**
5. **Type of Filter/Canister Required**
6. **Location of Respirator or SCBA**
7. **Filter/Cartridge change out schedule**

The schedule will be reviewed and updated at least annually and whenever any changes are made in the work environments, machinery, equipment, or processes or if different respirator models are introduced or existing models are removed.

Permanent Respirator Schedule Assignments Are:
Each person who engages in welding will have their own Employer provided dust-mist-fume filter APR. This respirator will be worn during all welding operations.

Physical and Medical Qualifications
Records of medical evaluations must be retained and made available in accordance with 29 CFR 1910.1020.

Medical Evaluation Required
Using a respirator may place a physiological burden on employees that varies with the type of respirator worn, the job and workplace conditions in which the respirator is used, and the medical status of the employee. The Employer is required to provide a medical evaluation to determine the employee's ability to use a respirator before the employee is fit tested or required to use the respirator in the workplace.

Medical Evaluation Procedures
The employee will be provided a medical questionnaire by the designated Occupational Health Care Provider



Follow-up Medical Examination

The Employer shall ensure that a follow-up medical examination is provided for an employee who gives a positive response to any question among questions in Part B of the questionnaire or whose initial medical examination demonstrates the need for a follow-up medical examination. The follow-up medical examination shall include any medical tests, consultations, or diagnostic procedures that the Physician deems necessary to make a final determination.

Administration of the Medical Questionnaire and Examinations
The medical questionnaire and examinations shall be administered confidentially during the employee's normal working hours or at a time and place convenient to the employee. The medical questionnaire shall be administered in a manner that ensures that the employee understands its content. The Employer shall provide the employee with an opportunity to discuss the questionnaire and examination results with the Physician.

Supplemental Information for the Physician
The following information must be provided to the Physician before the Physician makes a recommendation concerning an employee's ability to use a respirator;

- The type and weight of the respirator to be used by the employee.
- The duration and frequency of respirator use (including use for rescue and escape).
- The expected physical work effort.
- Additional protective clothing and equipment to be worn.
- Temperature and humidity extremes that may be encountered.
- Any supplemental information provided previously to the Physician regarding an employee need not be provided for a subsequent medical evaluation if the information and the Physician remain the same

The Employer has provided the Physician with a copy of the written respiratory protection program and a copy of the OSHA Standard 1910.134



Half face type respirator.

Medical Determination

In determining the employee's ability to use a respirator, the Employer shall:
Obtain a written recommendation regarding the employee's ability to use the respirator from the Physician. The recommendation shall provide only the following information:

- Any limitations on respirator use related to the medical condition of the employee, or relating to the workplace conditions in which the respirator will be used, including whether or not the employee is medically able to use the respirator.
- The need, if any, for follow-up medical evaluations.
- A statement that the Physician has provided the employee with a copy of the Physician's written recommendation.
- If the respirator is a negative pressure respirator and the Physician finds a medical condition that may place the employee's health at increased risk if the respirator is used, the Employer shall provide a APR if the Physician's medical evaluation finds that the employee can use such a respirator; if a subsequent medical evaluation finds that the employee is medically able to use a negative pressure respirator, then the Employer is no longer required to provide a APR.

Additional Medical Evaluations
At a minimum, the Employer shall provide additional medical evaluations that comply with the requirements of this section if:

- An employee reports medical signs or symptoms that are related to ability to use a respirator.
- A Physician, supervisor, or the respirator program administrator informs the Employer that an employee needs to be reevaluated.
- Information from the respiratory protection program, including observations made during fit testing and program evaluation, indicates a need for employee reevaluation.
- A change occurs in workplace conditions (e.g., physical work effort, protective clothing, and temperature) that may result in a substantial increase in the physiological burden placed on an employee.

Respirator Fit Testing *(see Appendix A for more information)*
Before an employee is required to use any respirator with a negative or positive pressure tight-fitting face piece, the employee must be fit tested with the same make, model, style, and size of respirator that will be used. The Employer shall ensure that an employee using a tight-fitting face piece respirator is fit tested prior to initial use of the respirator, whenever a different respirator face piece (size, style, model or make) is used, and at least annually thereafter
The Employer has established a record of the qualitative and quantitative fit tests administered to employees including:

- The name or identification of the employee tested.
- Type of fit test performed.
- Specific make, model, style, and size of respirator tested.
- Date of test.
- The pass/fail results for QLFTs or the fit factor and strip chart recording or other recording of the test results for QNFTs.

Additional fit tests will be conducted whenever the employee reports, or the Employer, Physician, supervisor, or program administrator makes visual observations of, changes in the employee's physical condition that could affect respirator fit. Such conditions include, but are not limited to, facial scarring, dental changes, cosmetic surgery, or an obvious change in body weight.

If after passing a QLFT or QNFT, the employee notifies the Employer's program administrator, supervisor, or Physician that the fit of the respirator is unacceptable, the employee shall be given a reasonable opportunity to select a different respirator face piece and to be retested.

Types of Fit Tests

The fit test shall be administered using an OSHA-accepted QLFT or QNFT protocol. The OSHA-accepted QLFT and QNFT protocols and procedures are contained in Appendix A of OSHA Standard 1910.134.

- QLFT may only be used to fit test negative pressure air-purifying respirators that must achieve a fit factor of 100 or less.
- If the fit factor, as determined through an OSHA-accepted QNFT protocol, is equal to or greater than 100 for tight-fitting half face pieces, or equal to or greater than 500 for tight-fitting full face pieces, the QNFT has been passed with that respirator.
- Fit testing of tight-fitting atmosphere-supplying respirators and tight-fitting powered air-purifying respirators shall be accomplished by performing quantitative or qualitative fit testing in the negative pressure mode, regardless of the mode of operation (negative or positive pressure) that is used for respiratory protection.
- Qualitative fit testing of these respirators shall be accomplished by temporarily converting the respirator user's actual face piece into a negative pressure respirator with appropriate filters, or by using an identical negative pressure air-purifying respirator face piece with the same sealing surfaces as a surrogate for the atmosphere-supplying or powered air-purifying respirator face piece.
- Quantitative fit testing of these respirators shall be accomplished by modifying the face piece to allow sampling inside the face piece in the breathing zone of the user, midway between the nose and mouth. This requirement shall be accomplished by installing a permanent sampling probe onto a surrogate face piece, or by using a sampling adapter designed to temporarily provide a means of sampling air from inside the face piece.
- Any modifications to the respirator face piece for fit testing shall be completely removed, and the face piece restored to NIOSH approved configuration, before that face piece can be used in the workplace.

Fit test records shall be retained for respirator users until the next fit test is administered. Written materials required to be retained shall be made available upon request to affected employees.

Respirator Operation and Use

Respirators will only be used following the respiratory protection safety procedures established in this program. The Operations and Use Manuals for each type of respirator will be maintained by the Program Administrator and be available to all qualified users. Surveillance by the direct supervisor shall be maintained of work area conditions and degree of employee exposure or stress. When there is a change in work area conditions or degree of employee exposure or stress that may affect respirator effectiveness, the Employer shall reevaluate the continued effectiveness of the respirator.

For continued protection of respirator users, the following general use rules apply:

- Users shall not remove respirators while in a hazardous environment .
- Respirators are to be stored in sealed containers out of harmful atmospheres.
- Store respirators away from heat and moisture.
- Store respirators such that the sealing area does not become distorted or warped
- Store respirators such that the face piece is protected.
- Face piece seal protection.

The Employer does not permit respirators with tight-fitting face pieces to be worn by employees who have:

- Facial hair that comes between the sealing surface of the face piece and the face or that interferes with valve function; or
- Any condition that interferes with the face-to-face piece seal or valve function.

If an employee wears corrective glasses or goggles or other personal protective equipment, the Employer shall ensure that such equipment is worn in a manner that does not interfere with the seal of the face piece to the face of the user.

Continuing Effectiveness of Respirators

The Employer shall ensure the following when employees leave the respirator use area:

- To wash their faces and respirator face pieces as necessary to prevent eye or skin irritation associated with respirator use.
- If they detect vapor or gas breakthrough, changes in breathing resistance, or leakage of the face piece.
- To replace the respirator or the filter, cartridge, or canister elements.

If the employee detects vapor or gas breakthrough, changes in breathing resistance, or leakage of the face piece, the Employer will replace or repair the respirator before allowing the employee to return to the work area.

Procedures for IDLH atmospheres

For all IDLH atmospheres, the Employer shall ensure that:

- One employee or, when needed, more than one employee is located outside the IDLH atmosphere.
- Visual, voice, or signal line communication is maintained between the employee(s) in the IDLH atmosphere and the employee(s) located outside the IDLH atmosphere.
- The employee(s) located outside the IDLH atmosphere are trained and equipped to provide effective emergency rescue.
- The Employer or designee is notified before the employee(s) located outside the IDLH atmosphere enter the IDLH atmosphere to provide emergency rescue.
- The Employer or designee authorized to do so by the Employer, once notified, provides necessary assistance appropriate to the situation.



Employee(s) located outside the IDLH atmospheres will be equipped with:

- Pressure demand or other positive pressure SCBAs, or a pressure demand or other positive pressure supplied-air respirator with auxiliary SCBA; and either
- Appropriate retrieval equipment for removing the employee(s) who enter(s) these hazardous atmospheres where retrieval equipment would contribute to the rescue of the employee(s) and would not increase the overall risk resulting from entry; or
- Equivalent means for rescue where retrieval equipment is not required.



A supervisor carefully checking his paperwork before allowing the Entrant in the Confined Space.

Gas and Vapor Contaminants

Gas and vapor contaminants can be classified according to their chemical characteristics. True gaseous contaminants are similar to air in that they possess the same ability to diffuse freely within an area or container. Nitrogen, chlorine, carbon monoxide, carbon dioxide and sulfur dioxide are examples.

Vapors are the gaseous state of substances that are liquids or solids at room temperature. They are formed when the solid or liquid evaporates. Gasoline, solvents and paint thinners are examples of liquids that evaporate easily, producing vapors.
In terms of chemical characteristics, gaseous contaminants may be classified as follows:

- **Inert Gases** —These include such true gases as helium, argon, neon, etc. Although they do not metabolize in the body, these gases represent a hazard because they can produce an oxygen deficiency by displacement of air.
- **Acidic Gases** —Often highly toxic, acidic gases exist as acids or produce acids by reaction with water. Sulfur dioxide, hydrogen sulfide and hydrogen chloride are examples.
- **Alkaline Gases** —These gases exist as alkalis or produce alkalis by reaction with water. Ammonia and phosphine are two examples.

In terms of chemical characteristics, vaporous contaminants may be classified as follows:

- **Organic Compounds** —Contaminants in this category can exist as true gases or vapors produced from organic liquids. Gasoline, solvents and paint thinners are examples.
- **Organometallic Compounds** —These are generally comprised of metals attached to organic groups. Tetraethyllead and organic phosphates are examples.

Hazard Assessment
Proper assessment of the hazard is the first important step to protection. This requires a thorough knowledge of processes, equipment, raw materials, end-products and by-products that can create an exposure hazard.

To determine an atmosphere's oxygen content or concentration levels of particulate and/or gaseous contaminants, air samples must be taken with proper sampling instruments during all conditions of operation. The sampling device and the type and frequency of sampling (spot testing or continuous monitoring) will be dictated by the exposure and operating conditions.

Breathing zone samples are recommended and sampling frequency should be sufficient to assess the average exposure under the variable operating and exposure conditions.
Should contaminant concentrations exceed exposure limits recommended by the American

Conference of Governmental Industrial Hygienists (**ACGIH**), OSHA or NIOSH, hazard control procedures must be implemented promptly.

Exposure monitoring plays a critical role in the respirator selection process. The results from such tests will help you determine whether respiratory protection is needed and, if it is, the type of respirator required.

Generally, respirator selection is based on three factors:

- The results of your atmospheric monitoring or sampling program;
- The accepted ACGIH, OSHA or NIOSH exposure limits for the substance(s) present;
- And the maximum use concentration (of a substance) for which a respirator can be used.

Exposure limits include ACGIH Threshold Limit Values (**TLVs**), OSHA Permissible Exposure Limits (**PELs**), NIOSH Recommended Exposure Levels (**RELs**) and AIHA Workplace Environmental Exposure Levels (**WEELs**). These values are guides for exposure concentrations that healthy individuals can normally tolerate for eight hours a day, five days a week without harmful effects. Unless otherwise noted, exposure limits are eight-hour, time-weighted-average (**TWA**) concentrations.

In general, gas and vapor exposure limits are expressed in ppm by volume (parts of contaminant per million parts of air), while particulate concentrations are expressed as mg/m^3 (milligrams of concentrations per cubic meter of air). For substances that can exist in more than one form (particulate or gaseous), concentrations are expressed in both values.

It is important to note that exposure limits and other exposure standards are constantly changing as more data is gathered about specific chemicals and substances. As such, you must be certain that you are using the most recent data when determining allowable exposure levels for employees.

Hazard Control
Hazard control should start at the process, equipment and plant design levels where contaminants can be effectively controlled at the outset. With operating processes, the problem becomes more difficult. In all cases, however, consideration should be given to the use of effective engineering controls to eliminate and/or reduce exposures to respiratory hazards.

This includes consideration of process encapsulation or isolation, use of less toxic materials in the process and suitable exhaust ventilation, filters and scrubbers to control the effluents.

Because it is sometimes not practical to maintain engineering controls that eliminate all airborne concentrations of contaminants, proper respiratory protective devices should be used whenever such protection is required.

Hazard Assessment or Hazard Certification sheet example is on the following page.
Even if you have a written RP Program and complete training records, OSHA will ask for a hazard certification or assessment form on where or why you need RP.

For example, if you were required to don SCBA to change a chlorine cylinder once a week, OSHA would request to see how that task was evaluated and certified.

HAZARD ASSESSMENT SURVEY DATA SHEET	
DATE OF EVALUATION:	**DATE LAST EVALUATED**: N/A
DEPARTMENT:	
DEPARTMENT:	**TELEPHONE:**
ADDRESS/LOCATION:	**NAME OF SECTION SUPERVISOR**:
Actions are required by: 29 CFR 1910.1200, 29 CFR 1910.146 and Town of Payson	**NAME OF HAZARD COMMUNICATION PROGRAM MONITOR:**
NON-ROUTINE POTENTIAL HAZARDS (Describe the Process) (Who, What, When, Where, How, Why) (Review SDS)	
EVALUATION (Your Findings/Discrepancies)	
CONTROLS (Existing or Recommended Protective Equipment, Engineering or Administrative Controls)	
Existing: **Recommended:**	
Surveyed By:	**Reviewed By:**



The revised Hazard Communication Standard specifically requires every hazardous chemical container to have a label. Labels are part of the company's Hazard Communication Program, which gives employees the Right to Understand about the chemical hazards they face on the job.

Each label will tell the worker

- ✓ ***What a chemical's identity is***
- ✓ ***Who made it***
- ✓ ***Why it's hazardous***
- ✓ ***How to protect himself or herself.***

Cleaning and Disinfecting *(See Appendix B for more information)*

The Employer shall provide each respirator user with a respirator that is clean, sanitary, and in good working order. The Employer shall ensure that respirators are cleaned and disinfected using the Standard Operating Procedure SOP: Cleaning and Disinfecting.

The Respirators Shall be Cleaned and Disinfected when:

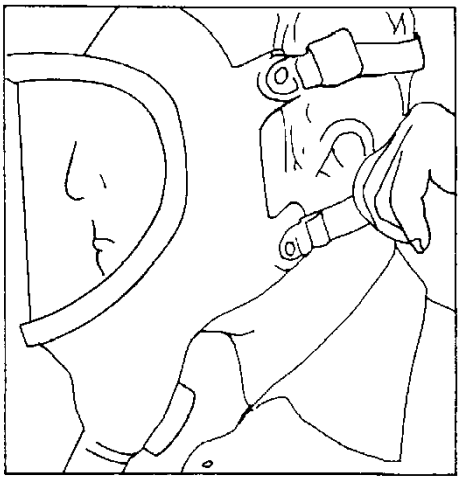
- Respirators issued for the exclusive use of an employee shall be cleaned and disinfected as often as necessary to be maintained in a sanitary condition.
- Respirators issued to more than one employee shall be cleaned and disinfected before being worn by different individuals.
- Respirators maintained for emergency use shall be cleaned and disinfected after each use.
- Respirators used in fit testing and training shall be cleaned and disinfected after each use.

Cleaning and Storage of respirators assigned to specific employees is the responsibility of that Employee.

Respirator Inspection

All respirators/SCBAs, both available for "**General Use**" and those on "**Permanent Check-out**", will be inspected after each use and at least monthly. Should any defects be noted, the respirator/SCBA will be taken to the program Administrator. Damaged Respirators will be either repaired or replaced. The inspection of respirators loaned on "**Permanent Check-out**" is the responsibility of that trained Employee.

Respirators Shall be Inspected as Follows:



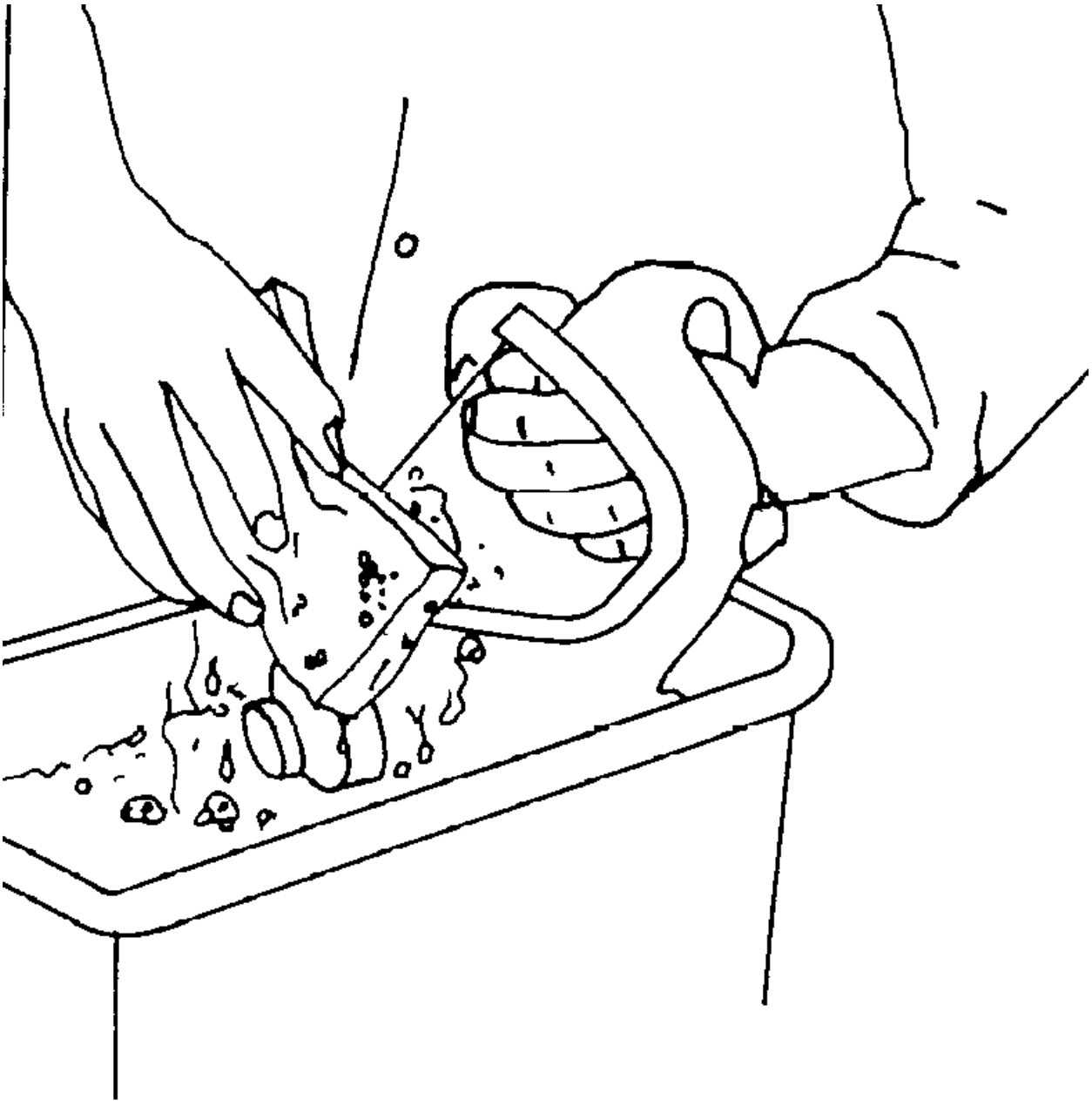
- All respirators used in routine situations shall be inspected before each use and during cleaning.
- All respirators maintained for use in emergency situations shall be inspected at least monthly and in accordance with the manufacturer's recommendations, and shall be checked for proper function before and after each use.
- Emergency escape-only respirators shall be inspected before being carried into the workplace for use.

Respirator Inspections Include the Following:

- A check of respirator function, tightness of connections, and the condition of the various parts including, but not limited to, the face piece, head straps, valves, connecting tube, and cartridges, canisters or filters.
- Check of elastomeric parts for pliability and signs of deterioration.
- Self-contained breathing apparatus shall be inspected monthly. Air and oxygen cylinders shall be maintained in a fully charged state and shall be recharged when the pressure falls to 90% of the manufacturer's recommended pressure level. The Employer shall determine that the regulator and warning devices function properly.

For Emergency Use Respirators the additional requirements apply:

- Certify the respirator by documenting the date the inspection was performed, the name (or signature) of the person who made the inspection, the findings, required remedial action, and a serial number or other means of identifying the inspected respirator.
- Provide this information on a tag or label that is attached to the storage compartment for the respirator, is kept with the respirator, or is included in inspection reports stored as paper or electronic files. This information shall be maintained until replaced following a subsequent certification.



Cleaning and Storage of respirators should be assigned to specific employees is the responsibility of that Employee.

Respirator Storage Section

Respirators are to be stored as follows:

- All respirators shall be stored to protect them from damage, contamination, dust, sunlight, extreme temperatures, excessive moisture, and damaging chemicals, and they shall be packed or stored to prevent deformation of the face piece and exhalation valve.

Emergency Respirators shall be:

- Kept accessible to the work area;
- Stored in compartments or in covers that are clearly marked as containing emergency respirators; and stored in accordance with any applicable manufacturer instructions.

Repair of Respirators

Respirators that fail an inspection or are otherwise found to be defective will be removed from service to be discarded, repaired or adjusted in accordance with the following procedures:

- Repairs or adjustments to respirators are to be made only by persons appropriately trained to perform such operations and shall use only the respirator manufacturer's NIOSH-approved parts designed for the respirator;
- Repairs shall be made according to the manufacturer's recommendations and specifications for the type and extent of repairs to be performed; and
- Reducing and admission valves, regulators, and alarms shall be adjusted or repaired only by the manufacturer or a technician trained by the manufacturer.

Breathing Air Quality and Use

The Employer shall ensure that compressed air, compressed oxygen, liquid air, and liquid oxygen used for respiration accords with the following specifications:

- Compressed and liquid oxygen shall meet the United States Pharmacopoeia requirements for medical or breathing oxygen; and
- Compressed breathing air shall meet at least the requirements for Grade D breathing air described in ANSI/Compressed Gas Association Commodity Specification for Air, G-7.1-1989, to include:
- Oxygen content (**v/v**) of 19.5-23.5%;
- Hydrocarbon (condensed) content of 5 milligrams per cubic meter of air or less;
- Carbon monoxide (**CO**) content of 10 ppm or less;
- Carbon dioxide content of 1,000 ppm or less; and
- Lack of noticeable odor.
- Compressed oxygen will not be used in atmosphere-supplying respirators that have previously used compressed air.
- Oxygen concentrations greater than 23.5% are used only in equipment designed for oxygen service or distribution.

Cylinders used to supply breathing air to respirators meet the following requirements.

- Cylinders are tested and maintained as prescribed in the Shipping Container Specification Regulations of the Department of Transportation (49 CFR part 173 and part 178).
- Cylinders of purchased breathing air have a certificate of analysis from the supplier that the breathing air meets the requirements for Grade D breathing air.
- Moisture content in breathing air cylinders does not exceed a dew point of -50 deg. F (-45.6 deg. C) at 1 atmosphere pressure.

- Breathing air couplings are incompatible with outlets for non-respirable worksite air or other gas systems. No asphyxiating substance shall be introduced into breathing air lines.
- Breathing gas containers shall be marked in accordance with the NIOSH respirator certification standard, 42 CFR part 84.

Summary

Following this training session, employees should

- Wear the respirator assigned to him or her.
- Always check for fit before wearing.
- Always check for damage and deterioration before wearing.
- Know when to replace canisters and cartridges.
- Practice maneuvering with a respirator.
- Store carefully in the proper location.



If you want to keep free from OSHA fines and possible employee death, rescue training and regular RP training is essential to your operation.

SCBA Section
SCBA (Self-Contained Breathing Apparatus)



The self-contained breathing apparatus that Firefighters carry on Fire trucks are literally the only thing that keeps Firefighters alive in smoke and fire situations. On the fireground, Firefighters face a wide variety of toxic gasses. These gases range from Carbon Monoxide, the largest killer, which combines with the blood 200 times easier than Oxygen and can be lethal at a concentration of only five hundredths of one percent, to phosgene, which reacts with the moisture in a person's lungs and forms hydrochloric acid. Besides toxic gases, SCBA's also protect Firefighters' lungs from being burned by the superheated air inside a burning structure. These temperatures can commonly reach over 1200 degrees Fahrenheit.

The SCBA mask provides protection from facial and respiratory burns, and holds in the cool breathing air. These masks are considered pressure demand breathing systems. This means that air is supplied only when the person wearing the mask inhales. Also, because the mask is pressurized, the mask will "**free-flow**", or flow air through any spaces between the mask and the wearer's face if the seal with the face is broken. This prevents any toxic gases from entering the mask if the seal is broken.







Firefighters will generally carry two types of air tanks on the trucks. The first type (white tank) is a low pressure tank. This tank will hold 2,216 PSI of compressed breathing air. The other type of tank (black) is a high pressure tank, which holds 4,500 PSI of air. The Captains are the only ones who use the high pressure tanks. Both air tanks are rated to last 30 minutes. However, under heavy exertion common in firefighting operations, these tanks will only last 20 minutes or less, depending on the person. The only difference in these two tanks is that the high pressure tanks are smaller and weigh less.



Donning Practice

SCBA and SCUBA Do's and Don'ts

Do...

- ✓ Do Visually inspect every cylinder at least once each year.
- ✓ Do perform a formal visual inspection of any SCBA wrapped cylinder in heavy use (5 or more fills per week) at least yearly.
- ✓ Do visually inspect all cylinders in accordance with the PSI 18 Step Protocol.
- ✓ Do hydrostatically retest SCUBA cylinders every 5 years.
- ✓ Do hydrostatically retest Composite SCBA cylinders every 3 years.
- ✓ Do fill cylinders slowly, at a rate of 300-600 psig per minute.
- ✓ Do rinse cylinders in fresh water after every day of diving giving particular attention to the cylinder valve and under the boot.
- ✓ Do store cylinders for prolonged periods secured in a vertical position with only about 50 psig pressure inside.
- ✓ Do transport cylinders so as to best protect the valve from damage.
- ✓ Do keep Evidence of Inspection (**EOI**) stickers in a secure location and away from unauthorized personnel.
- ✓ Do ensure documented training (e.g., PSI training) for all users, handlers and fillers of compressed gas cylinders (**OSHA requirement**).

Don'ts...

- ✓ Don't connect Titanium regulators to oxygen cylinders. The risk of explosion and fire is great.
  Don't fill cylinders beyond their stamped service pressure.
- ✓ Don't store Cylinders for a prolonged period with more that about 50 psig pressure.
- ✓ Don't charge cylinders cleaned and marked for EANx service with normal SCUBA air.
- ✓ Don't fill any cylinder that is beyond its visual inspection or hydrostatic test/retest period.
- ✓ Don't fill any cylinder that shows signs of excessive damage or mistreatment.
- ✓ Don't fill any cylinder with defects in excess of those established by CGA and/or PSI.
- ✓ Don't subject steel cylinder to temperatures in excess of 600^{o}F.
- ✓ Don't subject aluminum cylinders to temperatures in excess of 350^{o}F.
- ✓ Don't attempt to put a metrically threaded valve into an NGS threaded cylinder.
- ✓ Don't fill ANY cylinders marked **SP6688** or **SP6576**.
- ✓ Don't fill any cylinder that has internal noise or is abnormally heavy.
- ✓ Don't permit any untrained persons to visually inspect cylinders.
- ✓ Don't fill any composite cylinders which are beyond their 15 year service life (or five hydro test/retests + 3 years).
- ✓ Don't fill any cylinders with altered markings.

Use and Maintenance of Pressure-Demand

Self-contained Breathing Apparatus

Since July 1, 1983, the Occupational Safety and Health Administration (**OSHA**) Fire Brigade Standard, Title 29, Code of Federal Regulations, Part 1910.156, has required that pressure-demand or other positive pressure self-contained breathing apparatus be worn by fire brigade members performing interior structural firefighting.

Although this standard is only applicable to all industrial fire brigades and to municipal fire departments in states with state-OSHA plans, other fire service organizations and industrial users of self-contained breathing apparatus (**SCBA**) have also recognized the superior protective capabilities of positive-pressure SCBA. As a result, there has been a steady change from demand to pressure-demand SCBA in the United States.

To provide the increased respiratory protection afforded by pressure-demand SCBA, it is generally necessary to increase the static pressure within the facepiece. The complex mechanics necessary to maintain this increased pressure and to control air flow when the facepiece is removed, together with the wearer's physiological response to the pressure-demand system, have presented problems to SCBA users.

Pressure demand SCBA requires more careful maintenance and different training than is required for demand SCBA. Manufacturers have been providing maintenance and use instructions and training for purchasers of pressure-demand SCBA. The National Institute for Occupational Safety and Health (**NIOSH**) recommends that users of pressure-demand SCBA read those instructions, follow them carefully in apparatus use and maintenance, and take advantage of the manufacturer's training assistance. In addition to the manufacturers, training courses are offered by Fire Service organizations and by private organizations.



In the area of pressure-demand SCBA maintenance and repair, NIOSH strongly recommends that users have this service performed by a manufacturer-trained representative. This service is required to assure continued safe performance of pressure-demand SCBA.

Please advise NIOSH of any problems encountered in maintenance and use of pressure-demand self-contained breathing apparatus. Call the NIOSH Respirator Problem Coordinator, (304) 291-4595 (FTS 923-4595).

Reducing and admission valves, regulators, and alarms shall be adjusted or repaired only by the manufacturer or a technician trained by the manufacturer.

SCBA Donning

Each member of your department with emergency response duties shall be able to demonstrate a high level of proficiency with self-contained breathing apparatus (**SCBA**) under conditions comparable to those encountered in the performance of emergency work. Donning of the SCBA within a 35 second time limit is required of most members.

The following guidelines can be used when demonstrating compliance with the following donning policy:

A. The member will be wearing full protective clothing including helmet or Hard-hat. (The donning of gloves is not a part of the procedure that will be timed.)

B. Cylinder valve must be turned off prior to starting.

C. The SCBA will be donned from the ground. Timing will begin when the low pressure warning bell rings as the cylinder valve is opened

NOTE: Members have the option of donning their facepiece first. This option may be used only if the wearer's SCBA is stored with the regulator attached to the storage bracket on the waist belt. If this option is used, time will start at #10 below in the donning sequence.



D. Any air loss during the donning procedure will constitute a failure. Each member will be given three tries to successfully don their SCBA in 35 seconds or less.



E. The donning sequence is as follows:

1. Place SCBA on the ground, cylinder down, harness up, with cylinder valve away from body.



2. Open cylinder valve completely.

3. Grasp cylinder or harness at the center with both hands.

4. Lift SCBA above head - make sure straps are not tangled.

5. Bend forward at 45^{o} angle.

6. Lay SCBA on back, with arms inside shoulder straps.



7. Fasten chest strap.

8. Pull shoulder strap, adjusting tabs down as you straighten up.

9. Fasten waist belt.

10. Fully extend chin strap of helmet and slide helmet off head to the side, with chin strap around neck.

11. Remove facepiece from cover.

NOTE: If you do not store your regulator attached to the storage bracket, you may leave the regulator attached to your facepiece. The facepiece shall be stored in its protective pouch.



12. Don facepiece, chin first.

13. Adjust harness and the two neck straps.

14. Place helmet on head and tighten chin strap.

15. Quick connect the regulator to the facepiece and inhale sharply.





A different view of correct Donning SCBA.



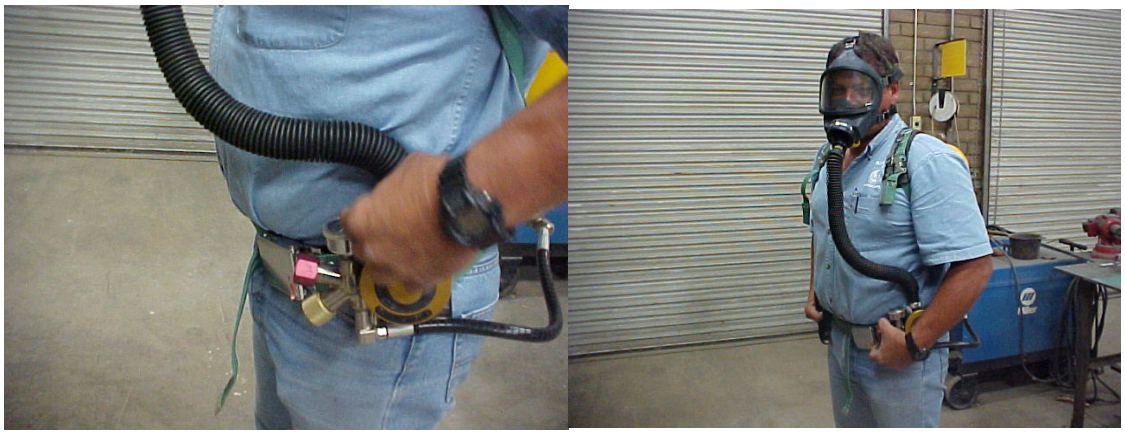
Fastening Waist belt and securing chest straps.



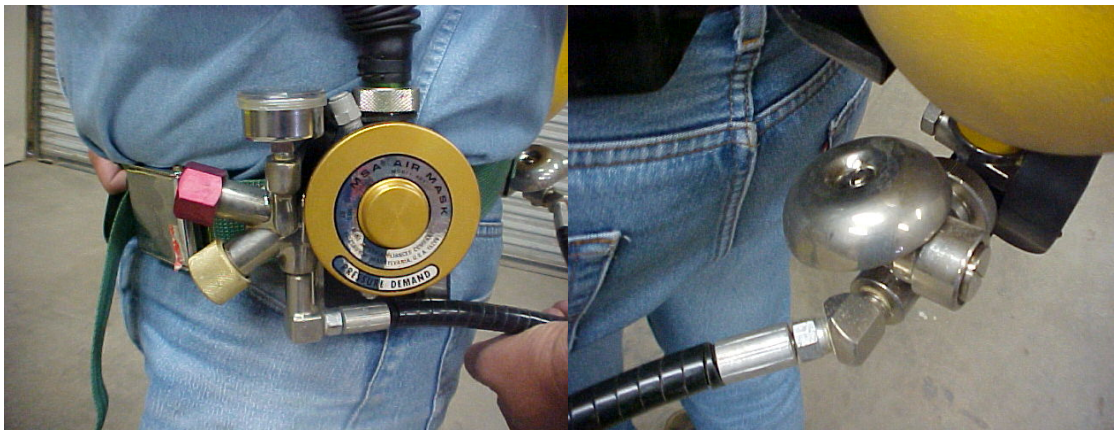
Grabbing face straps or spiders and putting on mask.



Drawing spiders tight and checking seal on facemask.



Connecting hose to regulator and turning on air on tank valve.



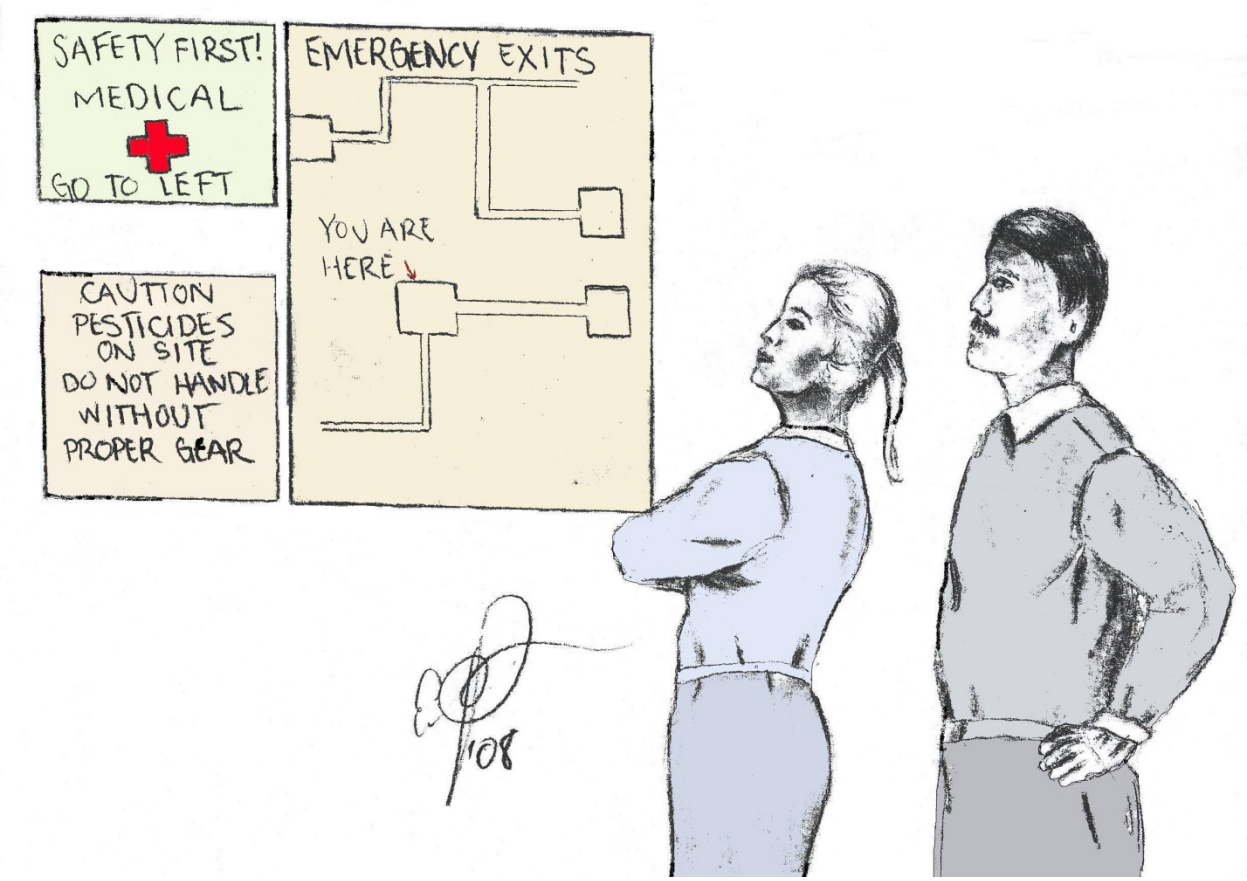
Regulator Valve and Warning Bell to let you know that the air is on and will ring again when the tank is getting low.

SCBA Spare Bottle Inspection Report Example
Inspect Monthly & After Each Use

Location: _____ **Date** _____

	Bottle Number			Inspector Initials
Cylinder: No dents or gouges, fits tightly in band				
Cylinder Hydro Test Date				
Gauge: Cylinder full, check gauge face, indicator				

If any defects are found DO NOT USE BOTTLE.
Turn in the Bottle to your supervisor immediately for repair or replacement.



Personal Protective Equipment Example Section

Purpose

Your Employer is required to provide all Employees with required PPE to suit the task and known hazards. This Chapter covers the requirements for Personal Protective Equipment with the exception of PPE used for respiratory protection or PPE required for hazardous material response to spills or releases. Applicable OSHA Standards are 1910 Subpart 1 App B and 1910.120 App B, 132, 133, 136, and 138.

General Rules Design

All personal protective equipment shall be of safe design and construction for the work to be performed.

Hazard Assessment and Equipment Selection

Hazard analysis procedures shall be used to assess the workplace to determine if hazards are present, or are likely to be present, which necessitate the use of personal protective equipment (PPE). If such hazards are present, or likely to be present, the following actions will be taken:

1) Select, and have each affected Employee use, the proper PPE.
2) Communicate selection decisions to each affected Employee.
3) Select PPE that properly fits each affected employee.

Defective and Damaged Equipment.

Defective or damaged personal protective equipment shall not be used.

Training

All Employees who are required to use PPE shall be trained to know at least the following:

1) When PPE is necessary;
2) What PPE is necessary;
3) How to properly don, remove, adjust, and wear PPE;
4) The limitations of the PPE
5) The proper care, maintenance, useful life and disposal of the PPE.

Each affected Employee shall demonstrate an understanding of the training and the ability to use PPE properly, before being allowed to perform work requiring the use of PPE.



Certification of training for PPE is required by OSHA and shall be accomplished by using the Job Safety Checklist to verify that each affected Employee has received and understood the required PPE training.

Personal Protective Equipment Selection

Controlling Hazards

PPE devices alone should not be relied on to provide protection against hazards, but should be used in conjunction with guards, engineering controls, and sound manufacturing practices.

Selection Guidelines

The general procedure for selection of protective equipment is to:

a) Become familiar with the potential hazards and the type of protective equipment that is available, and what it can do; i.e., splash protection, impact protection, etc.

b) Compare the hazards associated with the environment (i.e., impact velocities, masses, projectile shape, radiation intensities) with the capabilities of the available protective equipment;

c) Select the protective equipment which ensures a level of protection greater than the minimum required to protect employees from the hazards;

d) Fit the user with the protective device and give instructions on care and use of the PPE. It is very important that end users be made aware of all warning labels for and limitations of their PPE.

Fitting the Device

Careful consideration must be given to comfort and fit. PPE that fits poorly will not afford the necessary protection. Continued wearing of the device is more likely if it fits the wearer comfortably. Protective devices are generally available in a variety of sizes. Care should be taken to ensure that the right size is selected.

Devices with Adjustable Features

Adjustments should be made on an individual basis for a comfortable fit that will maintain the protective device in the proper position. Particular care should be taken in fitting devices for eye protection against dust and chemical splash to ensure that the devices are sealed to the face. In addition, proper fitting of helmets is important to ensure that it will not fall off during work operations.

In some cases a chin strap may be necessary to keep the helmet on an employee's head. (Chin straps should break at a reasonably low force, however, so as to prevent a strangulation hazard). Where manufacturer's instructions are available, they should be followed carefully.

Eye and Face Protection

Each affected employee shall use appropriate eye or face protection when exposed to eye or face hazards from flying particles, molten metal, liquid chemicals, acids or caustic liquids, chemical gases or vapors, or potentially injurious light radiation.

Each affected employee shall use eye protection that provides side protection when there is a hazard from flying objects. Detachable side protectors are acceptable.

Each affected employee who wears prescription lenses while engaged in operations that involve eye hazards shall wear eye protection that incorporates the prescription in its design, or shall wear eye protection that can be worn over the prescription lenses without disturbing the proper position of the prescription lenses or the protective lenses.

Eye and face PPE shall be distinctly marked to facilitate identification of the manufacturer.

Each affected employee shall use equipment with filter lenses that have a shade number appropriate for the work being performed for protection from injurious light radiation. The following is a listing of appropriate shade numbers for various operations.

Filter Lenses for Protection Against Radiant Energy			
Operations	**Electrode Size 1/32 in**	**Arc Current**	**Protective Shade**
Shielded metal arc welding	Less than 3	Less than 60	7
	3-5	60-160	8
	5-8	160-250	10
	More than 8	250-550	11
Torch brazing			3
Torch soldering			2
Note: as a rule of thumb, start with a shade that is too dark to see the weld zone. Then go to a lighter shade which gives sufficient view of the weld zone without going below the minimum. In oxyfuel gas welding or cutting where the torch produces a high yellow light, it is desirable to use a filter lens that absorbs the yellow or sodium line in the visible light of the (spectrum) operation.			



Always utilize a chemical fume hood and ventilation system when working or testing Chlorine.

Selection Chart Guidelines for Eye and Face Protection		
The following chart provides general guidance for the proper selection of eye and face protection to protect against hazards associated with the listed hazard "source" operations.		
Source	**Hazard**	**Protection**
IMPACT - Chipping, grinding machining, masonry work, woodworking, sawing, drilling, chiseling, powered fastening, riveting, and sanding	Flying fragments, objects, large chips, particles, sand, dirt, etc.	Spectacles with side protection, goggles, face shield For severe exposure, use face shield
HEAT - Furnace operation and arc welding	Hot sparks	Face shields, spectacles with side. For severe exposure use faceshield.
CHEMICALS - Acid and chemical handling, degreasing, plating	Splash	Goggles, eyecup and cover types. For severe exposure, use face shield.
DUST - Woodworking, buffing, general, buffing, general dusty conditions.	Nuisance dust	Goggles, eye cup and cover type







Selection Guidelines for Head Protection

All head protection is designed to provide protection from impact and penetration hazards caused by falling objects. Head protection is also available which provides protection from electric shock and burn.

When selecting head protection, knowledge of potential electrical hazards is important.

Class A helmets, in addition to impact and penetration resistance, provide electrical protection from low-voltage conductors (they are proof tested to 2,200 volts).

Class B helmets, in addition to impact and penetration resistance; provide electrical protection from high-voltage conductors (they are proof tested to 20,000 volts).

Class C helmets provide impact and penetration resistance (they are usually made of aluminum which conducts electricity), and should not be used around electrical hazards.

Where falling object hazards are present, helmets must be worn. Some examples include: working below other workers who are using tools and materials which could fall; working around or under conveyor belts which are carrying parts or materials; working below machinery or processes which might cause material or objects to fall; and working on exposed energized conductors.

Foot Protection Section

General Requirements
Each affected employee shall wear protective footwear when working in areas where there is a danger of foot injuries due to falling or rolling objects, or objects piercing the sole, and where employee's feet are exposed to electrical hazards.

Selection Guidelines for Foot Protection
Safety shoes and boots provide both impact and compression protection. Where necessary, safety shoes can be obtained which provide puncture protection. In some work situations, metatarsal protection should be provided, and in other special situations electrical conductive or insulating safety shoes would be appropriate.

Safety shoes or boots with impact protection would be required for carrying or handling materials such as packages, objects, parts or heavy tools, which could be dropped; and, for other activities where objects might fall onto the feet.

Safety shoes or boots with compression protection would be required for work activities involving skid trucks (manual material handling carts) around bulk rolls (such as paper rolls) and around heavy pipes, all of which could potentially roll over an employee's feet.

Safety shoes or boots with puncture protection would be required where sharp objects such as nails, wire, tacks, screws, large staples, scrap metal etc., could be stepped on by employees causing a foot injury.

Hand Protection

General Requirements
Hand protection is required when employees' hands are exposed to hazards such as those from skin absorption of harmful substances; severe cuts or lacerations; severe abrasions; punctures; chemical or thermal burns; and harmful temperature extremes.

Selection Guidelines for Hand Protection
Selection of hand PPE shall be based on an evaluation of the performance characteristics of the hand protection relative to the task(s) to be performed, conditions present, duration of use, and the hazards and potential hazards identified. Gloves are often relied upon to prevent cuts, abrasions, burns, and skin contact with chemicals that are capable of causing local or systemic effects following dermal exposure.



There is no glove that provides protection against all potential hand hazards, and commonly available glove materials provide only limited protection against many chemicals. Therefore, it is important to select the most appropriate glove for a particular application and to determine how long it can be worn and whether it can be reused.

It is also important to know the performance characteristics of gloves relative to the specific hazard anticipated; e.g., chemical hazards, cut hazards, flame hazards, etc. Before purchasing gloves, request documentation from the manufacturer that the gloves meet the appropriate test standard(s) for the hazard(s) anticipated.

Other Factors to be Considered for Glove Selection in General Include:

(A) As long as the performance characteristics are acceptable, in certain circumstances it may be more cost effective to regularly change cheaper gloves than to reuse more expensive types.
(B) The work activities of the employee should be studied to determine the degree of dexterity required, the duration, frequency, and degree of exposure of the hazard, and the physical stresses that will be applied.

Selection of Gloves for Protection Against Chemical Hazards:

(A) The toxic properties of the chemical(s) must be determined; in particular, the ability of the chemical to cause local effects on the skin and/or to pass through the skin and cause systemic effects.

(B) Generally, any "**chemical resistant**" glove can be used for dry powders;

(C) For mixtures and formulated products (unless specific test data are available), a glove should be selected on the basis of the chemical component with the shortest breakthrough time, since it is possible for solvents to carry active ingredients through polymeric materials.

(D) Employees must be able to remove the gloves in such a manner as to prevent skin contamination.

Protective Clothing Applications

A. The purpose of chemical protective clothing and equipment is to shield or isolate individuals from the chemical, physical, and biological hazards that may be encountered during hazardous materials operations. During chemical operations, it is not always apparent when exposure occurs. Many chemicals pose invisible hazards and offer no warning properties.

B. These guidelines describe the various types of clothing that are appropriate for use in various chemical operations, and provides recommendations in their selection and use. The final paragraph discusses heat stress and other key physiological factors that must be considered in connection with protective clothing use.

C. It is important that protective clothing users realize that no single combination of protective equipment and clothing is capable of protecting you against all hazards. Thus protective clothing should be used in conjunction with other protective methods. For example, engineering or administrative controls to limit chemical contact with personnel should always be considered as an alternative measure for preventing chemical exposure.

The use of protective clothing can itself create significant wearer hazards, such as heat stress, physical and psychological stress, in addition to impaired vision, mobility, and communication. In general, the greater the level of chemical protective clothing, the greater the associated risks. For any given situation, equipment and clothing should be selected that provide an adequate level of protection. Overprotection as well as under-protection can be hazardous and should be avoided.

Protective Clothing Applications

1. Protective clothing must be worn whenever the wearer faces potential hazards arising from chemical exposure. Some examples include:

- Emergency response;
- Chemical manufacturing and process industries;
- Hazardous waste site cleanup and disposal;
- Asbestos removal and other particulate operations; and
- Agricultural application of pesticides.

2. Within each application, there are several operations which require chemical protective clothing. For example, in emergency response, the following activities dictate chemical protective clothing use:

- ✓ **Site Survey**: The initial investigation of a hazardous materials incident; these situations are usually characterized by a large degree of uncertainty and mandate the highest levels of protection.

- ✓ **Rescue**: Entering a hazardous materials area for the purpose of removing an exposure victim; special considerations must be given to how the selected protective clothing may affect the ability of the wearer to carry out the rescue and to the contamination of the victim.

- ✓ **Spill Mitigation:** Entering a hazardous materials area to prevent a potential spill or to reduce the hazards from an existing spill (i.e., applying a chlorine kit on railroad tank car). Protective clothing must accommodate the required tasks without sacrificing adequate protection.

- ✓ **Emergency Monitoring**: Outfitting personnel in protective clothing for the primary purpose of observing a hazardous materials incident without entry into the spill site. This may be applied to monitoring contract activity for spill cleanup.

- ✓ **Decontamination:** Applying decontamination procedures to personnel or equipment leaving the site; in general a lower level of protective clothing is used by personnel involved in decontamination.

The Clothing Ensemble. The approach in selecting personal protective clothing must encompass an "**ensemble**" of clothing and equipment items which are easily integrated to provide both an appropriate level of protection and still allow one to carry out activities involving chemicals.

In many cases, simple protective clothing by itself may be sufficient to prevent chemical exposure, such as wearing gloves in combination with a splash apron and faceshield (or safety goggles).

1. The following is a checklist of components that may form the chemical protective ensemble:
   - **Protective clothing (suit, coveralls, hoods, gloves, boots);**
   - **Respiratory equipment (SCBA, combination SCBA/SAR, air purifying respirators);**
   - **Cooling system (ice vest, air circulation, water circulation);**
   - **Communications device;**
   - **Head protection;**
   - **Eye protection;**
   - **Ear protection;**
   - **Inner garment; and**
   - **Outer protection (overgloves, overboots, flashcover).**

2. **Factors that affect the selection of ensemble components include:**

- ✓ How each item accommodates the integration of other ensemble components. Some ensemble components may be incompatible due to how they are worn (e.g., some SCBA's may not fit within a particular chemical protective suit or allow acceptable mobility when worn).

- ✓ The ease of interfacing ensemble components without sacrificing required performance (e.g. a poorly fitting overglove that greatly reduces wearer dexterity).

- ✓ Limiting the number of equipment items to reduce donning time and complexity (e.g. some communications devices are built into SCBA's which as a unit are NIOSH certified).

Level of Protection

1. Table VIII:1-1 lists ensemble components based on the widely used EPA Levels of Protection: Levels A, B, C, and D. These lists can be used as the starting point for ensemble creation; however, each ensemble must be tailored to the specific situation in order to provide the most appropriate level of protection.

For example, if an emergency response activity involves a highly contaminated area or if the potential of contamination is high, it may be advisable to wear a disposable covering such as Tyvek coveralls or PVC splash suits, over the protective ensemble.

TABLE VIII:1-1. EPA's Levels of Protection

Level A:

Vapor protective suit (meets NFPA 1991)
Pressure-demand, full-face SCBA
Inner chemical-resistant gloves, chemical-resistant safety boots, two-way radio communication
Optional: Cooling system, outer gloves, hard hat
Protection Provided: Highest available level of respiratory, skin, and eye protection from solid, liquid and gaseous chemicals.
Used When: The chemical(s) have been identified and have high level of hazards to respiratory system, skin and eyes. Substances are present with known or suspected skin toxicity or carcinogenity. Operations must be conducted in confined or poorly ventilated areas.
Limitations: Protective clothing must resist permeation by the chemical or mixtures present. Ensemble items must allow integration without loss of performance.

Level B:

Liquid splash-protective suit (meets NFPA 1992)
Pressure-demand, full-facepiece SCBA
Inner chemical-resistant gloves, chemical-resistant safety boots, two-way radio communications and Hard hat.
Optional:: Cooling system, outer gloves
Protection Provided: Provides same level of respiratory protection as Level A, but less skin protection. Liquid splash protection, but no protection against chemical vapors or gases.
Used When: The chemical(s) have been identified but do not require a high level of skin protection. Initial site surveys are required until higher levels of hazards are identified. The primary hazards associated with site entry are from liquid and not vapor contact.
Limitations: Protective clothing items must resist penetration by the chemicals or mixtures present. Ensemble items must allow integration without loss of performance.

Level C:

Support Function Protective Garment (meets NFPA 1993)
Full-facepiece, air-purifying, canister-equipped respirator
Chemical resistant gloves and safety boots
Two-way communications system, hard hat
Optional: Faceshield, escape SCBA
Protection Provided: The same level of skin protection as Level B, but a lower level of respiratory protection. Liquid splash protection but no protection against chemical vapors or gases.
Used When: Contact with site chemical(s) will not affect the skin. Air contaminants have been identified and concentrations measured. A canister is available which can remove the contaminant. The site and its hazards have been completely characterized.

Limitations: Protective clothing items must resist penetration by the chemical or mixtures present. Chemical airborne concentration must be less than IDLH levels. The atmosphere must contain at least 19.5% oxygen.
Not Acceptable for Chemical Emergency Response

Level D:
Coveralls, safety boots/shoes, safety glasses or chemical splash goggles
Optional: Gloves, escape SCBA, face-shield
Protection Provided: No respiratory protection, minimal skin protection.
Used When: The atmosphere contains no known hazard. Work functions preclude splashes, immersion, potential for inhalation, or direct contact with hazard chemicals.
Limitations: This level should not be worn in the Hot Zone. The atmosphere must contain at least 19.5% oxygen.
Not Acceptable for Chemical Emergency Response

2. The type of equipment used and the overall level of protection should be reevaluated periodically as the amount of information about the chemical situation or process increases, and when workers are required to perform different tasks. Personnel should upgrade or downgrade their level of protection only with concurrence with the site supervisor, safety officer, or plant industrial hygienist.

3. The recommendations in Table VIII:1-1 serve only as guidelines. It is important for you to realize that selecting items by how they are designed or configured alone is not sufficient to ensure adequate protection. In other words, just having the right components to form an ensemble is not enough. The EPA levels of protection do not define what performance the selected clothing or equipment must offer. Many of these considerations are described in the ***"limiting criteria***" column of Table VIII: 1-1. Additional factors relevant to the various clothing and equipment items are described in subsequent Paragraphs.

Ensemble Selection Factors.
1. **Chemical Hazards**. Chemicals present a variety of hazards such as toxicity, corrosiveness, flammability, reactivity, and oxygen deficiency. Depending on the chemicals present, any combination of hazards may exist.

2. **Physical Environment.** Chemical exposure can happen anywhere: in industrial settings, on the highways, or in residential areas.

It may occur either indoors or outdoors; the environment may be extremely hot, cold, or moderate; the exposure site may be relatively uncluttered or rugged, presenting a number of physical hazards; chemical handling activities may involve entering confined spaces, heavy lifting, climbing a ladder, or crawling on the ground. The choice of ensemble components must account for these conditions.

3. **Duration of Exposure**. The protective qualities of ensemble components may be limited to certain exposure levels (e.g. material chemical resistance, air supply). The decision for ensemble use time must be made assuming the worst case exposure so that safety margins can be applied to increase the protection available to the worker.

4. **Protective Clothing or Equipment Available.** Hopefully, an array of different clothing or equipment is available to workers to meet all intended applications. Reliance on one particular clothing or equipment item may severely limit a facility's ability to handle a broad range of chemical exposures. In its acquisition of equipment and clothing, the safety department or other responsible authority should attempt to provide a high degree of flexibility while choosing protective clothing and equipment that is easily integrated and provides protection against each conceivable hazard.

Classification of Protective Clothing.
Personal protective clothing includes the following:
* Fully encapsulating suits;
* Nonencapsulating suits;
* Gloves, boots, and hoods;
* Firefighter's protective clothing;
* Proximity, or approach clothing;
* Blast or fragmentation suits; and
* Radiation-protective suits.

1. Firefighter turnout clothing, proximity gear, blast suits, and radiation suits by themselves are not acceptable for providing adequate protection from hazardous chemicals.

2. Table VIII:1-2 describes various types of protection clothing available, details the type of protection they offer, and lists factors to consider in their selection and use.

TABLE VIII: 1-2. Types of Protective Clothing for Full Body Protection

One-piece garment. Boots and gloves may be integral, attached and replaceable, or separate.
- Protects against splashes, dust gases, and vapors.

- Does not allow body heat to escape. May contribute to heat stress in wearer, particularly if worn in conjunction with a closed-circuit SCBA; a cooling garment may be needed. Impairs worker mobility, vision, and communication.
  Nonencapsulating suit

Jacket, hood, pants or bib overalls, and one-piece coveralls.
Protects against splashes, dust, and other materials but not against gases and vapors. Does not protect parts of head or neck.

- Do not use where gas-tight or pervasive splashing protection is required. May contribute to heat stress in wearer. Tape-seal connections between pant cuffs and boots and between gloves and sleeves.

Aprons, leggings, and sleeve protectors
- Fully sleeved and gloved apron. Separate coverings for arms and legs. Commonly worn over nonencapsulating suit.

- Provides additional splash protection of chest, forearms, and legs.

- Whenever possible, should be used over a non-encapsulating suit to minimize potential heat stress. Useful for sampling, labeling, and analysis operations. Should be used only when there is a low probability of total body contact with contaminants.

Firefighters' Protective Clothing

- Gloves, helmet, running or bunker coat, running or bunker pants (NFPA No. 1971, 1972, 1973, and boots (1974).

- Protects against heat, hot water, and some particles. Does not protect against gases and vapors, or chemical permeation or degradation. NFPA Standard No. 1971 specifies that a garment consists of an outer shell, an inner liner and a vapor barrier with a minimum water penetration of 25 lb/in2 (1.8 kg/cm2) to prevent passage of hot water.

- Decontamination is difficult. Should not be worn in areas where protection against gases, vapors, chemical splashes or permeation is required.

Proximity Garment (Approach Suit)

- One- or two-piece overgarment with boot covers, gloves, and hood of aluminized nylon or cotton fabric. Normally worn over other protective clothing, firefighters' bunker gear, or flame-retardant coveralls.

- Protects against splashes, dust, gases, and vapors.

- Does not allow body heat to escape. May contribute to heat stress in wearer, particularly if worn in conjunction with a closed-circuit SCBA; a cooling garment may be needed. Impairs worker mobility, vision, and communication.

Blast and Fragmentation Suit

- Blast and fragmentation vests and clothing, bomb blankets, and bomb carriers.

- Provides some protection against very small detonations. Bomb blankets and baskets can help redirect a blast.

- Does not provide for hearing protection.

Radiation-Contamination Protective Suit

- Various types of protective clothing designed to prevent contamination of the body by radioactive particles.

- Protects against alpha and beta particles. Does not protect against gamma radiation.

- Designed to prevent skin contamination. If radiation is detected on site, consult an experienced radiation expert and evacuate personnel until the radiation hazard has been evaluated.

Flame/Fire Retardant Coveralls.

- Normally worn as an undergarment.

- Provides protection from flash fires; adds bulk and may exacerbate heat stress problems and impair mobility.

Glossary of Respiratory Protection Terms

The following definitions are important terms used in the respiratory protection standard and terms that will assist in the understanding and the application of the NIOSH decision logic.

Air-Purifying Respirator: A respirator with an air-purifying filter, cartridge, or canister that removes specific air contaminants by passing ambient air through the air-purifying element. OSHA Definition

Assigned Protection Factor (**APF**): See ***PROTECTION FACTOR***. NIOSH Definition

Atmosphere-Supplying Respirator: A respirator that supplies the respirator user with breathing air from a source independent of the ambient atmosphere, and includes supplied-air respirators (SARs) and self-contained breathing apparatus (SCBA) units. OSHA Definition

Breakthrough: The penetration of challenge material(s) through a gas or a vapor air-purifying element. The quantity or extent of breakthrough during service life testing is often referred to as the percentage of the input concentration. NIOSH Definition

Canister or Cartridge: A container with a filter, sorbent, or catalyst, or combination of these items, which removes specific contaminants from the air passed through the container. OSHA Definition

Demand Respirator: An atmosphere-supplying respirator that admits breathing air to the facepiece only when a negative pressure is created inside the facepiece by inhalation. OSHA Definition

Disposable Respirators: A respirator that is discarded after the end of its recommended period of use, after excessive resistance or physical damage, or when odor breakthrough or other warning indicators render the respirator unsuitable for further use. NIOSH Definition

Dust: A solid, mechanically produced particle with a size ranging from submicroscopic to macroscopic. NIOSH Definition

Emergency Respirator Use Situation: A situation that requires the use of respirators due to the unplanned generation of a hazardous atmosphere (often of unknown composition) caused by an accident, mechanical failure, or other means and that requires evacuation of personnel or immediate entry for rescue or corrective action. NIOSH Definition

Emergency Situation: Any occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment that may or does result in an uncontrolled significant release of an airborne contaminant. OSHA Definition

Employee Exposure: Exposure to a concentration of an airborne contaminant that would occur if the employee were not using respiratory protection. OSHA Definition

End-Of-Service-Life Indicator (**ESLI**): A system that warns the respirator user of the approach of the end of adequate respiratory protection; for example, that the sorbent is approaching saturation or is no longer effective. OSHA Definition

Escape Gas Mask: A gas mask that consists of a half-mask facepiece or mouthpiece, a canister, and associated connections, and that is designed for use during escape-only from hazardous atmospheres. NIOSH Definition

Escape Only Respirator: Respiratory devices that are designed for use only during escape from hazardous atmospheres. NIOSH Definition

Escape-Only Respirator: A respirator intended to be used only for emergency exit. OSHA Definition

Filter or Air-Purifying Element: A component used in respirators to remove solid or liquid aerosols from the inspired air. OSHA Definition

Filtering Facepiece: A particulate respirator with a filter as an integral part of the facepiece or with the entire facepiece composed of the filtering medium. (See ***SINGLE-USE DUST or DUST and MIST RESPIRATORS and DISPOSABLE RESPIRATORS***.) NIOSH Definition

Filtering Facepiece (Dust Mask): A negative pressure particulate respirator with a filter as an integral part of the facepiece or with the entire facepiece composed of the filtering medium. OSHA Definition

Fit Factor: A quantitative measure of the fit of a specific respirator facepiece to a particular individual. NIOSH Definition

Fit Factor: A quantitative estimate of the fit of a particular respirator to a specific individual, and typically estimates the ratio of the concentration of a substance in ambient air to its concentration inside the respirator when worn. OSHA Definition

Fit Test: Means the use of a protocol to qualitatively or quantitatively evaluate the fit of a respirator on an individual. (See also Qualitative fit test QLFT and Quantitative fit test QNFT.) OSHA Definition

Fume: A solid condensation particulate, usually of a vaporized metal. NIOSH Definition

Gas: An aeriform fluid that is in a gaseous state at standard temperature and pressure. NIOSH Definition

Helmet: A rigid respiratory inlet covering that also provides head protection against impact and penetration. OSHA Definition

High-Efficiency Particulate Air (Hepa) Filter: A filter that is at least 99.97% efficient in removing monodisperse particles of 0.3 micrometers in diameter. The equivalent NIOSH 42 CFR 84 particulate filters are the N100, R100, and P100 filters. OSHA Definition

Hood: Means a respiratory inlet covering that completely covers the head and neck and may also cover portions of the shoulders and torso. OSHA Definition

Immediately Dangerous to Life or Health (IDLH): Acute respiratory exposure that poses an immediate threat of loss of life, immediate or delayed irreversible adverse effects on health, or acute eye exposure that would prevent escape from a hazardous atmosphere. NIOSH Definition

Immediately Dangerous to Life or Health (**IDLH**): An atmosphere that poses an immediate threat to life, would cause irreversible adverse health effects, or would impair an individual's ability to escape from a dangerous atmosphere. OSHA Definition

Interior Structural Firefighting: The physical activity of fire suppression, rescue or both, inside of buildings or enclosed structures which are involved in a fire situation beyond the incipient stage. (See 29 CFR 1910.155) OSHA Definition

Loose-Fitting Facepiece: A respiratory inlet covering that is designed to form a partial seal with the face. OSHA Definition

Maximum Use Concentration (MUC): [Reserved] OSHA Definition

Mist: A liquid condensation particulate. NIOSH Definition

Negative Pressure Respirator (Tight Fitting): A respirator in which the air pressure inside the facepiece is negative during inhalation with respect to the ambient air pressure outside the respirator. OSHA Definition

Orinasal Respirator: A respirator that covers the nose and mouth and that generally consists of a quarter- or half-facepiece. NIOSH Definition

Oxygen Deficient Atmosphere: An atmosphere with an oxygen content below 19.5% by volume. OSHA Definition

Physician or Other Licensed Health Care Professional (PLHCP): Means an individual whose legally permitted scope of practice (i.e., license, registration, or certification) allows him or her to independently provide, or be delegated the responsibility to provide, some or all of the health care services required by paragraph (e) of this section. OSHA Definition

Planned or Unplanned Entry into an IDLH Environment, an Environment of Unknown Concentration of Hazardous Contaminant, or an Environment of Unknown Composition: A situation in which respiratory devices are recommended to provide adequate protection to workers entering an area where the contaminant concentration is above the IDLH or is unknown. NIOSH Definition

Positive Pressure Respirator: A respirator in which the pressure inside the respiratory inlet covering exceeds the ambient air pressure outside the respirator. OSHA Definition

Potential Occupational Carcinogen: Any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory, or dermal exposure, or any other exposure which results in the induction of tumors at a site other than the site of administration. This definition also includes any substance that is metabolized into one or more potential occupational carcinogens by mammals (29 CFR 1990.103, OSHA Cancer Policy). NIOSH Definition

Powered Air-Purifying Respirator (PAPR): An air-purifying respirator that uses a blower to force the ambient air through air-purifying elements to the inlet covering. OSHA Definition

Pressure Demand Respirator: A positive pressure atmosphere-supplying respirator that admits breathing air to the facepiece when the positive pressure is reduced inside the facepiece by inhalation. OSHA Definition

Protection Factors: NIOSH Definition

Assigned Protection Factor (APF): The minimum anticipated protection provided by a properly functioning respirator or class of respirators to a given percentage of properly fitted and trained users.

Simulated Workplace Protection Factor (SWPF): A surrogate measure of the workplace protection provided by a respirator.

Workplace Protection Factor (WPF): A measure of the protection provided in the workplace by a properly functioning respirator when correctly worn and used.

Qualitative Fit Test (QLFT): A pass/fail fit test to assess the adequacy of respirator fit that relies on the individual's response to the test agent. OSHA Definition

Quantitative Fit Test (QNFT): Means an assessment of the adequacy of respirator fit by numerically measuring the amount of leakage into the respirator. OSHA Definition

Recommended Exposure Limit (REL): An 8- or 10-hour time-weighted average (TWA) or ceiling (C) exposure concentration recommended by NIOSH that is based on an evaluation of the health effects data. NIOSH Definition

Respiratory Inlet Covering: The portion of a respirator that forms the protective barrier between the user's respiratory tract and an air-purifying device or breathing air source, or both. It may be a facepiece, a helmet, a hood, a suit, or a mouthpiece respirator with nose clamp. OSHA Definition

Self-Contained Breathing Apparatus (SCBA): An atmosphere-supplying respirator for which the breathing air source is designed to be carried by the user. OSHA Definition

Service Life: The length of time required for an air-purifying element to reach a specific effluent concentration. Service life is determined by the type of substance being removed, the concentration of the substance, the ambient temperature, the specific element being tested (cartridge or canister), the flow rate resistance, and the selected breakthrough value. The service life for a self-contained breathing apparatus (SCBA) is the period of time, as determined by the NIOSH certification tests, in which adequate breathing gas is supplied. NIOSH Definition

Service Life: The period of time that a respirator, filter or sorbent, or other respiratory equipment provides adequate protection to the wearer. OSHA Definition

Single-Use Dust or Dust and Mist Respirators: Respirators approved for use against dusts or mists that may cause pneumoconiosis and fibrosis. NIOSH Definition

Supplied-Air Respirator (SAR) or Airline Respirator: An atmosphere-supplying respirator for which the source of breathing air is not designed to be carried by the user. OSHA Definition

This Section: This respiratory protection standard. OSHA Definition

Tight-Fitting Facepiece: A respiratory inlet covering that forms a complete seal with the face. OSHA Definition

User Seal Check: An action conducted by the respirator user to determine if the respirator is properly seated to the face. OSHA Definition

Vapor: The gaseous state of a substance that is solid or liquid at temperatures and pressures normally encountered. NIOSH Definition



Exhaust Ventilation

- Use exhaust ventilation to capture dust/fumes whenever possible;
- HEPA vacuum dust covered work surfaces; dry sweeping or blowing is prohibited; wet methods may be used;
- Do not eat, drink, smoke or apply cosmetics in areas where lead is present;
- Wash hands and face after working with toxic materials.



Here are a few photographs from one of TLC's hands on chlorine class. Instructor Jeff Durbin is shown in top photograph.



§ 1910.134 Respiratory Protection Rule

[PUBLISHER'S NOTE: This section was added at 63 FR 1152, 1270, Jan. 8, 1998, effective April 8, 1998. Paragraphs (c), (e)(3) through (e)(6), (f)(1), (i)(4), (m)(1) through (m)(2) and (m)(4) contain information collection requirements and will not become effective until the Office of Management and Budget approves them. A document will be published in the Federal Register once approval has been obtained.]

This section applies to General Industry (part 1910), Shipyards (part 1915), Marine Terminals (part 1917), Longshoring (part 1918), and Construction (part 1926).

(a) Permissible practice. (1) In the control of those occupational diseases caused by breathing air contaminated with harmful dusts, fogs, fumes, mists, gases, smokes, sprays, or vapors, the primary objective shall be to prevent atmospheric contamination. This shall be accomplished as far as feasible by accepted engineering control measures (for example, enclosure or confinement of the operation, general and local ventilation, and substitution of less toxic materials). When effective engineering controls are not feasible, or while they are being instituted, appropriate respirators shall be used pursuant to this section.

(2) Respirators shall be provided by the employer when such equipment is necessary to protect the health of the employee. The employer shall provide the respirators which are applicable and suitable for the purpose intended. The employer shall be responsible for the establishment and maintenance of a respiratory protection program which shall include the requirements outlined in paragraph (c) of this section.

(b) Definitions. The following definitions are important terms used in the respiratory protection standard in this section.

Air-purifying respirator means a respirator with an air-purifying filter, cartridge, or canister that removes specific air contaminants by passing ambient air through the air-purifying element.

Assigned protection factor (APF) [Reserved]

Atmosphere-supplying respirator means a respirator that supplies the respirator user with breathing air from a source independent of the ambient atmosphere, and includes supplied-air respirators (SARs) and self-contained breathing apparatus (SCBA) units.

Canister or cartridge means a container with a filter, sorbent, or catalyst, or combination of these items, which removes specific contaminants from the air passed through the container.

Demand respirator means an atmosphere-supplying respirator that admits breathing air to the facepiece only when a negative pressure is created inside the facepiece by inhalation.

Emergency situation means any occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment that may or does result in an uncontrolled significant release of an airborne contaminant.

Employee exposure means exposure to a concentration of an airborne contaminant that would occur if the employee were not using respiratory protection.

End-of-service-life indicator (ESLI) means a system that warns the respirator user of the approach of the end of adequate respiratory protection, for example, that the sorbent is approaching saturation or is no longer effective.

Escape-only respirator means a respirator intended to be used only for emergency exit.

Filter or air purifying element means a component used in respirators to remove solid or liquid aerosols from the inspired air.

Filtering facepiece (dust mask) means a negative pressure particulate respirator with a filter as an integral part of the facepiece or with the entire facepiece composed of the filtering medium.

Fit factor means a quantitative estimate of the fit of a particular respirator to a specific individual, and typically estimates the ratio of the concentration of a substance in ambient air to its concentration inside the respirator when worn.

Fit test means the use of a protocol to qualitatively or quantitatively evaluate the fit of a respirator on an individual. (See also Qualitative fit test QLFT and Quantitative fit test QNFT.)
Helmet means a rigid respiratory inlet covering that also provides head protection against impact and penetration.
High efficiency particulate air (HEPA) filter means a filter that is at least 99.97% efficient in removing monodisperse particles of 0.3 micrometers in diameter. The equivalent NIOSH 42 CFR 84 particulate filters are the N100, R100, and P100 filters.
Hood means a respiratory inlet covering that completely covers the head and neck and may also cover portions of the shoulders and torso.
Immediately dangerous to life or health (IDLH) means an atmosphere that poses an immediate threat to life, would cause irreversible adverse health effects, or would impair an individual's ability to escape from a dangerous atmosphere.
Interior structural firefighting means the physical activity of fire suppression, rescue or both, inside of buildings or enclosed structures which are involved in a fire situation beyond the incipient stage. (See 29 CFR 1910.155)
Loose-fitting facepiece means a respiratory inlet covering that is designed to form a partial seal with the face.
Maximum use concentration (MUC) [Reserved].
Negative pressure respirator (tight fitting) means a respirator in which the air pressure inside the facepiece is negative during inhalation with respect to the ambient air pressure outside the respirator.
Oxygen deficient atmosphere means an atmosphere with an oxygen content below 19.5% by volume.
Physician or other licensed health care professional (PLHCP) means an individual whose legally permitted scope of practice (i.e., license, registration, or certification) allows him or her to independently provide, or be delegated the responsibility to provide, some or all of the health care services required by paragraph (e) of this section.
Positive pressure respirator means a respirator in which the pressure inside the respiratory inlet covering exceeds the ambient air pressure outside the respirator.
Powered air-purifying respirator (PAPR) means an air-purifying respirator that uses a blower to force the ambient air through air-purifying elements to the inlet covering.
Pressure demand respirator means a positive pressure atmosphere-supplying respirator that admits breathing air to the facepiece when the positive pressure is reduced inside the facepiece by inhalation.
Qualitative fit test (QLFT) means a pass/fail fit test to assess the adequacy of respirator fit that relies on the individual's response to the test agent.
Quantitative fit test (QNFT) means an assessment of the adequacy of respirator fit by numerically measuring the amount of leakage into the respirator.
Respiratory inlet covering means that portion of a respirator that forms the protective barrier between the user's respiratory tract and an air-purifying device or breathing air source, or both. It may be a facepiece, helmet, hood, suit, or a mouthpiece respirator with nose clamp.
Self-contained breathing apparatus (SCBA) means an atmosphere-supplying respirator for which the breathing air source is designed to be carried by the user.
Service life means the period of time that a respirator, filter or sorbent, or other respiratory equipment provides adequate protection to the wearer.
Supplied-air respirator (SAR) or airline respirator means an atmosphere-supplying respirator for which the source of breathing air is not designed to be carried by the user.
This section means this respiratory protection standard.
Tight-fitting facepiece means a respiratory inlet covering that forms a complete seal with the face.

User seal check means an action conducted by the respirator user to determine if the respirator is properly seated to the face.
(c) Respiratory protection program. This paragraph requires the employer to develop and implement a written respiratory protection program with required worksite-specific procedures and elements for required respirator use. The program must be administered by a suitably trained program administrator. In addition, certain program elements may be required for voluntary use to prevent potential hazards associated with the use of the respirator. The Small Entity Compliance Guide contains criteria for the selection of a program administrator and a sample program that meets the requirements of this paragraph. Copies of the Small Entity Compliance Guide will be available on or about April 8, 1998 from the Occupational Safety and Health Administration's Office of Publications, Room N 3101, 200 Constitution Avenue, NW, Washington, DC, 20210 (202-219-4667).
(1) In any workplace where respirators are necessary to protect the health of the employee or whenever respirators are required by the employer, the employer shall establish and implement a written respiratory protection program with worksite-specific procedures. The program shall be updated as necessary to reflect those changes in workplace conditions that affect respirator use. The employer shall include in the program the following provisions of this section, as applicable:
(i) Procedures for selecting respirators for use in the workplace;
(ii) Medical evaluations of employees required to use respirators;
(iii) Fit testing procedures for tight-fitting respirators;
(iv) Procedures for proper use of respirators in routine and reasonably foreseeable emergency situations;
(v) Procedures and schedules for cleaning, disinfecting, storing, inspecting, repairing, discarding, and otherwise maintaining respirators;
(vi) Procedures to ensure adequate air quality, quantity, and flow of breathing air for atmosphere-supplying respirators;
(vii) Training of employees in the respiratory hazards to which they are potentially exposed during routine and emergency situations;
(viii) Training of employees in the proper use of respirators, including putting on and removing them, any limitations on their use, and their maintenance; and
(ix) Procedures for regularly evaluating the effectiveness of the program.
(2) Where respirator use is not required:
(i) An employer may provide respirators at the request of employees or permit employees to use their own respirators, if the employer determines that such respirator use will not in itself create a hazard. If the employer determines that any voluntary respirator use is permissible, the employer shall provide the respirator users with the information contained in Appendix D to this section ("Information for Employees Using Respirators When Not Required Under the Standard"); and
(ii) In addition, the employer must establish and implement those elements of a written respiratory protection program necessary to ensure that any employee using a respirator voluntarily is medically able to use that respirator, and that the respirator is cleaned, stored, and maintained so that its use does not present a health hazard to the user. Exception: Employers are not required to include in a written respiratory protection program those employees whose only use of respirators involves the voluntary use of filtering facepieces (dust masks).
(3) The employer shall designate a program administrator who is qualified by appropriate training or experience that is commensurate with the complexity of the program to administer or oversee the respiratory protection program and conduct the required evaluations of program effectiveness.
(4) The employer shall provide respirators, training, and medical evaluations at no cost to the employee.

(d) Selection of respirators. This paragraph requires the employer to evaluate respiratory hazard(s) in the workplace, identify relevant workplace and user factors, and base respirator selection on these factors. The paragraph also specifies appropriately protective respirators for use in IDLH atmospheres, and limits the selection and use of air-purifying respirators.
(1) General requirements. (i) The employer shall select and provide an appropriate respirator based on the respiratory hazard(s) to which the worker is exposed and workplace and user factors that affect respirator performance and reliability.
(ii) The employer shall select a NIOSH-certified respirator. The respirator shall be used in compliance with the conditions of its certification.
(iii) The employer shall identify and evaluate the respiratory hazard(s) in the workplace; this evaluation shall include a reasonable estimate of employee exposures to respiratory hazard(s) and an identification of the contaminant's chemical state and physical form. Where the employer cannot identify or reasonably estimate the employee exposure, the employer shall consider the atmosphere to be IDLH.
(iv) The employer shall select respirators from a sufficient number of respirator models and sizes so that the respirator is acceptable to, and correctly fits, the user.
(2) Respirators for IDLH atmospheres. (i) The employer shall provide the following respirators for employee use in IDLH atmospheres:
(A) A full facepiece pressure demand SCBA certified by NIOSH for a minimum service life of thirty minutes, or
(B) A combination full facepiece pressure demand supplied-air respirator (SAR) with auxiliary self-contained air supply.
(ii) Respirators provided only for escape from IDLH atmospheres shall be NIOSH-certified for escape from the atmosphere in which they will be used.
(iii) All oxygen-deficient atmospheres shall be considered IDLH. Exception: If the employer demonstrates that, under all foreseeable conditions, the oxygen concentration can be maintained within the ranges specified in Table II of this section (i.e., for the altitudes set out in the table), then any atmosphere-supplying respirator may be used.
(3) Respirators for atmospheres that are not IDLH. (i) The employer shall provide a respirator that is adequate to protect the health of the employee and ensure compliance with all other OSHA statutory and regulatory requirements, under routine and reasonably foreseeable emergency situations.
(A) Assigned Protection Factors (APFs) [Reserved]
(B) Maximum Use Concentration (MUC) [Reserved]
(ii) The respirator selected shall be appropriate for the chemical state and physical form of the contaminant.
(iii) For protection against gases and vapors, the employer shall provide:
(A) An atmosphere-supplying respirator, or
(B) An air-purifying respirator, provided that:
(1) The respirator is equipped with an end-of-service-life indicator (ESLI) certified by NIOSH for the contaminant; or
(2) If there is no ESLI appropriate for conditions in the employer's workplace, the employer implements a change schedule for canisters and cartridges that is based on objective information or data that will ensure that canisters and cartridges are changed before the end of their service life. The employer shall describe in the respirator program the information and data relied upon and the basis for the canister and cartridge change schedule and the basis for reliance on the data.
(iv) For protection against particulates, the employer shall provide:
(A) An atmosphere-supplying respirator; or

(B) An air-purifying respirator equipped with a filter certified by NIOSH under 30 CFR part 11 as a high efficiency particulate air (HEPA) filter, or an air-purifying respirator equipped with a filter certified for particulates by NIOSH under 42 CFR part 84; or
(C) For contaminants consisting primarily of particles with mass median aerodynamic diameters (MMAD) of at least 2 micrometers, an air-purifying respirator equipped with any filter certified for particulates by NIOSH.

Table I.--Assigned Protection Factors [Reserved]

Table II

Altitude (ft.)	Oxygen deficient Atmospheres (% 0[2]) for which the employer may rely on atmosphere- supplying respirators
Less than 3,001	16.0-19.5
3,001-4,000	16.4-19.5
4,001-5,000	17.1-19.5
5,001-6,000	17.8-19.5
6,001-7,000	18.5-19.5
7,001-8,000 fn1	19.3-19.5.

Notes
fn1 Above 8,000 feet the exception does not apply. Oxygen-enriched breathing air must be supplied above 14,000 feet.

(e) Medical evaluation. Using a respirator may place a physiological burden on employees that varies with the type of respirator worn, the job and workplace conditions in which the respirator is used, and the medical status of the employee. Accordingly, this paragraph specifies the minimum requirements for medical evaluation that employers must implement to determine the employee's ability to use a respirator.
(1) General. The employer shall provide a medical evaluation to determine the employee's ability to use a respirator, before the employee is fit tested or required to use the respirator in the workplace. The employer may discontinue an employee's medical evaluations when the employee is no longer required to use a respirator.
(2) Medical evaluation procedures. (i) The employer shall identify a physician or other licensed health care professional (PLHCP) to perform medical evaluations using a medical questionnaire or an initial medical examination that obtains the same information as the medical questionnaire.
(ii) The medical evaluation shall obtain the information requested by the questionnaire in Sections 1 and 2, Part A of Appendix C of this section.
(3) Follow-up medical examination. (i) The employer shall ensure that a follow-up medical examination is provided for an employee who gives a positive response to any question among questions 1 through 8 in Section 2, Part A of Appendix C or whose initial medical examination demonstrates the need for a follow-up medical examination.
(ii) The follow-up medical examination shall include any medical tests, consultations, or diagnostic procedures that the PLHCP deems necessary to make a final determination.
(4) Administration of the medical questionnaire and examinations. (i) The medical questionnaire and examinations shall be administered confidentially during the employee's normal working hours or at a time and place convenient to the employee. The medical questionnaire shall be administered in a manner that ensures that the employee understands its content.
(ii) The employer shall provide the employee with an opportunity to discuss the questionnaire and examination results with the PLHCP.

(5) Supplemental information for the PLHCP. (i) The following information must be provided to the PLHCP before the PLHCP makes a recommendation concerning an employee's ability to use a respirator:
(A) The type and weight of the respirator to be used by the employee;
(B) The duration and frequency of respirator use (including use for rescue and escape);
(C) The expected physical work effort;
(D) Additional protective clothing and equipment to be worn; and
(E) Temperature and humidity extremes that may be encountered.
(ii) Any supplemental information provided previously to the PLHCP regarding an employee need not be provided for a subsequent medical evaluation if the information and the PLHCP remain the same.
(iii) The employer shall provide the PLHCP with a copy of the written respiratory protection program and a copy of this section.

Note to Paragraph (e)(5)(iii): When the employer replaces a PLHCP, the employer must ensure that the new PLHCP obtains this information, either by providing the documents directly to the PLHCP or having the documents transferred from the former PLHCP to the new PLHCP. However, OSHA does not expect employers to have employees medically reevaluated solely because a new PLHCP has been selected.
(6) Medical determination. In determining the employee's ability to use a respirator, the employer shall:
(i) Obtain a written recommendation regarding the employee's ability to use the respirator from the PLHCP. The recommendation shall provide only the following information:
(A) Any limitations on respirator use related to the medical condition of the employee, or relating to the workplace conditions in which the respirator will be used, including whether or not the employee is medically able to use the respirator;
(B) The need, if any, for follow-up medical evaluations; and
(C) A statement that the PLHCP has provided the employee with a copy of the PLHCP's written recommendation.
(ii) If the respirator is a negative pressure respirator and the PLHCP finds a medical condition that may place the employee's health at increased risk if the respirator is used, the employer shall provide a PAPR if the PLHCP's medical evaluation finds that the employee can use such a respirator; if a subsequent medical evaluation finds that the employee is medically able to use a negative pressure respirator, then the employer is no longer required to provide a PAPR.
(7) Additional medical evaluations. At a minimum, the employer shall provide additional medical evaluations that comply with the requirements of this section if:
(i) An employee reports medical signs or symptoms that are related to ability to use a respirator;
(ii) A PLHCP, supervisor, or the respirator program administrator informs the employer that an employee needs to be reevaluated;
(iii) Information from the respiratory protection program, including observations made during fit testing and program evaluation, indicates a need for employee reevaluation; or
(iv) A change occurs in workplace conditions (e.g., physical work effort, protective clothing, and temperature) that may result in a substantial increase in the physiological burden placed on an employee.
(f) Fit testing. This paragraph requires that, before an employee may be required to use any respirator with a negative or positive pressure tight-fitting facepiece, the employee must be fit tested with the same make, model, style, and size of respirator that will be used. This paragraph specifies the kinds of fit tests allowed, the procedures for conducting them, and how the results of the fit tests must be used.
(1) The employer shall ensure that employees using a tight-fitting facepiece respirator pass an appropriate qualitative fit test (QLFT) or quantitative fit test (QNFT) as stated in this paragraph.

(2) The employer shall ensure that an employee using a tight-fitting facepiece respirator is fit tested prior to initial use of the respirator, whenever a different respirator facepiece (size, style, model or make) is used, and at least annually thereafter.
(3) The employer shall conduct an additional fit test whenever the employee reports, or the employer, PLHCP, supervisor, or program administrator makes visual observations of, changes in the employee's physical condition that could affect respirator fit. Such conditions include, but are not limited to, facial scarring, dental changes, cosmetic surgery, or an obvious change in body weight.
(4) If after passing a QLFT or QNFT, the employee subsequently notifies the employer, program administrator, supervisor, or PLHCP that the fit of the respirator is unacceptable, the employee shall be given a reasonable opportunity to select a different respirator facepiece and to be retested.
(5) The fit test shall be administered using an OSHA-accepted QLFT or QNFT protocol. The OSHA-accepted QLFT and QNFT protocols and procedures are contained in Appendix A of this section.
(6) QLFT may only be used to fit test negative pressure air-purifying respirators that must achieve a fit factor of 100 or less.
(7) If the fit factor, as determined through an OSHA-accepted QNFT protocol, is equal to or greater than 100 for tight-fitting half facepieces, or equal to or greater than 500 for tight-fitting full facepieces, the QNFT has been passed with that respirator.
(8) Fit testing of tight-fitting atmosphere-supplying respirators and tight-fitting powered air-purifying respirators shall be accomplished by performing quantitative or qualitative fit testing in the negative pressure mode, regardless of the mode of operation (negative or positive pressure) that is used for respiratory protection.
(i) Qualitative fit testing of these respirators shall be accomplished by temporarily converting the respirator user's actual facepiece into a negative pressure respirator with appropriate filters, or by using an identical negative pressure air-purifying respirator facepiece with the same sealing surfaces as a surrogate for the atmosphere-supplying or powered air-purifying respirator facepiece.
(ii) Quantitative fit testing of these respirators shall be accomplished by modifying the facepiece to allow sampling inside the facepiece in the breathing zone of the user, midway between the nose and mouth. This requirement shall be accomplished by installing a permanent sampling probe onto a surrogate facepiece, or by using a sampling adapter designed to temporarily provide a means of sampling air from inside the facepiece.
(iii) Any modifications to the respirator facepiece for fit testing shall be completely removed, and the facepiece restored to NIOSH-approved configuration, before that facepiece can be used in the workplace.
(g) Use of respirators. This paragraph requires employers to establish and implement procedures for the proper use of respirators. These requirements include prohibiting conditions that may result in facepiece seal leakage, preventing employees from removing respirators in hazardous environments, taking actions to ensure continued effective respirator operation throughout the work shift, and establishing procedures for the use of respirators in IDLH atmospheres or in interior structural firefighting situations.
(1) Facepiece seal protection. (i) The employer shall not permit respirators with tight-fitting facepieces to be worn by employees who have:
(A) Facial hair that comes between the sealing surface of the facepiece and the face or that interferes with valve function; or
(B) Any condition that interferes with the face-to-facepiece seal or valve function.

(ii) If an employee wears corrective glasses or goggles or other personal protective equipment, the employer shall ensure that such equipment is worn in a manner that does not interfere with the seal of the facepiece to the face of the user.
(iii) For all tight-fitting respirators, the employer shall ensure that employees perform a user seal check each time they put on the respirator using the procedures in Appendix B-1 or procedures recommended by the respirator manufacturer that the employer demonstrates are as effective as those in Appendix B-1 of this section.
(2) Continuing respirator effectiveness. (i) Appropriate surveillance shall be maintained of work area conditions and degree of employee exposure or stress. When there is a change in work area conditions or degree of employee exposure or stress that may affect respirator effectiveness, the employer shall reevaluate the continued effectiveness of the respirator.
(ii) The employer shall ensure that employees leave the respirator use area:
(A) To wash their faces and respirator facepieces as necessary to prevent eye or skin irritation associated with respirator use; or
(B) If they detect vapor or gas breakthrough, changes in breathing resistance, or leakage of the facepiece; or
(C) To replace the respirator or the filter, cartridge, or canister elements.
(iii) If the employee detects vapor or gas breakthrough, changes in breathing resistance, or leakage of the facepiece, the employer must replace or repair the respirator before allowing the employee to return to the work area.
(3) Procedures for IDLH atmospheres. For all IDLH atmospheres, the employer shall ensure that:
(i) One employee or, when needed, more than one employee is located outside the IDLH atmosphere;
(ii) Visual, voice, or signal line communication is maintained between the employee(s) in the IDLH atmosphere and the employee(s) located outside the IDLH atmosphere;
(iii) The employee(s) located outside the IDLH atmosphere are trained and equipped to provide effective emergency rescue;
(iv) The employer or designee is notified before the employee(s) located outside the IDLH atmosphere enter the IDLH atmosphere to provide emergency rescue;
(v) The employer or designee authorized to do so by the employer, once notified, provides necessary assistance appropriate to the situation;
(vi) Employee(s) located outside the IDLH atmospheres are equipped with:
(A) Pressure demand or other positive pressure SCBAs, or a pressure demand or other positive pressure supplied-air respirator with auxiliary SCBA; and either
(B) Appropriate retrieval equipment for removing the employee(s) who enter(s) these hazardous atmospheres where retrieval equipment would contribute to the rescue of the employee(s) and would not increase the overall risk resulting from entry; or
(C) Equivalent means for rescue where retrieval equipment is not required under paragraph (g)(3)(vi)(B).
(4) Procedures for interior structural firefighting. In addition to the requirements set forth under paragraph (g)(3), in interior structural fires, the employer shall ensure that:
(i) At least two employees enter the IDLH atmosphere and remain in visual or voice contact with one another at all times;
(ii) At least two employees are located outside the IDLH atmosphere; and
(iii) All employees engaged in interior structural firefighting use SCBAs.
Note 1 to paragraph (g): One of the two individuals located outside the IDLH atmosphere may be assigned to an additional role, such as incident commander in charge of the emergency or safety officer, so long as this individual is able to perform assistance or rescue activities without jeopardizing the safety or health of any firefighter working at the incident.

Note 2 to paragraph (g): Nothing in this section is meant to preclude firefighters from performing emergency rescue activities before an entire team has assembled.
(h) Maintenance and care of respirators. This paragraph requires the employer to provide for the cleaning and disinfecting, storage, inspection, and repair of respirators used by employees.
(1) Cleaning and disinfecting. The employer shall provide each respirator user with a respirator that is clean, sanitary, and in good working order. The employer shall ensure that respirators are cleaned and disinfected using the procedures in Appendix B-2 of this section, or procedures recommended by the respirator manufacturer, provided that such procedures are of equivalent effectiveness. The respirators shall be cleaned and disinfected at the following intervals:
(i) Respirators issued for the exclusive use of an employee shall be cleaned and disinfected as often as necessary to be maintained in a sanitary condition;
(ii) Respirators issued to more than one employee shall be cleaned and disinfected before being worn by different individuals;
(iii) Respirators maintained for emergency use shall be cleaned and disinfected after each use; and
(iv) Respirators used in fit testing and training shall be cleaned and disinfected after each use.
(2) Storage. The employer shall ensure that respirators are stored as follows:
(i) All respirators shall be stored to protect them from damage, contamination, dust, sunlight, extreme temperatures, excessive moisture, and damaging chemicals, and they shall be packed or stored to prevent deformation of the facepiece and exhalation valve.
(ii) In addition to the requirements of paragraph (h)(2)(i) of this section, emergency respirators shall be:
(A) Kept accessible to the work area;
(B) Stored in compartments or in covers that are clearly marked as containing emergency respirators; and
(C) Stored in accordance with any applicable manufacturer instructions.
(3) Inspection. (i) The employer shall ensure that respirators are inspected as follows:
(A) All respirators used in routine situations shall be inspected before each use and during cleaning;
(B) All respirators maintained for use in emergency situations shall be inspected at least monthly and in accordance with the manufacturer's recommendations, and shall be checked for proper function before and after each use; and
(C) Emergency escape-only respirators shall be inspected before being carried into the workplace for use.
(ii) The employer shall ensure that respirator inspections include the following:
(A) A check of respirator function, tightness of connections, and the condition of the various parts including, but not limited to, the facepiece, head straps, valves, connecting tube, and cartridges, canisters or filters; and
(B) A check of elastomeric parts for pliability and signs of deterioration.
(iii) In addition to the requirements of paragraphs (h)(3)(i) and (ii) of this section, self-contained breathing apparatus shall be inspected monthly. Air and oxygen cylinders shall be maintained in a fully charged state and shall be recharged when the pressure falls to 90% of the manufacturer's recommended pressure level. The employer shall determine that the regulator and warning devices function properly.
(iv) For respirators maintained for emergency use, the employer shall:
(A) Certify the respirator by documenting the date the inspection was performed, the name (or signature) of the person who made the inspection, the findings, required remedial action, and a serial number or other means of identifying the inspected respirator; and
(B) Provide this information on a tag or label that is attached to the storage compartment for the respirator, is kept with the respirator, or is included in inspection reports stored as paper or

electronic files. This information shall be maintained until replaced following a subsequent certification.
(4) Repairs. The employer shall ensure that respirators that fail an inspection or are otherwise found to be defective are removed from service, and are discarded or repaired or adjusted in accordance with the following procedures:
(i) Repairs or adjustments to respirators are to be made only by persons appropriately trained to perform such operations and shall use only the respirator manufacturer's NIOSH-approved parts designed for the respirator;
(ii) Repairs shall be made according to the manufacturer's recommendations and specifications for the type and extent of repairs to be performed; and
(iii) Reducing and admission valves, regulators, and alarms shall be adjusted or repaired only by the manufacturer or a technician trained by the manufacturer.
(i) Breathing air quality and use. This paragraph requires the employer to provide employees using atmosphere-supplying respirators (supplied-air and SCBA) with breathing gases of high purity.
(1) The employer shall ensure that compressed air, compressed oxygen, liquid air, and liquid oxygen used for respiration accords with the following specifications:
(i) Compressed and liquid oxygen shall meet the United States Pharmacopoeia requirements for medical or breathing oxygen; and
(ii) Compressed breathing air shall meet at least the requirements for Grade D breathing air described in ANSI/Compressed Gas Association Commodity Specification for Air, G-7.1-1989, to include:
(A) Oxygen content (v/v) of 19.5-23.5%;
(B) Hydrocarbon (condensed) content of 5 milligrams per cubic meter of air or less;
(C) Carbon monoxide (CO) content of 10 ppm or less;
(D) Carbon dioxide content of 1,000 ppm or less; and
(E) Lack of noticeable odor.
(2) The employer shall ensure that compressed oxygen is not used in atmosphere-supplying respirators that have previously used compressed air.
(3) The employer shall ensure that oxygen concentrations greater than 23.5% are used only in equipment designed for oxygen service or distribution.
(4) The employer shall ensure that cylinders used to supply breathing air to respirators meet the following requirements:
(i) Cylinders are tested and maintained as prescribed in the Shipping Container Specification Regulations of the Department of Transportation (49 CFR part 173 and part 178);
(ii) Cylinders of purchased breathing air have a certificate of analysis from the supplier that the breathing air meets the requirements for Grade D breathing air; and
(iii) The moisture content in the cylinder does not exceed a dew point of -50 [degrees] F (-45.6 [degrees] C) at 1 atmosphere pressure.
(5) The employer shall ensure that compressors used to supply breathing air to respirators are constructed and situated so as to:
(i) Prevent entry of contaminated air into the air-supply system;
(ii) Minimize moisture content so that the dew point at 1 atmosphere pressure is 10 degrees F (5.56 [degrees] C) below the ambient temperature;
(iii) Have suitable in-line air-purifying sorbent beds and filters to further ensure breathing air quality. Sorbent beds and filters shall be maintained and replaced or refurbished periodically following the manufacturer's instructions.
(iv) Have a tag containing the most recent change date and the signature of the person authorized by the employer to perform the change. The tag shall be maintained at the compressor.

(6) For compressors that are not oil-lubricated, the employer shall ensure that carbon monoxide levels in the breathing air do not exceed 10 ppm.
(7) For oil-lubricated compressors, the employer shall use a high-temperature or carbon monoxide alarm, or both, to monitor carbon monoxide levels. If only high-temperature alarms are used, the air supply shall be monitored at intervals sufficient to prevent carbon monoxide in the breathing air from exceeding 10 ppm.
(8) The employer shall ensure that breathing air couplings are incompatible with outlets for nonrespirable worksite air or other gas systems. No asphyxiating substance shall be introduced into breathing air lines.
(9) The employer shall use breathing gas containers marked in accordance with the NIOSH respirator certification standard, 42 CFR part 84.
(j) Identification of filters, cartridges, and canisters. The employer shall ensure that all filters, cartridges and canisters used in the workplace are labeled and color coded with the NIOSH approval label and that the label is not removed and remains legible.
(k) Training and information. This paragraph requires the employer to provide effective training to employees who are required to use respirators. The training must be comprehensive, understandable, and recur annually and more often if necessary. This paragraph also requires the employer to provide the basic information on respirators in Appendix D of this section to employees who wear respirators when not required by this section or by the employer to do so.
(1) The employer shall ensure that each employee can demonstrate knowledge of at least the following:
(i) Why the respirator is necessary and how improper fit, usage, or maintenance can compromise the protective effect of the respirator;
(ii) What the limitations and capabilities of the respirator are;
(iii) How to use the respirator effectively in emergency situations, including situations in which the respirator malfunctions;
(iv) How to inspect, put on and remove, use, and check the seals of the respirator;
(v) What the procedures are for maintenance and storage of the respirator;
(vi) How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators; and
(vii) The general requirements of this section.
(2) The training shall be conducted in a manner that is understandable to the employee.
(3) The employer shall provide the training prior to requiring the employee to use a respirator in the workplace.
(4) An employer who is able to demonstrate that a new employee has received training within the last 12 months that addresses the elements specified in paragraph (k)(1)(i) through (vii) is not required to repeat such training provided that, as required by paragraph (k)(1), the employee can demonstrate knowledge of those element(s). Previous training not repeated initially by the employer must be provided no later than 12 months from the date of the previous training.
(5) Retraining shall be administered annually, and when the following situations occur:
(i) Changes in the workplace or the type of respirator render previous training obsolete;
(ii) Inadequacies in the employee's knowledge or use of the respirator indicate that the employee has not retained the requisite understanding or skill; or
(iii) Any other situation arises in which retraining appears necessary to ensure safe respirator use.
(6) The basic advisory information on respirators, as presented in Appendix D of this section, shall be provided by the employer in any written or oral format, to employees who wear respirators when such use is not required by this section or by the employer.
(l) Program evaluation. This section requires the employer to conduct evaluations of the workplace to ensure that the written respiratory protection program is being properly implemented, and to consult employees to ensure that they are using the respirators properly.

(1) The employer shall conduct evaluations of the workplace as necessary to ensure that the provisions of the current written program are being effectively implemented and that it continues to be effective.
(2) The employer shall regularly consult employees required to use respirators to assess the employees' views on program effectiveness and to identify any problems. Any problems that are identified during this assessment shall be corrected. Factors to be assessed include, but are not limited to:
(i) Respirator fit (including the ability to use the respirator without interfering with effective workplace performance);
(ii) Appropriate respirator selection for the hazards to which the employee is exposed;
(iii) Proper respirator use under the workplace conditions the employee encounters; and
(iv) Proper respirator maintenance.
(m) Recordkeeping. This section requires the employer to establish and retain written information regarding medical evaluations, fit testing, and the respirator program. This information will facilitate employee involvement in the respirator program, assist the employer in auditing the adequacy of the program, and provide a record for compliance determinations by OSHA.
(1) Medical evaluation. Records of medical evaluations required by this section must be retained and made available in accordance with 29 CFR 1910.1020.
(2) Fit testing. (i) The employer shall establish a record of the qualitative and quantitative fit tests administered to an employee including:
(A) The name or identification of the employee tested;
(B) Type of fit test performed;
(C) Specific make, model, style, and size of respirator tested;
(D) Date of test; and
(E) The pass/fail results for QLFTs or the fit factor and strip chart recording or other recording of the test results for QNFTs.
(ii) Fit test records shall be retained for respirator users until the next fit test is administered.
(3) A written copy of the current respirator program shall be retained by the employer.
(4) Written materials required to be retained under this paragraph shall be made available upon request to affected employees and to the Assistant Secretary or designee for examination and copying.
(n) Dates. (1) Effective date. This section is effective April 8, 1998. The obligations imposed by this section commence on the effective date unless otherwise noted in this paragraph. Compliance with obligations that do not commence on the effective date shall occur no later than the applicable start-up date.
(2) Compliance dates. All obligations of this section commence on the effective date except as follows:
(i) The determination that respirator use is required (paragraph (a)) shall be completed no later than September 8, 1998.
(ii) Compliance with provisions of this section for all other provisions shall be completed no later than October 5, 1998.
(3) The provisions of 29 CFR 1910.134 and 29 CFR 1926.103, contained in the 29 CFR parts 1900 to 1910.99 and the 29 CFR part 1926 editions, revised as of July 1, 1997, are in effect and enforceable until October 5, 1998, or during any administrative or judicial stay of the provisions of this section.

Appendix A to § 1910.134: Fit Testing Procedures (Mandatory)
Part I. OSHA-Accepted Fit Test Protocols
A. Fit Testing Procedures-General Requirements

The employer shall conduct fit testing using the following procedures. The requirements in this appendix apply to all OSHA-accepted fit test methods, both QLFT and QNFT.

1. The test subject shall be allowed to pick the most acceptable respirator from a sufficient number of respirator models and sizes so that the respirator is acceptable to, and correctly fits, the user.

2. Prior to the selection process, the test subject shall be shown how to put on a respirator, how it should be positioned on the face, how to set strap tension and how to determine an acceptable fit. A mirror shall be available to assist the subject in evaluating the fit and positioning of the respirator. This instruction may not constitute the subject's formal training on respirator use, because it is only a review.

3. The test subject shall be informed that he/she is being asked to select the respirator that provides the most acceptable fit. Each respirator represents a different size and shape, and if fitted and used properly, will provide adequate protection.

4. The test subject shall be instructed to hold each chosen facepiece up to the face and eliminate those that obviously do not give an acceptable fit.

5. The more acceptable facepieces are noted in case the one selected proves unacceptable; the most comfortable mask is donned and worn at least five minutes to assess comfort. Assistance in assessing comfort can be given by discussing the points in the following item A.6. If the test subject is not familiar with using a particular respirator, the test subject shall be directed to don the mask several times and to adjust the straps each time to become adept at setting proper tension on the straps.

6. Assessment of comfort shall include a review of the following points with the test subject and allowing the test subject adequate time to determine the comfort of the respirator:

(a) Position of the mask on the nose

(b) Room for eye protection

(c) Room to talk

(d) Position of mask on face and cheeks

7. The following criteria shall be used to help determine the adequacy of the respirator fit:

(a) Chin properly placed;

(b) Adequate strap tension, not overly tightened;

(c) Fit across nose bridge;

(d) Respirator of proper size to span distance from nose to chin;

(e) Tendency of respirator to slip;

(f) Self-observation in mirror to evaluate fit and respirator position.

8. The test subject shall conduct a user seal check, either the negative and positive pressure seal checks described in Appendix B-1 of this section or those recommended by the respirator manufacturer which provide equivalent protection to the procedures in Appendix B-1. Before conducting the negative and positive pressure checks, the subject shall be told to seat the mask on the face by moving the head from side-to-side and up and down slowly while taking in a few slow deep breaths. Another facepiece shall be selected and retested if the test subject fails the user seal check tests.

9. The test shall not be conducted if there is any hair growth between the skin and the facepiece sealing surface, such as stubble beard growth, beard, mustache or sideburns which cross the respirator sealing surface. Any type of apparel which interferes with a satisfactory fit shall be altered or removed.

10. If a test subject exhibits difficulty in breathing during the tests, she or he shall be referred to a physician or other licensed health care professional, as appropriate, to determine whether the test subject can wear a respirator while performing her or his duties.
11. If the employee finds the fit of the respirator unacceptable, the test subject shall be given the opportunity to select a different respirator and to be retested.
12. Exercise regimen. Prior to the commencement of the fit test, the test subject shall be given a description of the fit test and the test subject's responsibilities during the test procedure. The description of the process shall include a description of the test exercises that the subject will be performing. The respirator to be tested shall be worn for at least 5 minutes before the start of the fit test.
13. The fit test shall be performed while the test subject is wearing any applicable safety equipment that may be worn during actual respirator use which could interfere with respirator fit.
14. Test Exercises. (a) The following test exercises are to be performed for all fit testing methods prescribed in this appendix, except for the CNP method. A separate fit testing exercise regimen is contained in the CNP protocol. The test subject shall perform exercises, in the test environment, in the following manner:
(1) Normal breathing. In a normal standing position, without talking, the subject shall breathe normally.
(2) Deep breathing. In a normal standing position, the subject shall breathe slowly and deeply, taking caution so as not to hyperventilate.
(3) Turning head side to side. Standing in place, the subject shall slowly turn his/her head from side to side between the extreme positions on each side. The head shall be held at each extreme momentarily so the subject can inhale at each side.
(4) Moving head up and down. Standing in place, the subject shall slowly move his/her head up and down. The subject shall be instructed to inhale in the up position (i.e., when looking toward the ceiling).
(5) Talking. The subject shall talk out loud slowly and loud enough so as to be heard clearly by the test conductor. The subject can read from a prepared text such as the Rainbow Passage, count backward from 100, or recite a memorized poem or song.

Rainbow Passage
When the sunlight strikes raindrops in the air, they act like a prism and form a rainbow. The rainbow is a division of white light into many beautiful colors. These take the shape of a long round arch, with its path high above, and its two ends apparently beyond the horizon. There is, according to legend, a boiling pot of gold at one end. People look, but no one ever finds it. When a man looks for something beyond reach, his friends say he is looking for the pot of gold at the end of the rainbow.
(6) Grimace. The test subject shall grimace by smiling or frowning. (This applies only to QNFT testing; it is not performed for QLFT)
(7) Bending over. The test subject shall bend at the waist as if he/she were to touch his/her toes. Jogging in place shall be substituted for this exercise in those test environments such as shroud type QNFT or QLFT units that do not permit bending over at the waist.
(8) Normal breathing. Same as exercise (1).
(b) Each test exercise shall be performed for one minute except for the grimace exercise which shall be performed for 15 seconds. The test subject shall be questioned by the test conductor regarding the comfort of the respirator upon completion of the protocol. If it has become unacceptable, another model of respirator shall be tried. The respirator shall not be adjusted once the fit test exercises begin. Any adjustment voids the test, and the fit test must be repeated.
B. Qualitative Fit Test (QLFT) Protocols
1. General

(a) The employer shall ensure that persons administering QLFT are able to prepare test solutions, calibrate equipment and perform tests properly, recognize invalid tests, and ensure that test equipment is in proper working order.
(b) The employer shall ensure that QLFT equipment is kept clean and well maintained so as to operate within the parameters for which it was designed.
2. Isoamyl Acetate Protocol
Note: This protocol is not appropriate to use for the fit testing of particulate respirators. If used to fit test particulate respirators, the respirator must be equipped with an organic vapor filter.
(a) Odor Threshold Screening
Odor threshold screening, performed without wearing a respirator, is intended to determine if the individual tested can detect the odor of isoamyl acetate at low levels.
(1) Three 1 liter glass jars with metal lids are required.
(2) Odor-free water (e.g., distilled or spring water) at approximately 25 [degrees] C (77 [degrees] F) shall be used for the solutions.
(3) The isoamyl acetate (IAA) (also known at isopentyl acetate) stock solution is prepared by adding 1 ml of pure IAA to 800 ml of odor-free water in a 1 liter jar, closing the lid and shaking for 30 seconds. A new solution shall be prepared at least weekly.
(4) The screening test shall be conducted in a room separate from the room used for actual fit testing. The two rooms shall be well-ventilated to prevent the odor of IAA from becoming evident in the general room air where testing takes place.
(5) The odor test solution is prepared in a second jar by placing 0.4 ml of the stock solution into 500 ml of odor-free water using a clean dropper or pipette. The solution shall be shaken for 30 seconds and allowed to stand for two to three minutes so that the IAA concentration above the liquid may reach equilibrium. This solution shall be used for only one day.
(6) A test blank shall be prepared in a third jar by adding 500 cc of odor-free water.
(7) The odor test and test blank jar lids shall be labeled (e.g., 1 and 2) for jar identification. Labels shall be placed on the lids so that they can be peeled off periodically and switched to maintain the integrity of the test.
(8) The following instruction shall be typed on a card and placed on the table in front of the two test jars (i.e., 1 and 2): "The purpose of this test is to determine if you can smell banana oil at a low concentration. The two bottles in front of you contain water. One of these bottles also contains a small amount of banana oil. Be sure the covers are on tight, then shake each bottle for two seconds. Unscrew the lid of each bottle, one at a time, and sniff at the mouth of the bottle. Indicate to the test conductor which bottle contains banana oil."
(9) The mixtures used in the IAA odor detection test shall be prepared in an area separate from where the test is performed, in order to prevent olfactory fatigue in the subject.
(10) If the test subject is unable to correctly identify the jar containing the odor test solution, the IAA qualitative fit test shall not be performed.
(11) If the test subject correctly identifies the jar containing the odor test solution, the test subject may proceed to respirator selection and fit testing.
(b) Isoamyl Acetate Fit Test
(1) The fit test chamber shall be a clear 55-gallon drum liner suspended inverted over a 2-foot diameter frame so that the top of the chamber is about 6 inches above the test subject's head. If no drum liner is available, a similar chamber shall be constructed using plastic sheeting. The inside top center of the chamber shall have a small hook attached.
(2) Each respirator used for the fitting and fit testing shall be equipped with organic vapor cartridges or offer protection against organic vapors.
(3) After selecting, donning, and properly adjusting a respirator, the test subject shall wear it to the fit testing room. This room shall be separate from the room used for odor threshold screening and respirator selection, and shall be well-ventilated, as by an exhaust fan or lab hood, to prevent general room contamination.

(4) A copy of the test exercises and any prepared text from which the subject is to read shall be taped to the inside of the test chamber.
(5) Upon entering the test chamber, the test subject shall be given a 6-inch by 5-inch piece of paper towel, or other porous, absorbent, single-ply material, folded in half and wetted with 0.75 ml of pure IAA. The test subject shall hang the wet towel on the hook at the top of the chamber. An IAA test swab or ampule may be substituted for the IAA wetted paper towel provided it has been demonstrated that the alternative IAA source will generate an IAA test atmosphere with a concentration equivalent to that generated by the paper towel method.
(6) Allow two minutes for the IAA test concentration to stabilize before starting the fit test exercises. This would be an appropriate time to talk with the test subject; to explain the fit test, the importance of his/her cooperation, and the purpose for the test exercises; or to demonstrate some of the exercises.
(7) If at any time during the test, the subject detects the banana-like odor of IAA, the test is failed. The subject shall quickly exit from the test chamber and leave the test area to avoid olfactory fatigue.
(8) If the test is failed, the subject shall return to the selection room and remove the respirator. The test subject shall repeat the odor sensitivity test, select and put on another respirator, return to the test area and again begin the fit test procedure described in (b) (1) through (7) above. The process continues until a respirator that fits well has been found. Should the odor sensitivity test be failed, the subject shall wait at least 5 minutes before retesting. Odor sensitivity will usually have returned by this time.
(9) If the subject passes the test, the efficiency of the test procedure shall be demonstrated by having the subject break the respirator face seal and take a breath before exiting the chamber.
(10) When the test subject leaves the chamber, the subject shall remove the saturated towel and return it to the person conducting the test, so that there is no significant IAA concentration buildup in the chamber during subsequent tests. The used towels shall be kept in a self-sealing plastic bag to keep the test area from being contaminated.
3. Saccharin Solution Aerosol Protocol
The entire screening and testing procedure shall be explained to the test subject prior to the conduct of the screening test.
(a) Taste threshold screening. The saccharin taste threshold screening, performed without wearing a respirator, is intended to determine whether the individual being tested can detect the taste of saccharin.
(1) During threshold screening as well as during fit testing, subjects shall wear an enclosure about the head and shoulders that is approximately 12 inches in diameter by 14 inches tall with at least the front portion clear and that allows free movements of the head when a respirator is worn. An enclosure substantially similar to the 3M hood assembly, parts # FT 14 and # FT 15 combined, is adequate.
(2) The test enclosure shall have a 3/4-inch (1.9 cm) hole in front of the test subject's nose and mouth area to accommodate the nebulizer nozzle.
(3) The test subject shall don the test enclosure. Throughout the threshold screening test, the test subject shall breathe through his/her slightly open mouth with tongue extended. The subject is instructed to report when he/she detects a sweet taste.
(4) Using a DeVilbiss Model 40 Inhalation Medication Nebulizer or equivalent, the test conductor shall spray the threshold check solution into the enclosure. The nozzle is directed away from the nose and mouth of the person. This nebulizer shall be clearly marked to distinguish it from the fit test solution nebulizer.
(5) The threshold check solution is prepared by dissolving 0.83 gram of sodium saccharin USP in 100 ml of warm water. It can be prepared by putting 1 ml of the fit test solution (see (b)(5) below) in 100 ml of distilled water.

(6) To produce the aerosol, the nebulizer bulb is firmly squeezed so that it collapses completely, then released and allowed to fully expand.
(7) Ten squeezes are repeated rapidly and then the test subject is asked whether the saccharin can be tasted. If the test subject reports tasting the sweet taste during the ten squeezes, the screening test is completed. The taste threshold is noted as ten regardless of the number of squeezes actually completed.
(8) If the first response is negative, ten more squeezes are repeated rapidly and the test subject is again asked whether the saccharin is tasted. If the test subject reports tasting the sweet taste during the second ten squeezes, the screening test is completed. The taste threshold is noted as twenty regardless of the number of squeezes actually completed.
(9) If the second response is negative, ten more squeezes are repeated rapidly and the test subject is again asked whether the saccharin is tasted. If the test subject reports tasting the sweet taste during the third set of ten squeezes, the screening test is completed. The taste threshold is noted as thirty regardless of the number of squeezes actually completed.
(10) The test conductor will take note of the number of squeezes required to solicit a taste response.
(11) If the saccharin is not tasted after 30 squeezes (step 10), the test subject is unable to taste saccharin and may not perform the saccharin fit test.
Note to paragraph 3. (a): If the test subject eats or drinks something sweet before the screening test, he/she may be unable to taste the weak saccharin solution.
(12) If a taste response is elicited, the test subject shall be asked to take note of the taste for reference in the fit test.
(13) Correct use of the nebulizer means that approximately 1 ml of liquid is used at a time in the nebulizer body.
(14) The nebulizer shall be thoroughly rinsed in water, shaken dry, and refilled at least each morning and afternoon or at least every four hours.
(b) Saccharin solution aerosol fit test procedure.
(1) The test subject may not eat, drink (except plain water), smoke, or chew gum for 15 minutes before the test.
(2) The fit test uses the same enclosure described in 3. (a) above.
(3) The test subject shall don the enclosure while wearing the respirator selected in section I. A. of this appendix. The respirator shall be properly adjusted and equipped with a particulate filter(s).
(4) A second DeVilbiss Model 40 Inhalation Medication Nebulizer or equivalent is used to spray the fit test solution into the enclosure. This nebulizer shall be clearly marked to distinguish it from the screening test solution nebulizer.
(5) The fit test solution is prepared by adding 83 grams of sodium saccharin to 100 ml of warm water.
(6) As before, the test subject shall breathe through the slightly open mouth with tongue extended, and report if he/she tastes the sweet taste of saccharin.
(7) The nebulizer is inserted into the hole in the front of the enclosure and an initial concentration of saccharin fit test solution is sprayed into the enclosure using the same number of squeezes (either 10, 20 or 30 squeezes) based on the number of squeezes required to elicit a taste response as noted during the screening test. A minimum of 10 squeezes is required.
(8) After generating the aerosol, the test subject shall be instructed to perform the exercises in section I. A. 14. of this appendix.
(9) Every 30 seconds the aerosol concentration shall be replenished using one half the original number of squeezes used initially (e.g., 5, 10 or 15).
(10) The test subject shall indicate to the test conductor if at any time during the fit test the taste of saccharin is detected. If the test subject does not report tasting the saccharin, the test is passed.

(11) If the taste of saccharin is detected, the fit is deemed unsatisfactory and the test is failed. A different respirator shall be tried and the entire test procedure is repeated (taste threshold screening and fit testing).
(12) Since the nebulizer has a tendency to clog during use, the test operator must make periodic checks of the nebulizer to ensure that it is not clogged. If clogging is found at the end of the test session, the test is invalid.
4. Bitrex<TM> (Denatonium Benzoate) Solution Aerosol Qualitative Fit Test Protocol
The Bitrex<TM> (Denatonium benzoate) solution aerosol QLFT protocol uses the published saccharin test protocol because that protocol is widely accepted. Bitrex is routinely used as a taste aversion agent in household liquids which children should not be drinking and is endorsed by the American Medical Association, the National Safety Council, and the American Association of Poison Control Centers. The entire screening and testing procedure shall be explained to the test subject prior to the conduct of the screening test.
(a) Taste Threshold Screening.
The Bitrex taste threshold screening, performed without wearing a respirator, is intended to determine whether the individual being tested can detect the taste of Bitrex.
(1) During threshold screening as well as during fit testing, subjects shall wear an enclosure about the head and shoulders that is approximately 12 inches (30.5 cm) in diameter by 14 inches (35.6 cm) tall. The front portion of the enclosure shall be clear from the respirator and allow free movement of the head when a respirator is worn. An enclosure substantially similar to the 3M hood assembly, parts # FT 14 and # FT 15 combined, is adequate.
(2) The test enclosure shall have a 3/4 inch (1.9 cm) hole in front of the test subject's nose and mouth area to accommodate the nebulizer nozzle.
(3) The test subject shall don the test enclosure. Throughout the threshold screening test, the test subject shall breathe through his or her slightly open mouth with tongue extended. The subject is instructed to report when he/she detects a bitter taste.
(4) Using a DeVilbiss Model 40 Inhalation Medication Nebulizer or equivalent, the test conductor shall spray the Threshold Check Solution into the enclosure. This Nebulizer shall be clearly marked to distinguish it from the fit test solution nebulizer.
(5) The Threshold Check Solution is prepared by adding 13.5 milligrams of Bitrex to 100 ml of 5% salt (NaCl) solution in distilled water.
(6) To produce the aerosol, the nebulizer bulb is firmly squeezed so that the bulb collapses completely, and is then released and allowed to fully expand.
(7) An initial ten squeezes are repeated rapidly and then the test subject is asked whether the Bitrex can be tasted. If the test subject reports tasting the bitter taste during the ten squeezes, the screening test is completed. The taste threshold is noted as ten regardless of the number of squeezes actually completed.
(8) If the first response is negative, ten more squeezes are repeated rapidly and the test subject is again asked whether the Bitrex is tasted. If the test subject reports tasting the bitter taste during the second ten squeezes, the screening test is completed. The taste threshold is noted as twenty regardless of the number of squeezes actually completed.
(9) If the second response is negative, ten more squeezes are repeated rapidly and the test subject is again asked whether the Bitrex is tasted. If the test subject reports tasting the bitter taste during the third set of ten squeezes, the screening test is completed. The taste threshold is noted as thirty regardless of the number of squeezes actually completed.
(10) The test conductor will take note of the number of squeezes required to solicit a taste response.
(11) If the Bitrex is not tasted after 30 squeezes (step 10), the test subject is unable to taste Bitrex and may not perform the Bitrex fit test.
(12) If a taste response is elicited, the test subject shall be asked to take note of the taste for reference in the fit test.

(13) Correct use of the nebulizer means that approximately 1 ml of liquid is used at a time in the nebulizer body.
(14) The nebulizer shall be thoroughly rinsed in water, shaken to dry, and refilled at least each morning and afternoon or at least every four hours.
(b) Bitrex Solution Aerosol Fit Test Procedure.
(1) The test subject may not eat, drink (except plain water), smoke, or chew gum for 15 minutes before the test.
(2) The fit test uses the same enclosure as that described in 4. (a) above.
(3) The test subject shall don the enclosure while wearing the respirator selected according to section I. A. of this appendix. The respirator shall be properly adjusted and equipped with any type particulate filter(s).
(4) A second DeVilbiss Model 40 Inhalation Medication Nebulizer or equivalent is used to spray the fit test solution into the enclosure. This nebulizer shall be clearly marked to distinguish it from the screening test solution nebulizer.
(5) The fit test solution is prepared by adding 337.5 mg of Bitrex to 200 ml of a 5% salt (NaCl) solution in warm water.
(6) As before, the test subject shall breathe through his or her slightly open mouth with tongue extended, and be instructed to report if he/she tastes the bitter taste of Bitrex..
(7) The nebulizer is inserted into the hole in the front of the enclosure and an initial concentration of the fit test solution is sprayed into the enclosure using the same number of squeezes (either 10, 20 or 30 squeezes) based on the number of squeezes required to elicit a taste response as noted during the screening test.
(8) After generating the aerosol, the test subject shall be instructed to perform the exercises in section I. A. 14. of this appendix.
(9) Every 30 seconds the aerosol concentration shall be replenished using one half the number of squeezes used initially (e.g., 5, 10 or 15).
(10) The test subject shall indicate to the test conductor if at any time during the fit test the taste of Bitrex is detected. If the test subject does not report tasting the Bitrex, the test is passed.
(11) If the taste of Bitrex is detected, the fit is deemed unsatisfactory and the test is failed. A different respirator shall be tried and the entire test procedure is repeated (taste threshold screening and fit testing).
5. Irritant Smoke (Stannic Chloride) Protocol
This qualitative fit test uses a person's response to the irritating chemicals released in the "smoke" produced by a stannic chloride ventilation smoke tube to detect leakage into the respirator.
(a) General Requirements and Precautions
(1) The respirator to be tested shall be equipped with high efficiency particulate air (HEPA) or P100 series filter(s).
(2) Only stannic chloride smoke tubes shall be used for this protocol.
(3) No form of test enclosure or hood for the test subject shall be used.
(4) The smoke can be irritating to the eyes, lungs, and nasal passages. The test conductor shall take precautions to minimize the test subject's exposure to irritant smoke. Sensitivity varies, and certain individuals may respond to a greater degree to irritant smoke. Care shall be taken when performing the sensitivity screening checks that determine whether the test subject can detect irritant smoke to use only the minimum amount of smoke necessary to elicit a response from the test subject.
(5) The fit test shall be performed in an area with adequate ventilation to prevent exposure of the person conducting the fit test or the build-up of irritant smoke in the general atmosphere.
(b) Sensitivity Screening Check
The person to be tested must demonstrate his or her ability to detect a weak concentration of the irritant smoke.

(1) The test operator shall break both ends of a ventilation smoke tube containing stannic chloride, and attach one end of the smoke tube to a low flow air pump set to deliver 200 milliliters per minute, or an aspirator squeeze bulb. The test operator shall cover the other end of the smoke tube with a short piece of tubing to prevent potential injury from the jagged end of the smoke tube.
(2) The test operator shall advise the test subject that the smoke can be irritating to the eyes, lungs, and nasal passages and instruct the subject to keep his/her eyes closed while the test is performed.
(3) The test subject shall be allowed to smell a weak concentration of the irritant smoke before the respirator is donned to become familiar with its irritating properties and to determine if he/she can detect the irritating properties of the smoke. The test operator shall carefully direct a small amount of the irritant smoke in the test subject's direction to determine that he/she can detect it.
(c) Irritant Smoke Fit Test Procedure
(1) The person being fit tested shall don the respirator without assistance, and perform the required user seal check(s).
(2) The test subject shall be instructed to keep his/her eyes closed.
(3) The test operator shall direct the stream of irritant smoke from the smoke tube toward the faceseal area of the test subject, using the low flow pump or the squeeze bulb. The test operator shall begin at least 12 inches from the facepiece and move the smoke stream around the whole perimeter of the mask. The operator shall gradually make two more passes around the perimeter of the mask, moving to within six inches of the respirator.
(4) If the person being tested has not had an involuntary response and/or detected the irritant smoke, proceed with the test exercises.
(5) The exercises identified in section I.A. 14. of this appendix shall be performed by the test subject while the respirator seal is being continually challenged by the smoke, directed around the perimeter of the respirator at a distance of six inches.
(6) If the person being fit tested reports detecting the irritant smoke at any time, the test is failed. The person being retested must repeat the entire sensitivity check and fit test procedure.
(7) Each test subject passing the irritant smoke test without evidence of a response (involuntary cough, irritation) shall be given a second sensitivity screening check, with the smoke from the same smoke tube used during the fit test, once the respirator has been removed, to determine whether he/she still reacts to the smoke. Failure to evoke a response shall void the fit test.
(8) If a response is produced during this second sensitivity check, then the fit test is passed.
C. Quantitative Fit Test (QNFT) Protocols
The following quantitative fit testing procedures have been demonstrated to be acceptable: Quantitative fit testing using a non-hazardous test aerosol (such as corn oil, polyethylene glycol 400 [PEG 400], di-2-ethyl hexyl sebacate [DEHS], or sodium chloride) generated in a test chamber, and employing instrumentation to quantify the fit of the respirator; Quantitative fit testing using ambient aerosol as the test agent and appropriate instrumentation (condensation nuclei counter) to quantify the respirator fit; Quantitative fit testing using controlled negative pressure and appropriate instrumentation to measure the volumetric leak rate of a facepiece to quantify the respirator fit.
1. General
(a) The employer shall ensure that persons administering QNFT are able to calibrate equipment and perform tests properly, recognize invalid tests, calculate fit factors properly and ensure that test equipment is in proper working order.
(b) The employer shall ensure that QNFT equipment is kept clean, and is maintained and calibrated according to the manufacturer's instructions so as to operate at the parameters for which it was designed.
2. Generated Aerosol Quantitative Fit Testing Protocol

(a) Apparatus.
(1) Instrumentation. Aerosol generation, dilution, and measurement systems using particulates (corn oil, polyethylene glycol 400 [PEG 400], di-2-ethyl hexyl sebacate [DEHS] or sodium chloride) as test aerosols shall be used for quantitative fit testing.
(2) Test chamber. The test chamber shall be large enough to permit all test subjects to perform freely all required exercises without disturbing the test agent concentration or the measurement apparatus. The test chamber shall be equipped and constructed so that the test agent is effectively isolated from the ambient air, yet uniform in concentration throughout the chamber.
(3) When testing air-purifying respirators, the normal filter or cartridge element shall be replaced with a high efficiency particulate air (HEPA) or P100 series filter supplied by the same manufacturer.
(4) The sampling instrument shall be selected so that a computer record or strip chart record may be made of the test showing the rise and fall of the test agent concentration with each inspiration and expiration at fit factors of at least 2,000. Integrators or computers that integrate the amount of test agent penetration leakage into the respirator for each exercise may be used provided a record of the readings is made.
(5) The combination of substitute air-purifying elements, test agent and test agent concentration shall be such that the test subject is not exposed in excess of an established exposure limit for the test agent at any time during the testing process, based upon the length of the exposure and the exposure limit duration.
(6) The sampling port on the test specimen respirator shall be placed and constructed so that no leakage occurs around the port (e.g., where the respirator is probed), a free air flow is allowed into the sampling line at all times, and there is no interference with the fit or performance of the respirator. The in-mask sampling device (probe) shall be designed and used so that the air sample is drawn from the breathing zone of the test subject, midway between the nose and mouth and with the probe extending into the facepiece cavity at least 1/4 inch.
(7) The test setup shall permit the person administering the test to observe the test subject inside the chamber during the test.
(8) The equipment generating the test atmosphere shall maintain the concentration of test agent constant to within a 10 percent variation for the duration of the test.
(9) The time lag (interval between an event and the recording of the event on the strip chart or computer or integrator) shall be kept to a minimum. There shall be a clear association between the occurrence of an event and its being recorded.
(10) The sampling line tubing for the test chamber atmosphere and for the respirator sampling port shall be of equal diameter and of the same material. The length of the two lines shall be equal.
(11) The exhaust flow from the test chamber shall pass through an appropriate filter (i.e., high efficiency particulate filter) before release.
(12) When sodium chloride aerosol is used, the relative humidity inside the test chamber shall not exceed 50 percent.
(13) The limitations of instrument detection shall be taken into account when determining the fit factor.
(14) Test respirators shall be maintained in proper working order and be inspected regularly for deficiencies such as cracks or missing valves and gaskets.
(b) Procedural Requirements.
(1) When performing the initial user seal check using a positive or negative pressure check, the sampling line shall be crimped closed in order to avoid air pressure leakage during either of these pressure checks.
(2) The use of an abbreviated screening QLFT test is optional. Such a test may be utilized in order to quickly identify poor fitting respirators that passed the positive and/or negative pressure test and reduce the amount of QNFT time. The use of the CNC QNFT instrument in the count

mode is another optional method to obtain a quick estimate of fit and eliminate poor fitting respirators before going on to perform a full QNFT.
(3) A reasonably stable test agent concentration shall be measured in the test chamber prior to testing. For canopy or shower curtain types of test units, the determination of the test agent's stability may be established after the test subject has entered the test environment.
(4) Immediately after the subject enters the test chamber, the test agent concentration inside the respirator shall be measured to ensure that the peak penetration does not exceed 5 percent for a half mask or 1 percent for a full facepiece respirator.
(5) A stable test agent concentration shall be obtained prior to the actual start of testing.
(6) Respirator restraining straps shall not be over-tightened for testing. The straps shall be adjusted by the wearer without assistance from other persons to give a reasonably comfortable fit typical of normal use. The respirator shall not be adjusted once the fit test exercises begin.
(7) The test shall be terminated whenever any single peak penetration exceeds 5 percent for half masks and 1 percent for full facepiece respirators. The test subject shall be refitted and retested.
(8) Calculation of fit factors.
(i) The fit factor shall be determined for the quantitative fit test by taking the ratio of the average chamber concentration to the concentration measured inside the respirator for each test exercise except the grimace exercise.
(ii) The average test chamber concentration shall be calculated as the arithmetic average of the concentration measured before and after each test (i.e., 7 exercises) or the arithmetic average of the concentration measured before and after each exercise or the true average measured continuously during the respirator sample.
(iii) The concentration of the challenge agent inside the respirator shall be determined by one of the following methods:
(A) Average peak penetration method means the method of determining test agent penetration into the respirator utilizing a strip chart recorder, integrator, or computer. The agent penetration is determined by an average of the peak heights on the graph or by computer integration, for each exercise except the grimace exercise. Integrators or computers that calculate the actual test agent penetration into the respirator for each exercise will also be considered to meet the requirements of the average peak penetration method.
(B) Maximum peak penetration method means the method of determining test agent penetration in the respirator as determined by strip chart recordings of the test. The highest peak penetration for a given exercise is taken to be representative of average penetration into the respirator for that exercise.
(C) Integration by calculation of the area under the individual peak for each exercise except the grimace exercise. This includes computerized integration.
(D) The calculation of the overall fit factor using individual exercise fit factors involves first converting the exercise fit factors to penetration values, determining the average, and then converting that result back to a fit factor. This procedure is described in the following equation:

$$\text{Overall Fit Factor} = \frac{\text{Number of exercises}}{1/ff1+1/ff2+1/ff3+1/ff4+1/ff5+1/ff7+1/ff8}$$

Where ff[1], ff[2], ff[3], etc. are the fit factors for exercises 1, 2, 3, etc.
(9) The test subject shall not be permitted to wear a half mask or quarter facepiece respirator unless a minimum fit factor of 100 is obtained, or a full facepiece respirator unless a minimum fit factor of 500 is obtained.
(10) Filters used for quantitative fit testing shall be replaced whenever increased breathing resistance is encountered, or when the test agent has altered the integrity of the filter media.
3. Ambient aerosol condensation nuclei counter (CNC) quantitative fit testing protocol.

The ambient aerosol condensation nuclei counter (CNC) quantitative fit testing (Portacount<TM>) protocol quantitatively fit tests respirators with the use of a probe. The probed respirator is only used for quantitative fit tests. A probed respirator has a special sampling device, installed on the respirator that allows the probe to sample the air from inside the mask. A probed respirator is required for each make, style, model, and size that the employer uses and can be obtained from the respirator manufacturer or distributor. The CNC instrument manufacturer, TSI Inc., also provides probe attachments (TSI sampling adapters) that permit fit testing in an employee's own respirator. A minimum fit factor pass level of at least 100 is necessary for a half-mask respirator and a minimum fit factor pass level of at least 500 is required for a full facepiece negative pressure respirator. The entire screening and testing procedure shall be explained to the test subject prior to the conduct of the screening test.
(a) Portacount Fit Test Requirements.
(1) Check the respirator to make sure the sampling probe and line are properly attached to the facepiece and that the respirator is fitted with a particulate filter capable of preventing significant penetration by the ambient particles used for the fit test (e.g., NIOSH 42 CFR 84 series 100, series 99, or series 95 particulate filter) per manufacturer's instruction.
(2) Instruct the person to be tested to don the respirator for five minutes before the fit test starts. This purges the ambient particles trapped inside the respirator and permits the wearer to make certain the respirator is comfortable. This individual shall already have been trained on how to wear the respirator properly.
(3) Check the following conditions for the adequacy of the respirator fit: Chin properly placed; Adequate strap tension, not overly tightened; Fit across nose bridge; Respirator of proper size to span distance from nose to chin; Tendency of the respirator to slip; Self-observation in a mirror to evaluate fit and respirator position.
(4) Have the person wearing the respirator do a user seal check. If leakage is detected, determine the cause. If leakage is from a poorly fitting facepiece, try another size of the same model respirator, or another model of respirator.
(5) Follow the manufacturer's instructions for operating the Portacount and proceed with the test.
(6) The test subject shall be instructed to perform the exercises in section I. A. 14. of this appendix.
(7) After the test exercises, the test subject shall be questioned by the test conductor regarding the comfort of the respirator upon completion of the protocol. If it has become unacceptable, another model of respirator shall be tried.
(b) Portacount Test Instrument.
(1) The Portacount will automatically stop and calculate the overall fit factor for the entire set of exercises. The overall fit factor is what counts. The Pass or Fail message will indicate whether or not the test was successful. If the test was a Pass, the fit test is over.
(2) Since the pass or fail criterion of the Portacount is user programmable, the test operator shall ensure that the pass or fail criterion meet the requirements for minimum respirator performance in this Appendix.
(3) A record of the test needs to be kept on file, assuming the fit test was successful. The record must contain the test subject's name; overall fit factor; make, model, style, and size of respirator used; and date tested.
4. Controlled negative pressure (CNP) quantitative fit testing protocol.
The CNP protocol provides an alternative to aerosol fit test methods. The CNP fit test method technology is based on exhausting air from a temporarily sealed respirator facepiece to generate and then maintain a constant negative pressure inside the facepiece. The rate of air exhaust is controlled so that a constant negative pressure is maintained in the respirator during the fit test. The level of pressure is selected to replicate the mean inspiratory pressure that causes leakage into the respirator under normal use conditions. With pressure held constant, air

flow out of the respirator is equal to air flow into the respirator. Therefore, measurement of the exhaust stream that is required to hold the pressure in the temporarily sealed respirator constant yields a direct measure of leakage air flow into the respirator. The CNP fit test method measures leak rates through the facepiece as a method for determining the facepiece fit for negative pressure respirators. The CNP instrument manufacturer Dynatech Nevada also provides attachments (sampling manifolds) that replace the filter cartridges to permit fit testing in an employee's own respirator. To perform the test, the test subject closes his or her mouth and holds his/her breath, after which an air pump removes air from the respirator facepiece at a pre-selected constant pressure. The facepiece fit is expressed as the leak rate through the facepiece, expressed as milliliters per minute. The quality and validity of the CNP fit tests are determined by the degree to which the in-mask pressure tracks the test pressure during the system measurement time of approximately five seconds. Instantaneous feedback in the form of a real-time pressure trace of the in-mask pressure is provided and used to determine test validity and quality. A minimum fit factor pass level of 100 is necessary for a half-mask respirator and a minimum fit factor of at least 500 is required for a full facepiece respirator. The entire screening and testing procedure shall be explained to the test subject prior to the conduct of the screening test.

(a) CNP Fit Test Requirements.

(1) The instrument shall have a non-adjustable test pressure of 15.0 mm water pressure.

(2) The CNP system defaults selected for test pressure shall be set at -15 mm of water (-0.58 inches of water) and the modeled inspiratory flow rate shall be 53.8 liters per minute for performing fit tests.

(Note: CNP systems have built-in capability to conduct fit testing that is specific to unique work rate, mask, and gender situations that might apply in a specific workplace. Use of system default values, which were selected to represent respirator wear with medium cartridge resistance at a low-moderate work rate, will allow inter-test comparison of the respirator fit.)

(3) The individual who conducts the CNP fit testing shall be thoroughly trained to perform the test.

(4) The respirator filter or cartridge needs to be replaced with the CNP test manifold. The inhalation valve downstream from the manifold either needs to be temporarily removed or propped open.

(5) The test subject shall be trained to hold his or her breath for at least 20 seconds.

(6) The test subject shall don the test respirator without any assistance from the individual who conducts the CNP fit test.

(7) The QNFT protocol shall be followed according to section I. C. 1. of this appendix with an exception for the CNP test exercises.

(b) CNP Test Exercises.

(1) Normal breathing. In a normal standing position, without talking, the subject shall breathe normally for 1 minute. After the normal breathing exercise, the subject needs to hold head straight ahead and hold his or her breath for 10 seconds during the test measurement.

(2) Deep breathing. In a normal standing position, the subject shall breathe slowly and deeply for 1 minute, being careful not to hyperventilate. After the deep breathing exercise, the subject shall hold his or her head straight ahead and hold his or her breath for 10 seconds during test measurement.

(3) Turning head side to side. Standing in place, the subject shall slowly turn his or her head from side to side between the extreme positions on each side for 1 minute. The head shall be held at each extreme momentarily so the subject can inhale at each side. After the turning head side to side exercise, the subject needs to hold head full left and hold his or her breath for 10 seconds during test measurement. Next, the subject needs to hold head full right and hold his or her breath for 10 seconds during test measurement.

(4) Moving head up and down. Standing in place, the subject shall slowly move his or her head up and down for 1 minute. The subject shall be instructed to inhale in the up position (i.e., when looking toward the ceiling). After the moving head up and down exercise, the subject shall hold his or her head full up and hold his or her breath for 10 seconds during test measurement. Next, the subject shall hold his or her head full down and hold his or her breath for 10 seconds during test measurement.
(5) Talking. The subject shall talk out loud slowly and loud enough so as to be heard clearly by the test conductor. The subject can read from a prepared text such as the Rainbow Passage, count backward from 100, or recite a memorized poem or song for 1 minute. After the talking exercise, the subject shall hold his or her head straight ahead and hold his or her breath for 10 seconds during the test measurement.
(6) Grimace. The test subject shall grimace by smiling or frowning for 15 seconds.
(7) Bending Over. The test subject shall bend at the waist as if he or she were to touch his or her toes for 1 minute. Jogging in place shall be substituted for this exercise in those test environments such as shroud-type QNFT units that prohibit bending at the waist. After the bending over exercise, the subject shall hold his or her head straight ahead and hold his or her breath for 10 seconds during the test measurement.
(8) Normal Breathing. The test subject shall remove and re-don the respirator within a one-minute period. Then, in a normal standing position, without talking, the subject shall breathe normally for 1 minute. After the normal breathing exercise, the subject shall hold his or her head straight ahead and hold his or her breath for 10 seconds during the test measurement. After the test exercises, the test subject shall be questioned by the test conductor regarding the comfort of the respirator upon completion of the protocol. If it has become unacceptable, another model of a respirator shall be tried.
(c) CNP Test Instrument.
(1) The test instrument shall have an effective audio warning device when the test subject fails to hold his or her breath during the test. The test shall be terminated whenever the test subject failed to hold his or her breath. The test subject may be refitted and retested.
(2) A record of the test shall be kept on file, assuming the fit test was successful. The record must contain the test subject's name; overall fit factor; make, model, style and size of respirator used; and date tested.
Part II. New Fit Test Protocols
A. Any person may submit to OSHA an application for approval of a new fit test protocol. If the application meets the following criteria, OSHA will initiate a rulemaking proceeding under section 6(b)(7) of the OSH Act to determine whether to list the new protocol as an approved protocol in this Appendix A.
B. The application must include a detailed description of the proposed new fit test protocol. This application must be supported by either:
1. A test report prepared by an independent government research laboratory (e.g., Lawrence Livermore National Laboratory, Los Alamos National Laboratory, the National Institute for Standards and Technology) stating that the laboratory has tested the protocol and had found it to be accurate and reliable; or
2. An article that has been published in a peer-reviewed industrial hygiene journal describing the protocol and explaining how test data support the protocol's accuracy and reliability.
C. If OSHA determines that additional information is required before the Agency commences a rulemaking proceeding under this section, OSHA will so notify the applicant and afford the applicant the opportunity to submit the supplemental information. Initiation of a rulemaking proceeding will be deferred until OSHA has received and evaluated the supplemental information.



One Ton Chlorine Cylinders

These are now a target for terrorists to steal and use against human targets. You can best figure that this threat will increase the cost of chlorine and security also. A funny side note is every once in a while you will find a chlorine containers from World War II, used both for water disinfection and for a weapon, just in case. I guess history is repeating itself at the cost of civilians and taxpayers.

Appendix B-1 to § 1910.134: User Seal Check Procedures (Mandatory)

The individual who uses a tight-fitting respirator is to perform a user seal check to ensure that an adequate seal is achieved each time the respirator is put on. Either the positive and negative pressure checks listed in this appendix, or the respirator manufacturer's recommended user seal check method shall be used. User seal checks are not substitutes for qualitative or quantitative fit tests.

I. Facepiece Positive and/or Negative Pressure Checks

A. Positive pressure check. Close off the exhalation valve and exhale gently into the facepiece. The face fit is considered satisfactory if a slight positive pressure can be built up inside the facepiece without any evidence of outward leakage of air at the seal. For most respirators this method of leak testing requires the wearer to first remove the exhalation valve cover before closing off the exhalation valve and then carefully replacing it after the test.

B. Negative pressure check. Close off the inlet opening of the canister or cartridge(s) by covering with the palm of the hand(s) or by replacing the filter seal(s), inhale gently so that the facepiece collapses slightly, and hold the breath for ten seconds. The design of the inlet opening of some cartridges cannot be effectively covered with the palm of the hand. The test can be performed by covering the inlet opening of the cartridge with a thin latex or nitrile glove. If the facepiece remains in its slightly collapsed condition and no inward leakage of air is detected, the tightness of the respirator is considered satisfactory.

II. Manufacturer's Recommended User Seal Check Procedures

The respirator manufacturer's recommended procedures for performing a user seal check may be used instead of the positive and/or negative pressure check procedures provided that the employer demonstrates that the manufacturer's procedures are equally effective.

Appendix B-2 to § 1910.134: Respirator Cleaning Procedures (Mandatory)

These procedures are provided for employer use when cleaning respirators. They are general in nature, and the employer as an alternative may use the cleaning recommendations provided by the manufacturer of the respirators used by their employees, provided such procedures are as effective as those listed here in Appendix B-2. Equivalent effectiveness simply means that the procedures used must accomplish the objectives set forth in Appendix B-2, i.e., must ensure that the respirator is properly cleaned and disinfected in a manner that prevents damage to the respirator and does not cause harm to the user.

I. Procedures for Cleaning Respirators

A. Remove filters, cartridges, or canisters. Disassemble facepieces by removing speaking diaphragms, demand and pressure-demand valve assemblies, hoses, or any components recommended by the manufacturer. Discard or repair any defective parts.

B. Wash components in warm (43 [degrees] C [110 [degrees] F] maximum) water with a mild detergent or with a cleaner recommended by the manufacturer. A stiff bristle (not wire) brush may be used to facilitate the removal of dirt.

C. Rinse components thoroughly in clean, warm (43 [degrees] C [110 [degrees] F] maximum), preferably running water. Drain.

D. When the cleaner used does not contain a disinfecting agent, respirator components should be immersed for two minutes in one of the following:

1. Hypochlorite solution (50 ppm of chlorine) made by adding approximately one milliliter of laundry bleach to one liter of water at 43 [degrees] C (110 [degrees] F); or,

2. Aqueous solution of iodine (50 ppm iodine) made by adding approximately 0.8 milliliters of tincture of iodine (6-8 grams ammonium and/or potassium iodide/100 cc of 45% alcohol) to one liter of water at 43 [degrees] C (110 [degrees] F); or,

3. Other commercially available cleansers of equivalent disinfectant quality when used as directed, if their use is recommended or approved by the respirator manufacturer.

E. Rinse components thoroughly in clean, warm (43 [degrees] C [110 [degrees] F] maximum), preferably running water. Drain. The importance of thorough rinsing cannot be overemphasized. Detergents or disinfectants that dry on facepieces may result in dermatitis. In addition, some disinfectants may cause deterioration of rubber or corrosion of metal parts if not completely removed.
F. Components should be hand-dried with a clean lint-free cloth or air-dried.
G. Reassemble facepiece, replacing filters, cartridges, and canisters where necessary.
H. Test the respirator to ensure that all components work properly.

Appendix C to § 1910.134: OSHA Respirator Medical Evaluation Questionnaire (Mandatory)

To the employer: Answers to questions in Section 1, and to question 9 in Section 2 of Part A, do not require a medical examination.

To the employee:
Can you read (circle one): Yes/No
Your employer must allow you to answer this questionnaire during normal working hours, or at a time and place that is convenient to you. To maintain your confidentiality, your employer or supervisor must not look at or review your answers, and your employer must tell you how to deliver or send this questionnaire to the health care professional who will review it.
Part A. Section 1. (Mandatory) The following information must be provided by every employee who has been selected to use any type of respirator (please print).
1. Today's date: ----------
2. Your name: ---------- -
3. Your age (to nearest year): ----------
4. Sex (circle one): Male/Female
5. Your height: ----- ft. ----- in.
6. Your weight: ------ lbs.
7. Your job title: ----------
8. A phone number where you can be reached by the health care professional who reviews this questionnaire (include the Area Code): ----------
9. The best time to phone you at this number: --------
10. Has your employer told you how to contact the health care professional who will review this questionnaire (circle one): Yes/No
11. Check the type of respirator you will use (you can check more than one category):
a. --- N, R, or P disposable respirator (filter-mask, non-cartridge type only).
b. --- Other type (for example, half- or full-facepiece type, powered-air purifying, supplied-air, self-contained breathing apparatus).
12. Have you worn a respirator (circle one): Yes/No
If "yes," what type(s): ----------
---------- ----------

Part A. Section 2. (Mandatory) Questions 1 through 9 below must be answered by every employee who has been selected to use any type of respirator (please circle "yes" or "no").

1. Do you currently smoke tobacco, or have you smoked tobacco in the last month: Yes/No
2. Have you ever had any of the following conditions?
a. Seizures (fits): Yes/No
b. Diabetes (sugar disease): Yes/No
c. Allergic reactions that interfere with your breathing: Yes/No
d. Claustrophobia (fear of closed-in places): Yes/No
e. Trouble smelling odors: Yes/No
3. Have you ever had any of the following pulmonary or lung problems?
a. Asbestosis: Yes/No
b. Asthma: Yes/No
c. Chronic bronchitis: Yes/No

d. Emphysema: Yes/No
e. Pneumonia: Yes/No
f. Tuberculosis: Yes/No
g. Silicosis: Yes/No
h. Pneumothorax (collapsed lung): Yes/No
i. Lung cancer: Yes/No
j. Broken ribs: Yes/No
k. Any chest injuries or surgeries: Yes/No
l. Any other lung problem that you've been told about: Yes/No
4. Do you currently have any of the following symptoms of pulmonary or lung illness?
a. Shortness of breath: Yes/No
b. Shortness of breath when walking fast on level ground or walking up a slight hill or incline: Yes/No
c. Shortness of breath when walking with other people at an ordinary pace on level ground: Yes/No
d. Have to stop for breath when walking at your own pace on level ground: Yes/No
e. Shortness of breath when washing or dressing yourself: Yes/No
f. Shortness of breath that interferes with your job: Yes/No
g. Coughing that produces phlegm (thick sputum): Yes/No
h. Coughing that wakes you early in the morning: Yes/No
i. Coughing that occurs mostly when you are lying down: Yes/No
j. Coughing up blood in the last month: Yes/No
k. Wheezing: Yes/No
l. Wheezing that interferes with your job: Yes/No
m. Chest pain when you breathe deeply: Yes/No
n. Any other symptoms that you think may be related to lung problems: Yes/No
5. Have you ever had any of the following cardiovascular or heart problems?
a. Heart attack: Yes/No
b. Stroke: Yes/No
c. Angina: Yes/No
d. Heart failure: Yes/No
e. Swelling in your legs or feet (not caused by walking): Yes/No
f. Heart arrhythmia (heart beating irregularly): Yes/No
g. High blood pressure: Yes/No
h. Any other heart problem that you've been told about: Yes/No
6. Have you ever had any of the following cardiovascular or heart symptoms?
a. Frequent pain or tightness in your chest: Yes/No
b. Pain or tightness in your chest during physical activity: Yes/No
c. Pain or tightness in your chest that interferes with your job: Yes/No
d. In the past two years, have you noticed your heart skipping or missing a beat: Yes/No
e. Heartburn or indigestion that is not related to eating: Yes/No
f. Any other symptoms that you think may be related to heart or circulation problems: Yes/No
7. Do you currently take medication for any of the following problems?
a. Breathing or lung problems: Yes/No
b. Heart trouble: Yes/No

c. Blood pressure: Yes/No
d. Seizures (fits): Yes/No
8. If you've used a respirator, have you ever had any of the following problems? (If you've never used a respirator, check the following space and go to question 9:)
a. Eye irritation: Yes/No
b. Skin allergies or rashes: Yes/No
c. Anxiety: Yes/No
d. General weakness or fatigue: Yes/No
e. Any other problem that interferes with your use of a respirator: Yes/No
9. Would you like to talk to the health care professional who will review this questionnaire about your answers to this questionnaire: Yes/No
Questions 10 to 15 below must be answered by every employee who has been selected to use either a full-facepiece respirator or a self-contained breathing apparatus (SCBA). For employees who have been selected to use other types of respirators, answering these questions is voluntary.
10. Have you ever lost vision in either eye (temporarily or permanently): Yes/No
11. Do you currently have any of the following vision problems?
a. Wear contact lenses: Yes/No
b. Wear glasses: Yes/No
c. Color blind: Yes/No
d. Any other eye or vision problem: Yes/No
12. Have you ever had an injury to your ears, including a broken ear drum: Yes/No
13. Do you currently have any of the following hearing problems?
a. Difficulty hearing: Yes/No
b. Wear a hearing aid: Yes/No
c. Any other hearing or ear problem: Yes/No
14. Have you ever had a back injury: Yes/No
15. Do you currently have any of the following musculoskeletal problems?
a. Weakness in any of your arms, hands, legs, or feet: Yes/No
b. Back pain: Yes/No
c. Difficulty fully moving your arms and legs: Yes/No
d. Pain or stiffness when you lean forward or backward at the waist: Yes/No
e. Difficulty fully moving your head up or down: Yes/No
f. Difficulty fully moving your head side to side: Yes/No
g. Difficulty bending at your knees: Yes/No
h. Difficulty squatting to the ground: Yes/No
i. Climbing a flight of stairs or a ladder carrying more than 25 lbs: Yes/No
j. Any other muscle or skeletal problem that interferes with using a respirator: Yes/No
Part B Any of the following questions, and other questions not listed, may be added to the questionnaire at the discretion of the health care professional who will review the questionnaire.
1. In your present job, are you working at high altitudes (over 5,000 feet) or in a place that has lower than normal amounts of oxygen: Yes/No
If "yes," do you have feelings of dizziness, shortness of breath, pounding in your chest, or other symptoms when you're working under these conditions: Yes/No

2. At work or at home, have you ever been exposed to hazardous solvents, hazardous airborne chemicals (e.g., gases, fumes, or dust), or have you come into skin contact with hazardous chemicals: Yes/No
If "yes," name the chemicals if you know them: ----------
---------- ----------
---------- ----------
3. Have you ever worked with any of the materials, or under any of the conditions, listed below:
a. Asbestos: Yes/No
b. Silica (e.g., in sandblasting): Yes/No
c. Tungsten/cobalt (e.g., grinding or welding this material): Yes/No
d. Beryllium: Yes/No
e. Aluminum: Yes/No
f. Coal (for example, mining): Yes/No
g. Iron: Yes/No
h. Tin: Yes/No
i. Dusty environments: Yes/No
j. Any other hazardous exposures: Yes/No
If "yes," describe these exposures: ----------
---------- ----------
---------- ----------
4. List any second jobs or side businesses you have:----------
---------- ----------
5. List your previous occupations:----------
---------- ----------
6. List your current and previous hobbies:----------
---------- ----------
7. Have you been in the military services? Yes/No
If "yes," were you exposed to biological or chemical agents (either in training or combat): Yes/No
8. Have you ever worked on a HAZMAT team? Yes/No
9. Other than medications for breathing and lung problems, heart trouble, blood pressure, and seizures mentioned earlier in this questionnaire, are you taking any other medications for any reason (including over-the-counter medications): Yes/No
If "yes," name the medications if you know them: ----------
10. Will you be using any of the following items with your respirator(s)?
a. HEPA Filters: Yes/No
b. Canisters (for example, gas masks): Yes/No
c. Cartridges: Yes/No
11. How often are you expected to use the respirator(s) (circle "yes" or "no" for all answers that apply to you)?:
a. Escape only (no rescue): Yes/No
b. Emergency rescue only: Yes/No
c. Less than 5 hours per week: Yes/No
d. Less than 2 hours per day: Yes/No
e. 2 to 4 hours per day: Yes/No

f. Over 4 hours per day: Yes/No
12. During the period you are using the respirator(s), is your work effort:
a. Light (less than 200 kcal per hour): Yes/No
If "yes," how long does this period last during the average shift:------ hrs.------ mins.
Examples of a light work effort are sitting while writing, typing, drafting, or performing light assembly work; or standing while operating a drill press (1-3 lbs.) or controlling machines.
b. Moderate (200 to 350 kcal per hour): Yes/No
If "yes," how long does this period last during the average shift:------ hrs.------ mins.
Examples of moderate work effort are sitting while nailing or filing; driving a truck or bus in urban traffic; standing while drilling, nailing, performing assembly work, or transferring a moderate load (about 35 lbs.) at trunk level; walking on a level surface about 2 mph or down a 5-degree grade about 3 mph; or pushing a wheelbarrow with a heavy load (about 100 lbs.) on a level surface.
c. Heavy (above 350 kcal per hour): Yes/No
If "yes," how long does this period last during the average shift:------ hrs.------ mins.
Examples of heavy work are lifting a heavy load (about 50 lbs.) from the floor to your waist or shoulder; working on a loading dock; shoveling; standing while bricklaying or chipping castings; walking up an 8-degree grade about 2 mph; climbing stairs with a heavy load (about 50 lbs.).
13. Will you be wearing protective clothing and/or equipment (other than the respirator) when you're using your respirator: Yes/No
If "yes," describe this protective clothing and/or equipment:----------
---------- ----------
14. Will you be working under hot conditions (temperature exceeding 77 [degrees] F): Yes/No
15. Will you be working under humid conditions: Yes/No
16. Describe the work you'll be doing while you're using your respirator(s):
---------- ----------
---------- ----------
17. Describe any special or hazardous conditions you might encounter when you're using your respirator(s) (for example, confined spaces, life-threatening gases):
---------- ----------
---------- ----------
18. Provide the following information, if you know it, for each toxic substance that you'll be exposed to when you're using your respirator(s):
Name of the first toxic substance: ----------
Estimated maximum exposure level per shift: ----------
Duration of exposure per shift ----------
Name of the second toxic substance: ----------
Estimated maximum exposure level per shift: ----------
Duration of exposure per shift: ----------
Name of the third toxic substance: ----------
Estimated maximum exposure level per shift: ----------
Duration of exposure per shift: ----------

The name of any other toxic substances that you'll be exposed to while using your respirator:
---------- ----------
---------- ----------
---------- ----------
19. Describe any special responsibilities you'll have while using your respirator(s) that may affect the safety and well-being of others (for example, rescue, and security):

Appendix D to § 1910.134 (Mandatory)

Information for Employees Using Respirators When Not Required Under the Standard
Respirators are an effective method of protection against designated hazards when properly selected and worn. Respirator use is encouraged, even when exposures are below the exposure limit, to provide an additional level of comfort and protection for workers. However, if a respirator is used improperly or not kept clean, the respirator itself can become a hazard to the worker. Sometimes, workers may wear respirators to avoid exposures to hazards, even if the amount of hazardous substance does not exceed the limits set by OSHA standards. If your employer provides respirators for your voluntary use, or if you provide your own respirator, you need to take certain precautions to be sure that the respirator itself does not present a hazard.
You should do the following:
1. Read and heed all instructions provided by the manufacturer on use, maintenance, cleaning and care, and warnings regarding the respirators limitations.
2. Choose respirators certified for use to protect against the contaminant of concern. NIOSH, the National Institute for Occupational Safety and Health of the U.S. Department of Health and Human Services, certifies respirators. A label or statement of certification should appear on the respirator or respirator packaging. It will tell you what the respirator is designed for and how much it will protect you.
3. Do not wear your respirator into atmospheres containing contaminants for which your respirator is not designed to protect against. For example, a respirator designed to filter dust particles will not protect you against gases, vapors, or very small solid particles of fumes or smoke.
4. Keep track of your respirator so that you do not mistakenly use someone else's respirator



Summary

- Since the terrorist attacks of September 11th, water systems are giving greater attention to security at their facilities. These systems must consider threats such as chemical or biological contamination of the water supply, as well as the physical disruption of treatment or distribution systems. Chemical transportation, storage and handling may also pose security challenges as well.
- Most drinking water systems have completed or are in the process of conducting assessments of their vulnerability to terrorist attack or other security threats. Strengthening the physical security of treatment plants and distribution systems are top priorities.
- Several treatment plants in the United States have switched from chlorine gas to sodium hypochlorite disinfection. While change had been planned for some time, heightened security concerns following the terrorist attacks in September of 2001have led facility personnel to accelerate its implementation.
- The change to sodium hypochlorite requires a large capital investment for new equipment. In addition, the annual cost of purchasing chemicals has increased from gas chlorine for sodium hypochlorite.
- Local circumstances will dictate rather this is a good investment or not i.e. size of plant, amount of chlorine gas kept on site, surrounding community, how secure the plant is and etc.



Respiratory Protection Quiz

The following questions will come from the definitions section. This section is to test your respiratory protection knowledge. It is not part of your final assignment. Just an exercise to test your RP knowledge.

1. _____: A system that warns the respirator user of the approach of the end of adequate respiratory protection; for example, that the sorbent is approaching saturation or is no longer effective.

2. _____: A gas mask that consists of a half-mask facepiece or mouthpiece, a canister, and associated connections, and that is designed for use during escape-only from hazardous atmospheres.

3. _____: Respiratory devices that are designed for use only during escape from hazardous atmospheres.

4. _____: A respirator intended to be used only for emergency exit.

5. _____: The penetration of challenge material(s) through a gas or a vapor air-purifying element. The quantity or extent of _____ during service life testing is often referred to as the percentage of the input concentration.

6. _____: A component used in respirators to remove solid or liquid aerosols from the inspired air.

7. _____: A particulate respirator with a filter as an integral part of the facepiece or with the entire facepiece composed of the filtering medium.

8. _____: A negative pressure particulate respirator with a filter as an integral part of the facepiece or with the entire facepiece composed of the filtering medium.

9. _____: A container with a filter, sorbent, or catalyst, or combination of these items, which removes specific contaminants from the air passed through the container.

10. _____: An atmosphere-supplying respirator that admits breathing air to the facepiece only when a negative pressure is created inside the facepiece by inhalation.

11. _____: A respirator that is discarded after the end of its recommended period of use, after excessive resistance or physical damage, or when odor breakthrough or other warning indicators render the respirator unsuitable for further use.

12. _____: A respirator with an air-purifying filter, cartridge, or canister that removes specific air contaminants by passing ambient air through the air-purifying element.

13. _____: A respirator that supplies the respirator user with breathing air from a source independent of the ambient atmosphere, and includes supplied-air respirators (SARs) and self-contained breathing apparatus (SCBA) units.

14. _____: A solid, mechanically produced particle with a size ranging from submicroscopic to macroscopic.

15. _____: A situation that requires the use of respirators due to the unplanned generation of a hazardous atmosphere (often of unknown composition) caused by an accident, mechanical failure, or other means and that requires evacuation of personnel or immediate entry for rescue or corrective action.

16. _____: Any occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment that may or does result in an uncontrolled significant release of an airborne contaminant.

17. _____: Exposure to a concentration of an airborne contaminant that would occur if the employee was not using respiratory protection.

18. _____: A filter that is at least 99.97% efficient in removing monodisperse particles of 0.3 micrometers in diameter. The equivalent NIOSH 42 CFR 84 particulate filters are the N100, R100, and P100 filters.

19. _____: A respiratory inlet covering that completely covers the head and neck and may also cover portions of the shoulders and torso.

20. _____: Acute respiratory exposure that poses an immediate threat of loss of life, immediate or delayed irreversible adverse effects on health, or acute eye exposure that would prevent escape from a hazardous atmosphere.

21. _____: An atmosphere that poses an immediate threat to life, would cause irreversible adverse health effects, or would impair an individual's ability to escape from a dangerous atmosphere.

22. _____: The physical activity of fire suppression, rescue or both, inside of buildings or enclosed structures which are involved in a fire situation beyond the incipient stage.

23. _____: A respiratory inlet covering that is designed to form a partial seal with the face.

24. _____: A quantitative measure of the fit of a specific respirator facepiece to a particular individual.

25. _____: A quantitative estimate of the fit of a particular respirator to a specific individual, and typically estimates the ratio of the concentration of a substance in ambient air to its concentration inside the respirator when worn.

26. _____: Means the use of a protocol to qualitatively or quantitatively evaluate the fit of a respirator on an individual.

27. _____: A solid condensation particulate, usually of a vaporized metal.

28. _____: An aeriform fluid that is in a gaseous state and at standard temperature and pressure.

29. _____: A rigid respiratory inlet covering that also provides head protection against impact and penetration.

30. _____: A liquid condensation particulate.

31. _____: A respirator in which the air pressure inside the facepiece is negative during inhalation this is with respect to the ambient air pressure outside the respirator.

32. _____: A respirator that covers the nose and mouth and generally consists of a quarter- or half-facepiece.

33. _____: An atmosphere with an oxygen content below 19.5% by volume.

34. _____: An individual whose legally permitted scope of practice (i.e., license, registration, or certification) allows him or her to independently provide, or be delegated the responsibility to provide, some or all of the health care services required by paragraph (e) of this section.

35. A situation in which respiratory devices are recommended to provide adequate protection to workers entering an area where the contaminant concentration is above the_____ or is unknown.

36. _____: A respirator in which the pressure inside the respiratory inlet covering exceeds the ambient air pressure outside the respirator.

37. _____: Any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory, or dermal exposure, or any

other exposure which results in the induction of tumors at a site other than the site of administration.

38. _____: An air-purifying respirator that uses a blower to force the ambient air through air-purifying elements in to the inlet covering.

39. _____: A positive pressure atmosphere-supplying respirator that admits breathing air to the facepiece when the positive pressure is reduced inside the facepiece by inhalation.

40. _____: The minimum anticipated protection provided by a properly functioning respirator or class of respirators to a given percentage of properly fitted and trained users.

41. _____: A surrogate measure of the workplace protection provided by a respirator.

42. _____: A measure of the protection provided in the workplace by a properly functioning respirator when it is correctly worn and used.

43. _____: A pass/fail fit test to assess the adequacy of respirator fit that relies on the individual's response to the test agent.

44. _____: An assessment of the adequacy of respirator fit by numerically measuring the amount of leakage into the respirator.

45. _____: The portion of a respirator that forms the protective barrier between the user's respiratory tract and an air-purifying device or breathing air source, or both. It may be a facepiece, a helmet, a hood, a suit, or a mouthpiece respirator with nose clamp.

46. _____: An atmosphere-supplying respirator for which the breathing air source is designed to be carried by the user.

47. _____: The length of time required for an air-purifying element to reach a specific effluent concentration.

48. _____: The period of time that a respirator, filter or sorbent, or other respiratory equipment provides adequate protection to the wearer.

49. _____: Respirators approved for use against dusts or mists that may cause pneumoconiosis and fibrosis.

50. _____: An atmosphere-supplying respirator for which the source of breathing air is not designed to be carried by the user.

51. _____: A respiratory inlet covering that forms a complete seal with the face.

52. _____: An action conducted by the respirator user to determine if the respirator is properly seated to the face.

53. _____: The gaseous state of a substance that is solid or liquid at temperatures and pressures normally encountered.

True or False Section

54. The Employee is required to retain written information regarding medical evaluations, fit testing, and the respirator program.
A. True
B. False

55. This information may facilitate employee involvement in the respirator program, assist the Employee in auditing the adequacy of the program, and provide a record for compliance determinations by the EPA.
A. True
B. False

56. Effective training for employees who are required to use respirators is helpful.
A. True
B. False

57. The training may be comprehensive, understandable, and recur bi-annually and more often if necessary.
A. True
B. False

58. Training may be provided prior to requiring the employee to use a respirator in the workplace.
A. True
B. False

The training shall ensure that each employee can demonstrate knowledge of at least the following:

59. The respirator is necessary and how improper fit, usage, or maintenance can compromise the protective effect of the respirator.
A. True
B. False

60. The limitations and capabilities of OSHA.
A. True
B. False

61. How to use the respirator effectively in emergency situations, including situations in which the respirator malfunctions.
A. True
B. False

62. How to inspect, put on and remove, use, and check the seals of a transmission.
A. True
B. False

63. What the procedures are for maintenance and storage of the respirator.
A. True
B. False

64. How not to recognize medical signs and symptoms that may not limit or prevent the effective use of respirators.
A. True
B. False

Retraining shall be conducted annually and when:
65. Changes in the workplace or the type of respirator render previous training obsolete.
A. True
B. False

66. Adequacies in the employee's knowledge or use of the respirator indicate that the employee has retained the requisite understanding or skill.
A. True
B. False

67. Other situations arise in which retraining appears necessary to ensure safe respirator use.
A. True
B. False

68. Only authorized and trained Respirators may use Respirators.
A. True
B. False

69. Those Employees may use only the Respirator that they have been trained on and properly fitted to use.
A. True
B. False

70. Only Mentally Unqualified Employees may be trained and authorized to use Respirators.
A. True
B. False

71. A pre-authorization and annual certification by a qualified physician will be required and maintained. Any changes in an Employees health or physical characteristics will be reported to the Occupational Health Department and will be evaluated by a qualified physician.
A. True
B. False

72. Only the proper prescribed dust mask or OSHA may be used for the job or work environment.
A. True
B. False

73. Employees working in environments where a sudden release of a hazardous substance is likely will wear an appropriate respirator for that hazardous substance (example: Employees working in an ammonia compressor room will have an ammonia APR respirator on their person.).
A. True
B. False

74. Only SCBAs will be used in oxygen deficient environments, environments with an unknown hazardous substance or unknown quantity of a known hazardous substance or any environment that is determined "Immediately Dangerous to Life or Health" (**IDLH**).
A. True
B. False

75. Employees with respirators loaned on "permanent check out" will be responsible for the sanitation, proper storage and security.
A. True
B. False

76. Respirators damaged by abnormal wear will be repaired or replaced by the Employee when returned.
A. True
B. False

77. The first Employee using a respirator and/or SCBA that is available for general use will be responsible for proper storage and sanitation.
A. True
B. False

78. Monthly and after each use, all respirators will be inspected with documentation to assure its availability for use.
A. True
B. False

79. All respirators will be located in a clean, convenient and sanitary location.
A. True
B. False

80. In the event that Employees must enter a confined space, work in environments with hazardous substances that would be dangerous to life or health should an RPE fail (a SCBA is required in this environment), and/or conduct a HAZMAT entry, a "***buddy system***" detail will be used with a Safety Watchman with constant voice, visual or signal line communication.
A. True
B. False

81. Employees will follow the established Emergency Response Program and/or Confined Space Entry Program when applicable.
A. True
B. False

82. Management will establish and maintain surveillance of jobs and work place conditions and degree of Employee exposure or stress to maintain the proper procedures and to provide the necessary RPE.
A. True
B. False

83. The Employer is responsible and needs to have evaluated the respiratory hazard(s) in each workplace, identified relevant workplace and user factors and has based respirator selection on these factors. Also included are estimates of employee exposures to respiratory hazard(s) and an identification of the contaminant's chemical state and physical form.
A. True
B. False

Filter Classifications - These classifications are marked on the filter or filter package

84. **R-Series: Oil Resistant.** Approved for non-oil particulate contaminants
Examples: dust, fumes, mists not containing oil.
A. True
B. False



85. **N-Series: Not Oil Resistant**
Approved for all particulate contaminants, including those containing oil Examples: dusts, mists, fumes, Time restriction of 8 hours when oils are present .
A. True
B. False

86. **K-Series: Oil Proof**
Approved for all particulate contaminants including those containing oil
Examples: dust, fumes, mists, See Manufacturer's time use restrictions on packaging.
A. True
B. False

Respirators for IDLH atmospheres.
The following respirators will be used in IDLH atmospheres:

87. A full face piece pressure demand SCBA certified by OSHA for a minimum service life of thirty hours.
A. True
B. False

88. A combination full face piece pressure demand supplied-air respirator (SAR) with auxiliary self-contained air supply.
A. True
B. False

89. Respirators provided only for escape from PEL atmospheres shall be NIOSH-certified for escape from the atmosphere in which they will be used.
A. True
B. False

90. The respirators selected shall be adequate to protect the health of the employee and ensure compliance with all other OSHA statutory and regulatory requirements, under routine and reasonably foreseeable emergency situations.
A. True
B. False

91. The respirator selected shall be appropriate for the chemical state and physical form of the contaminant.
A. True
B. False

The respirators shall be cleaned and disinfected when:
92. Respirators issued for the exclusive use of an employee shall be cleaned and disinfected as often as necessary to be maintained in a sanitary condition.
A. True
B. False

93. Respirators issued to more than one employee do not need to be cleaned and disinfected before being worn by different individuals.
A. True
B. False

94. Respirators maintained for emergency use shall be cleaned and disinfected after each use.
A. True
B. False

95. Respirators used in fit testing and training shall be cleaned and disinfected after each use.
A. True
B. False

96. Cleaning and Storage of respirators assigned to specific employees is the responsibility of OSHA.
A. True
B. False

Respirator Inspection

97. All respirators/SCBAs, both available for "***General Use***" and those on "***Permanent Check-out***", will be inspected after each use and at least monthly.

A. True
B. False

98. Should any defects be noted, the respirator/SCBA will be taken to the program Administrator.

A. True
B. False

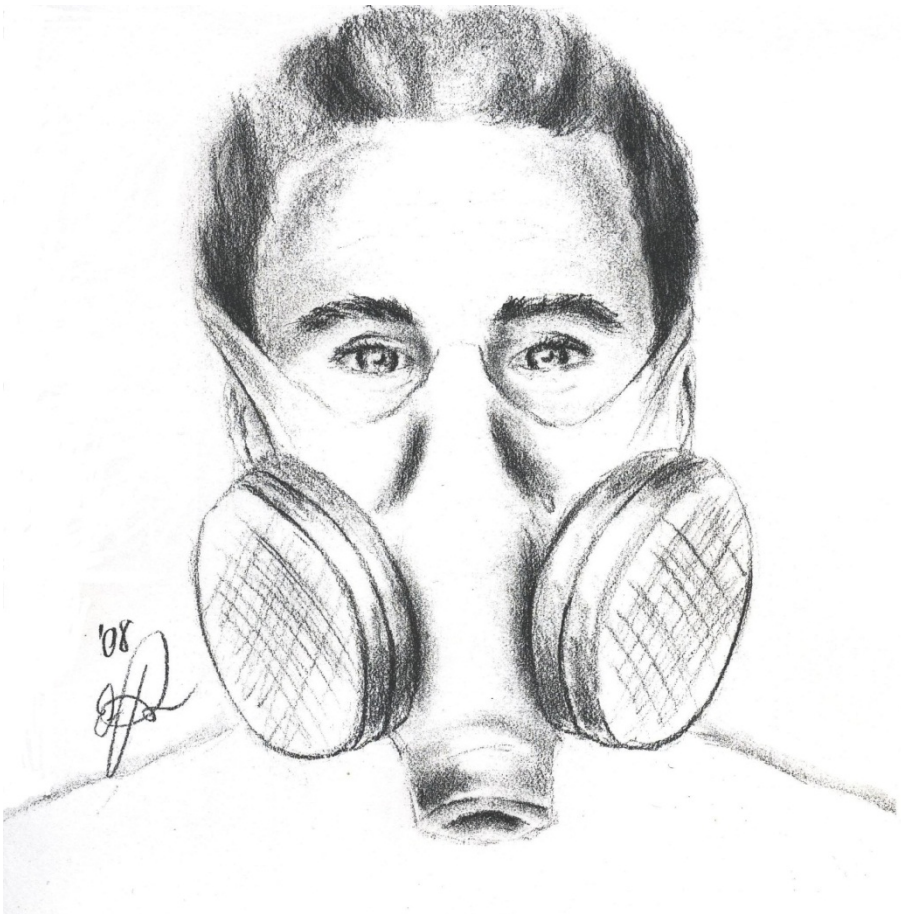
99. Damaged Respirators will be either repaired or replaced. The inspection of respirators loaned on "***Permanent Check-out***" is the responsibility of that trained Employee.

A. True
B. False

100. **Respirators shall be inspected as follows:**

All respirators used in routine situations shall be inspected before each use and during cleaning.

A. True
B. False



Disinfection Supplement

Chlorine

Upon adding chlorine to water, two chemical species, known together as free chlorine, are formed. These species, hypochlorous acid (HOCl, electrically neutral) and hypochlorite ion (OCl-, electrically negative), behave very differently. Hypochlorous acid is not only more reactive than the hypochlorite ion, but is also a stronger disinfectant and oxidant.

The ratio of hypochlorous acid to hypochlorite ion in water is determined by the pH. At low pH (higher acidity), hypochlorous acid dominates while at high pH hypochlorite ion dominates. Thus, the speed and efficacy of chlorine disinfection against pathogens may be affected by the pH of the water being treated. Fortunately, bacteria and viruses are relatively easy targets of chlorination over a wide range of pH. However, treatment operators of surface water systems treating raw water contaminated by the parasitic protozoan Giardia may take advantage of the pH-hypochlorous acid relationship and adjust the pH to be effective against Giardia, which is much more resistant to chlorination than either viruses or bacteria.

Another reason for maintaining a predominance of hypochlorous acid during treatment has to do with the fact that pathogen surfaces carry a natural negative electrical charge. These surfaces are more readily penetrated by the uncharged, electrically neutral hypochlorous acid than the negatively charged hypochlorite ion. Moving through slime coatings, cell walls and resistant shells of waterborne microorganisms, hypochlorous acid effectively destroys these pathogens. Water is made microbiologically safe as pathogens either die or are rendered incapable of reproducing. A typical bacterium has a negatively charged slime coating on its exterior cell wall, which is effectively penetrated by electrically neutral hypochlorous acid, favored by lower pH's.

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Chloramines

Chloramines are chemical compounds formed by combining a specific ratio of chlorine and ammonia in water. Because chloramines are relatively weak as a disinfectant, they are almost never used as a primary disinfectant. Chloramines provide a durable residual, and are often used as a secondary disinfectant for long distribution lines and where free chlorine demand is high. Chloramines may also be used instead of chlorine in order to reduce chlorinated byproduct formation and to remove some taste and odor problems.

Advantages

- Reduced formation of THMs, HAAs
- Will not oxidize bromide to bromine forming brominated byproducts
- More stable residual than free chlorine
- Excellent secondary disinfectant, has been found to be better than free chlorine at controlling coliform bacteria and biofilm growth
- Lower taste and odor than free chlorine

Limitations

- Weak disinfectant and oxidant
- Requires shipment and handling of ammonia or ammonia compounds as well as chlorinating chemicals
- Ammonia is toxic to fish, and may pose problems for aquarium owners
- Will cause problems for kidney dialysis if not removed from water

Chlorine Dioxide

Chlorine dioxide (ClO_2) is generated on-site at water treatment facilities. In most generators sodium chlorite and elemental chlorine are mixed in solution, which almost instantaneously forms chlorine dioxide. Chlorine dioxide characteristics are quite different from chlorine. In solution it is a dissolved gas, which makes it largely unaffected by pH but volatile and relatively easily stripped from solution. Chlorine dioxide is also a strong disinfectant and a selective oxidant. While chlorine dioxide does produce a residual it is only rarely used for this purpose.

Advantages

- ✓ Effective against Cryptosporidium
- ✓ Up to five times faster than chlorine at inactivating Giardia
- ✓ Disinfection is only moderately affected by pH
- ✓ Will not form chlorinated byproducts (THMs, HAAs)
- ✓ Does not oxidize bromide to bromine (can form bromate in sunlight)
- ✓ More effective than chlorine in treating some taste and odor problems
- ✓ Selective oxidant used for manganese oxidation and targeting some chlorine resistant organics

Limitations

- ✓ Inorganic byproduct formation (chlorite, chlorate)
- ✓ Highly volatile residuals
- ✓ Requires on-site generation equipment and handling of chemicals (chlorine and sodium chlorite)
- ✓ Requires a high level of technical competence to operate and monitoring equipment, product and residuals
- ✓ Occasionally poses unique odor and taste problems
- ✓ High operating cost (chlorite chemical cost is high)

Understanding Chlorine Basics

Chlorine is applied to water in one of three forms: elemental chlorine (chlorine gas), hypochlorite solution (bleach), or dry calcium hypochlorite. All three forms produce free chlorine in water.

Advantages

- ✓ Highly effective against most pathogens
- ✓ Provides a residual to protect against recontamination and to reduce bio-film growth in the distribution system
- ✓ Easily applied, controlled, and monitored
- ✓ Strong oxidant meeting most preoxidation objectives
- ✓ Operationally the most reliable
- ✓ The most cost-effective disinfectant

Limitations

- ✓ Byproduct formation (THMs, HAAs)
- ✓ Will oxidize bromide to bromine, forming brominated organic byproducts
- ✓ Not effective against Cryptosporidium
- ✓ Requires transport and storage of chemicals

Elemental Chlorine

Elemental chlorine is the most commonly used form of chlorine. It is transported and stored as a liquefied gas under pressure. Water treatment facilities typically use chlorine in 100 and 150-lb cylinders or one-ton containers. Some large systems use railroad tank cars or tanker trucks.

Advantages

- ✓ Lowest cost of chlorine forms
- ✓ Unlimited shelf-life

Limitations

- ✓ Hazardous gas requires special handling and operator training
- ✓ Additional regulatory requirements, including EPA's Risk Management Program and the Occupational Safety and Health Administration's Process Safety Management program

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Disinfection and Bioterrorism

Disinfection is crucial to water system security, providing the 'front line' of defense against biological contamination. Normal filtration and disinfection processes would dampen or remove

the threats posed by a number of potential bioterrorism agents. In addition, water systems should maintain an ability to increase disinfection doses in response to a particular threat.
However, conventional treatment barriers in no way guarantee safety from biological attacks. For many potential bioterrorism agents, there is little scientific information about what levels of reduction can be achieved with chlorine or other disinfectants. In addition, contamination of water after it is treated could overwhelm the residual disinfectant levels in distribution systems. Furthermore, typical water quality monitoring does not provide real-time data to warn of potential problems (Rose 2002). Additional research and funding are needed to improve prevention, detection, and responses to potential threats.

Protecting Chlorine and Other Treatment Chemicals
As part of its vulnerability assessment, each water system must consider its transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). For example, a release of chlorine gas would pose an immediate threat to system operators, and a large release may pose a danger to the surrounding community. As part of its vulnerability assessment, a water system using chlorine must determine if existing layers of protection are adequate. If not, a system should consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Possible measures to address chlorine security include: enhanced physical barriers (e.g., constructing secure chemical storage facilities), policy changes (e.g., tightening procedures for receiving chemical shipments), reducing quantities stored on site, or adopting alternative disinfection methods. These options must be weighed and prioritized, considering the unique characteristics and resources of each system. Water system officials must evaluate the risk-tradeoffs associated with each option. For example, reducing the chemical quantities on-site may reduce a system's ability to cope with an interruption of chemical supplies. Furthermore, changing disinfection technologies will not necessarily improve overall safety and security.

Understanding Calculation and Reporting of CT Data
Basically, log inactivation is a measurement of how effective a disinfection process is at killing microorganisms in a specific environment. Operationally, directly measuring log inactivation is not practical, but determining the microbial inactivation for an individual water treatment plant (WTP) can be achieved using the log inactivation calculations. The log inactivation calculation adjusts the WTP's CT value to account for the disinfection chemical reaction process variables that influence the disinfection process efficiency.

Log Inactivation
"Log inactivation" is a convenient way to express the number or percent of microorganisms inactivated (killed or unable to replicate) through the disinfection process. For example, a 3 log inactivation value means that 99.9% of microorganisms of interest have been inactivated. Log inactivation measures the effectiveness of the disinfection process, which is influenced by variables including disinfectant concentration, temperature, pH and disinfectant type (e.g., lower temperature results in less inactivation since the reactions slow down as temperature decreases).

CT and Log Inactivation Calculation Overview

This reference takes you step by step through the CT and log inactivation calculation procedure, through an example calculation, and presents the disinfection segment concept.

"CT" (minutes•mg/L) in the context of water treatment is defined as the product of: C, for "residual disinfectant concentration" in mg/L (determined before or at the first customer) and T, for the corresponding "disinfectant contact time" in minutes. CT is a measure of the disinfection process reaction time, but CT is only one of several variables that control the effectiveness of the disinfection
process.

CTCALC = Concentration Time, Calculated Value (minutes•mg/L)

C = Residual disinfectant concentration measured during peak flow (mg/L)

T = Actual Detention Time (minutes)

CTCALC = C × T

TDT = Theoretical Detention Time (minutes)

V = Volume, based on low water level (gallons)

Q = Peak hourly flow (gpm)

TDT = V/Q

Volume Equations:
Cylindrical: π x r2 x d Pipeline: π x r2 x l
Rectangular: l x w x d
d = minimum water depth
π = 3.1416

Disinfection Segments
Total inactivation = Σ log inactivation from each disinfection segment
Disinfection Profile
Almost all community and non-transient, non-community public water systems that use Surface Water or Ground Water Under the Direct Influence of Surface Water sources are required to develop a disinfection profile. Systems are required to retain the disinfection profile in graphic form and it must be available for review by the state as part of a sanitary survey.

Disinfection Profile and Benchmark
• A disinfection profile is a graphical representation of a system's level of *Giardia lamblia* or virus inactivation measured, at least weekly, during the course of a year.
• A benchmark is the lowest monthly average microbial inactivation during the disinfection profile time period.

The EPA has developed a disinfection profile spreadsheet calculator that calculates and graphs the disinfection profile for *Giardia* and viruses. The spreadsheet can be downloaded from: http://www.epa.gov/safewater/mdbp/lt1eswtr.html.

Understanding Chlorine Demand
The amount of chlorine used by reactions with substances that oxidize in the water before chlorine residual can be measured. It is the difference between the amount of chlorine added to wastewater and the amount of chlorine residual remaining after a given contact time. Chlorine demand may change with dosage, time, temperature, pH, and the type and amount of pollutants in the water.

The presence of chlorine residual in drinking water indicates that: 1) a sufficient amount of chlorine was added initially to the water to inactivate the bacteria and some viruses that cause diarrheal disease; and, 2) the water is protected from recontamination during storage. The presence of free residual chlorine in drinking water is correlated with the absence of disease-causing organisms, and thus is a measure of the potability of water.

While chlorine's most important attributes are its broad-spectrum germicidal potency and persistence in water distribution systems, its ability to efficiently and economically address many other water treatment concerns has also supported its wide use. Chlorine-based compounds are the only major disinfectants exhibiting lasting residual properties. Residual protection guards against microbial regrowth and prevents contamination of the water as it moves from the treatment plant to household taps.

Definitions
When chlorine is added to water, some of the chlorine reacts first with organic materials and metals in the water and is not available for disinfection (this is called the chlorine demand of the water). The remaining chlorine concentration after the chlorine demand is accounted for is called total chlorine. Total chlorine is further divided into: 1) the amount of chlorine that has reacted with nitrates and is unavailable for disinfection which is called combined chlorine and, 2) the free chlorine, which is the chlorine available to inactivate disease-causing organisms, and thus a measure to determine the potability of water.

For example, if using completing clean water the chlorine demand will be zero, and there will be no nitrates present, so no combined chlorine will be present. Thus, the free chlorine concentration will be equal to the concentration of chlorine initially added. In natural waters, especially surface water supplies such as rivers, organic material will exert a chlorine demand, and nitrates will form combined chlorine. Thus, the free chlorine concentration will be less than the concentration of chlorine initially added.

Chlorine Dose, Demand, and Residual
Most water treatment plants are required to disinfect the water, a process used to kill harmful bacteria. The most frequently used method of disinfection is the addition of chlorine. Here, we will briefly introduce three terms used during chlorination - chlorine dose, chlorine demand, and chlorine residual. These three characteristics are related to each other using the following equation:

(Chlorine demand) = (Chlorine dose) - (Chlorine residual)

The amount of chlorine added to the water is known as the chlorine dose. This is a measured quantity chosen by the operator and introduced into the water using a chlorinator or hypochlorinator.

As the chlorine reacts with bacteria and chemicals in the water, some of the chlorine is used up. The amount of chlorine used up by reacting with substances in the water is known as the chlorine demand. If nothing reacts with the chlorine (as would be the case in distilled water), then the chlorine demand is zero. However, in most cases the operator should count on some of the chlorine dose being used up when it reacts with substances in the water.

The amount of chlorine remaining in the water after some of the chlorine reacts with substances in the water is known as the chlorine residual. This lab introduces a test which can be used to calculate the chlorine residual. The chlorine residual is the most important of these three values - dose, demand, and residual - because it represents the actual amount of chlorine remaining in the water to act as a disinfectant.

The test for chlorine residual is performed frequently at most water treatment plants. Since regulations require a certain level of chlorine in water at the far ends of the distribution system, operators should be sure to test the chlorine residual in the distribution system as well as in the clear well.

Combined residual chlorination involves the addition of chlorine to water to produce, with natural ammonia present or with ammonia added, a combined available chlorine residual. Combined available chlorine forms have lower oxidation potentials than free available chlorine forms and are less effective as oxidants. They are also less effective as disinfectants. In fact, 25 times more combined available residual chlorine must be obtained to meet the same disinfectant level as a free available residual. The contact time has to be up to 100 times greater to obtain the same level of bacterial kill at the same pH and temperature conditions.

When a combined available chlorine residual is desired, the character of the water determines how it can be accomplished. These conditions may have to be considered:
1. If the water contains sufficient ammonia to produce the desired level of combined residual, the application of sufficient chlorine alone is all that is needed.
2. If the water contains too little or no ammonia, then addition of both chlorine and ammonia is required.
3. If the water has a free available chlorine, the addition of ammonia alone is all that is required. A combined chlorine residual should contain little or no free available chlorine.

The practice of combined residual chlorination is the most effective way of maintaining a stable residual throughout the distribution system to the point of consumer use. Combined residuals in the distribution system are generally longer-lasting and will carry farther into the system, but they are not as effective as free residuals are at disinfecting. The levels required by the regulatory agencies, when using combined residuals, is 1.0 ppm to 2.0 ppm.

Understanding Chlorine Residual
The amount of available chlorine present in wastewater after a given contact time (20 minutes at peak flow; 30 minutes at average flow), and under specific conditions including pH and temperature.

For effective water treatment, the water supply industry has recognized the need for adequate exposure to the disinfectant and sufficient disinfectant dosage for a certain amount of time. In the 1980s, the two functions were combined with the development of the CT values for various disinfectants.

CT represents the combination of the disinfectant dosage and the length of time water has been exposed to a minimum amount of the disinfectant residual.

Mathematically it is represented as CT = concentration x time
concentration = final disinfectant concentration in mg/l
time = minimum exposure time in minutes

In an assessment of disinfection effectiveness, two types of organisms have been chosen as disinfection surrogates – the protozoan Giardia and viruses. CT values established for disinfection of surface waters require treatment plants to achieve a three-log or 99.9% reduction in Giardia and a four-log or 99.99% virus reduction. It is important to recognize that the use of chlorine as the disinfectant is only one part of the treatment process. Equally important is the

need for improved filtration to remove organisms. A combination of proper disinfection and filtration is most effective in providing safe drinking water. Recent experiments in controlling Cryptosporidium also suggest the effectiveness of filtration in the water treatment process.

Free residual chlorination involves the application of chlorine to water to produce--either directly or by first destroying any naturally present ammonia--a free available chlorine residual and to maintain this residual through part or all of the water treatment plant and distribution system. Free available residual forms have higher oxidation potentials than combined available chlorine forms and are more effective as disinfectants.

When free available chlorine residuals are desired, the characteristics of the water will determine how this will be accomplished. This may have to be considered:
1. If the water contains no ammonia or other nitrogen compounds, any application of chlorine will yield a free residual once it has reacted with any bacteria, virus and other microorganisms present in the water.
2. If the water contains ammonia, it results in the formation of a combined residual, which must be destroyed by applying an excess of chlorine.

Breakpoint Chlorination
Breakpoint chlorination is the name of the process of adding chlorine to water until the chlorine demand has been satisfied. Chlorine demand equals the amount of chlorine used up before a free available chlorine residual is produced. Further additions of chlorine will result in a chlorine residual that is directly proportional to the amount of chlorine added beyond the breakpoint. Public water supplies normally chlorinate past the breakpoint.

When chlorine is initially added to water, the following may happen:
1. If the water contains some iron, manganese, organic matter, and ammonia, the chlorine reacts with these materials and no residual is formed, meaning that no disinfection has taken place.
2. If additional chlorine is added at this point, it will react with the organics and ammonia to form chloramines. The chloramines produce a combined chlorine residual. As the chlorine is combined with other substances, it loses some of the disinfection strength. Combined residuals have poor disinfection power and may be the cause of taste and odor problems.
3. With a little more chlorine added, the chloramines and some of the chlororganics are destroyed.
4. With still more chlorine added, a free chlorine residual is formed, free in the sense that it can react quickly.

Free available chlorine is the best residual for disinfection. It disinfects faster and without the swimming-pool odor of combined residual chlorine. The free available residual forms at the breakpoint; therefore, the process is called breakpoint chlorination. The common practice today is to go just beyond the breakpoint to a residual of about .2 to .5 ppm.

A variety of reactions take place during chlorination. When chlorine is added to a water containing ammonia (NH_3), the ammonia reacts with hypochlorous acid (HOCL) to form monochloramine, dichloramine, and trichloramine. The formation of these chloramines depends on the pH of the water and the initial chlorine-ammonia ratio.

Ammonia + Hypochlorous acid ----> Chloramine + Water
NH3 + HOC1----------> NH2C1 + H20 Monochloramine
NH2C1 + HOC1---------- > NHC12 + H20 Dichloramine
NHC12 + HOC1---------- > NC13 + H20 Trichloramine

At the pH of most natural water (pH 6.5 to 7.5), monochloramine and dichloramine exist together. At pH levels below 5.5, dichloramine exists by itself. Below pH 4.0, trichloramine is the only compound found. The monochloramine and dichloramine forms have a definite disinfection power. Dichloramine is a more effective disinfecting agent than monochloramine. However,

dichloramine is not recommended as a disinfectant due to the possibility of the formation of taste and odor compounds. Chlorine reacts with phenol and salicylic acid to form chlorophenol, which has an intense medicinal odor. This reaction is much slower in the presence of monochloramines.

Both the chlorine residual and the contact time are essential for effective disinfection. It is important to have complete mixing. The operator also needs to be aware that changes in the pH may affect the ability of the chlorine to disinfect the water. The operator must examine the application and select the best point of feed and the best contact time to achieve the results desired. The operator needs to consider:
1. Whether the injection point and the method of mixing is designed so that the disinfectant is able to get into contact with all of the water to be disinfected. This also depends on whether pre- and/or post-chlorination is being used.
2. Contact time. In situations of good initial mixing, the longer the contact time, the more effective the disinfection.
3. Effectiveness of upstream treatment processes. The lower the turbidity of the water, the more effective the disinfection.
4. Temperature. At higher temperatures the rate of disinfection is more rapid.
5. Dosage and type of chemical. Usually the higher the dose, the quicker the disinfection rate. The form of disinfectant (chloramine or free chlorine) and the type of chemical used influence the disinfection rate.
6. pH. The lower the pH, the better the disinfection.

Emergency Disinfection of Drinking Water
USE ONLY WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH
1. Use bottled water that has not been exposed to flood waters if it is available.
2. If you don't have bottled water, you should boil water to make it safe. Boiling water will kill most types of disease-causing organisms that may be present. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for boiling. Boil the water for one minute, let it cool, and store it in clean containers with covers.
3. If you can't boil water, you can disinfect it using household bleach. Bleach will kill some, but not all, types of disease-causing organisms that may be in the water. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for disinfection. Add 1/8 teaspoon (or 8 drops) of regular, unscented, liquid household bleach for each gallon of water, stir it well and let it stand for 30 minutes before you use it. Store disinfected water in clean containers with covers.
4. If you have a well that has been flooded, the water should be tested and disinfected after flood waters recede. If you suspect that your well may be contaminated, contact your local or state health department or agriculture extension agent for specific advice.
(U.S. federal agencies and the Red Cross recommend these same four steps to disinfect drinking water in an emergency. Please, read the text below for important details about disinfection.
More information about disinfection

- ✓ In times of crisis, follow advice from local officials. Local health departments or public water systems may urge consumers to use more caution or to follow additional measures than the information provided here.

- ✓ Look for other sources of potable water in and around your home.

- ✓ When your home water supply is interrupted by natural or other forms of disaster, you can obtain limited amounts of water by draining your hot water tank or melting ice cubes. In most cases, well water is the preferred source of drinking water. If it is not available and river or lake water must be used, avoid sources containing floating material and water with a dark color or an odor. Generally, flowing water is better quality than stagnant water.

Examine the physical condition of the water.
When emergency disinfection is necessary, disinfectants are less effective in cloudy, murky or colored water. Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter. After filtering until it is clear, or allowing all dirt and other particles to settle, draw off the clean and clear water for disinfection. Water prepared for disinfection should be stored only in clean, tightly covered, containers, not subject to corrosion.

- ✓ Choose a disinfection method.

- ✓ Boiling and chemical treatment are two general methods used to effectively disinfect small quantities of filtered and settled water.

Boiling
Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes. These disease-causing organisms are less likely to occur in well water (as long as it has not been affected by flood waters). If not treated properly and neutralized, Giardia may cause diarrhea, fatigue, and cramps after ingestion. Cryptosporidium is highly resistant to disinfection. It may cause diarrhea, nausea and/or stomach cramps. People with severely weakened immune systems are likely to have more severe and more persistent symptoms than healthy individuals.

Boil filtered and settled water vigorously for one minute (at altitudes above one mile, boil for three minutes). To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

If boiling is not possible, chemical disinfection of filtered and settled water collected from a well, spring, river, or other surface water body will still provide some health benefits and is better than no treatment at all.

Chemical Treatment
When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. For chemical disinfection to be effective, the water must be filtered and settled first. Chlorine and iodine are the two chemicals commonly used to treat water. They are somewhat effective in protecting against exposure to Giardia, but may not be effective in controlling more resistant organisms like Cryptosporidium. Chlorine is generally more effective than iodine in controlling Giardia, and both disinfectants work much better in warm water. You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. Do not use non-chlorine bleach to disinfect water. Typically, household chlorine bleaches will be 5.25% available chlorine. Follow the procedure written on the label. When the necessary procedure is not given, find the percentage of available chlorine on the label and use the information in the following table as a guide. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)

Available Chlorine

Drops per Quart/Gallon of Clear Water Drops per Liter of Clear Water
1% 10 per Quart - 40 per Gallon 10 per Liter
4-6% 2 per Quart - 8 per Gallon (1/8 teaspoon) 2 per Liter

7-10%
1 per Quart - 4 per Gallon
1 per Liter

(If the strength of the bleach is unknown, add ten drops per quart or liter of filtered and settled water. Double the amount of chlorine for cloudy, murky or colored water or water that is extremely cold.)

Mix the treated water thoroughly and allow it to stand, preferably covered, for 30 minutes. The water should have a slight chlorine odor. If not, repeat the dosage and allow the water to stand for an additional 15 minutes. If the treated water has too strong a chlorine taste, allow the water to stand exposed to the air for a few hours or pour it from one clean container to another several times.

You can use granular calcium hypochlorite to disinfect water.
Add and dissolve one heaping teaspoon of high-test granular calcium hypochlorite (approximately ¼ ounce) for each two gallons of water, or 5 milliliters (approximately 7 grams) per 7.5 liters of water. The mixture will produce a stock chlorine solution of approximately 500 milligrams per liter, since the calcium hypochlorite has available chlorine equal to 70 percent of its weight. To disinfect water, add the chlorine solution in the ratio of one part of chlorine solution to each 100 parts of water to be treated. This is roughly equal to adding 1 pint (16 ounces) of stock chlorine to each 12.5 gallons of water or (approximately ½ liter to 50 liters of water) to be disinfected. To remove any objectionable chlorine odor, aerate the disinfected water by pouring it back and forth from one clean container to another.

You can use chlorine tablets to disinfect filtered and settled water.

Chlorine tablets containing the necessary dosage for drinking water disinfection can be purchased in a commercially prepared form. These tablets are available from drug and sporting goods stores and should be used as stated in the instructions. When instructions are not available, use one tablet for each quart or liter of water to be purified.

You can use tincture of iodine to disinfect filtered and settled water.

Common household iodine from the medicine chest or first aid kit may be used to disinfect water. Add five drops of 2 percent U.S. or your country's approved Pharmacopeia tincture of iodine to each quart or liter of clear water. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

You can use iodine tablets to disinfect filtered and settled water.

Purchase commercially prepared iodine tablets containing the necessary dosage for drinking water disinfection at drug and sporting goods stores. Use as stated in instructions. When instructions are not available, use one tablet for each quart or liter of filtered and settled water to be purified.

ONLY USE WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH.

Summary and Illustration of Key Points

- ✓ Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter.

- ✓ Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes.

- ✓ To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

- ✓ When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. Chlorine (in the form of unscented bleach) and iodine are the two chemicals commonly used to treat water.

- ✓ You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)

- ✓ You can use tincture of iodine to disinfect filtered and settled water. Common household iodine from the medicine chest or first aid kit may be used to disinfect water.

- ✓ Tincture of iodine. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

Understanding Combined Chlorine Residual

The residual consisting of chlorine that is combined with ammonia, nitrogen, or nitrogenous compounds (chloramines).

Understanding Free Available Chlorine
The residual consisting of hypochlorite ions (OCl-), hypochlorous acid (HOCl) or a combination of the two. These are the most effective in killing bacteria.

Total Combined Chlorine Residual
The total amount of chlorine present in a sample. This is the sum of the free chlorine residual and the combined available chlorine residual.

Understanding Pre-Chlorination
Chlorination is the application of chlorine to water to accomplish some definite purpose. In this lesson, we will be concerned with the application of chlorine for the purpose of disinfection, but you should be aware that chlorination can also be used for taste and odor control, iron and manganese removal, and to remove some gases such as ammonia and hydrogen sulfide. Chlorination is currently the most frequently used form of disinfection in the water treatment field. However, other disinfection processes have been developed. These alternatives will be discussed at the end of this lesson.

Pre-Chlorination and Post-Chlorination
Like several other water treatment processes, chlorination can be used as a pretreatment process (prechlorination) or as part of the primary treatment of water (postchlorination). Treatment usually involves either postchlorination only or a combination of prechlorination and postchlorination.

Pre-chlorination is the act of adding chlorine to the raw water. The residual chlorine is useful in several stages of the treatment process - aiding in coagulation, controlling algae problems in basins, reducing odor problems, and controlling mudball formation. In addition, the chlorine has a much longer contact time when added at the beginning of the treatment process, so prechlorination increases safety in disinfecting heavily contaminated water.

Post-chlorination is the application of chlorine after water has been treated but before the water reaches the distribution system. At this stage, chlorination is meant to kill pathogens and to provide a chlorine residual in the distribution system. Post-chlorination is nearly always part of the treatment process, either used in combination with prechlorination or used as the sole disinfection process.

Until the middle of the 1970s, water treatment plants typically used both prechlorination and post-chlorination. However, the longer contact time provided by prechlorination allows the chlorine to react with the organics in the water and produce carcinogenic substances known as trihalomethanes. As a result of concerns over trihalomethanes, prechlorination has become much less common in the United States. Currently, prechlorination is only used in plants where trihalomethane formation is not a problem.

Understanding Breakpoint Chlorination
Addition of chlorine to water until the chlorine demand has been satisfied. Since ammonia is present in all domestic wastewaters, the reaction of ammonia with chlorine is a great significance. When chlorine is added to waters containing ammonia, the ammonia reacts with hypochlorous acid (HOCl) to form monochloramine, dichloramine and trichloramine. The formation of these chloramines depends on the pH of the solution and the initial chlorine-ammonia ratio.

Chlor-Alkali Membrane Process
The chloralkali process (also chlor-alkali and chlor alkali) is an industrial process for the electrolysis of sodium chloride solution (brine). Depending on the method, several products besides hydrogen can be produced. If the products are separated, chlorine and sodium hydroxide (caustic soda) are the products; by mixing, sodium hypochlorite or sodium chlorate are produced, depending on the temperature. Higher temperatures are needed for the production of sodium chlorate instead of sodium hypochlorite. Industrial scale production began in 1892. When using calcium chloride or potassium chloride, the products contain calcium or potassium instead of sodium.

The process has a high energy consumption, for example over 4 billion kWh per year in West Germany in 1985, and produces equal (molar) amounts of chlorine and sodium hydroxide, which makes it necessary to find a use for the product for which there is less demand, usually the chlorine. There are three production methods in use. While the mercury cell method produces chlorine-free sodium hydroxide, the use of several tons of mercury leads to serious environmental problems. In a normal production cycle a few hundred pounds of mercury per year are emitted, which accumulate in the environment. Additionally, the chlorine and sodium hydroxide produced via the mercury-cell chloralkali process are themselves contaminated with trace amounts of mercury. The membrane and diaphragm method use no mercury, but the sodium hydroxide contains chlorine, which must be removed.

Understanding Chlorine's Effectiveness
In 1881, German bacteriologist Robert Koch demonstrated under controlled laboratory conditions that pure cultures of bacteria could be destroyed by hypochlorite (bleach). The bulk of chlorine disinfection research, which was conducted from the 1940s to the 1970s with a focus on bacteria, provided observations as to how chlorine kills the microorganism. The observations that (1) bacterial cells dosed with chlorine release nucleic acids, proteins and potassium and (2) membrane functions such as respiration and active transport are affected more by chlorine than are cytoplasmic processes, directed researchers' attention to the surface of the bacterial cell. The hypothesis was that the bacterial cell wall, under environmental stress, could interact with chlorine.

Chlorine exposure appears to cause physical, chemical, and biochemical alterations to the cell wall, thus destroying the cell's protective barrier, terminating vital functions, resulting in death of the microorganism. A possible sequence of events during chlorination would be: (1) disruption of the cell wall barrier by reactions of chlorine with target sites at the cell surface, (2) release of vital cellular constituents from the cell, (3) termination of membrane-associated functions, and (4) termination of cellular functions within the cell. During the course of this sequence of events, the microorganism dies, meaning it is no longer capable of growing or causing disease.

Understanding Chlorine Solubility Effects
Chlorine is only slightly soluble in water; its maximum solubility is approximately one percent at 49° C. At temperatures below this point it combines with water to form chlorine ice, a crystalline substance. When the water supply to a gas chlorinator is below normal room temperature, it may cool the chlorine gas to the point at which chlorine ice is formed and accumulates on the needle valve and gas outlet tube, resulting in erratic feed results. Because the vapor pressure of chlorine increases with rising temperatures, its solubility also decreases. At 212° F. chlorine is insoluble in water.

Chlorine dissolved in water forms a weak corrosive mixture of hydrochloric and hypochlorous acid. The corrosivity of chlorine solutions in water creates problems in handling chlorine spills and chlorine containers. Chlorine reacts with many compounds. Because of its great affinity for hydrogen, it removes hydrogen from some compounds, such as hydrogen sulfide. It also reacts with ammonia or other nitrogen-containing compounds to form various mixtures of chloramines. It reacts with organic materials, sometimes with explosive violence.

Chemicals like chlorine, bromine, and ozone are examples of oxidizers. It is their ability to oxidize or steal electrons from other substances that makes them good water sanitizers. As soon as the oxidizing agent is added to the water, it begins to combine with microorganisms like bacteria, algae, and whatever else the water may contain.

Now the free and available oxidizer is combining with contaminants and its effectiveness is reduced according to how much combining took place. Although the hydrogen ion does not play a direct reduction role on copper surfaces, pH can influence copper corrosion by altering the equilibrium potential of the oxygen reduction half-reaction and by changing the speciation of copper in solution (Reiber, 1989). Copper corrosion increases rapidly as the pH drops below 6; in addition, uniform corrosion rates can be high at low pH values (below about pH 7), causing metal thinning. At higher pH values (above about pH 8), copper corrosion problems are almost always associated with non-uniform or pitting corrosion processes (Edwards et al., 1994a; Ferguson et al., 1996). Edwards et al. (1994b) found that for new copper surfaces exposed to simple solutions that contained bicarbonate, chloride, nitrate, perchlorate or sulphate, increasing the pH from 5.5 to 7.0 roughly halved corrosion rates, but further increases in pH yielded only subtle changes.

The prediction of copper levels in drinking water relies on the solubility and physical properties of the cupric oxide, hydroxide and basic carbonate solids that comprise most scales in copper water systems (Schock et al., 1995). In the cupric hydroxide model of Schock et al. (1995), a decrease in copper solubility with higher pH is evident. Above a pH of approximately 9.5, an upturn in solubility is predicted, caused by carbonate and hydroxide complexes increasing the solubility of cupric hydroxide. Examination of experience from 361 utilities reporting copper levels under the U.S. EPA Lead and Copper Rule revealed that the average 90th-percentile copper levels were highest in waters with pH below 7.4 and that no utilities with pH above 7.8 exceeded the U.S. EPA's action level for copper of 1.3 mg/L (Dodrill and Edwards, 1995). However, problems associated with copper solubility were also found to persist up to about pH 7.9 in cold, high-alkalinity and high-sulphate groundwater (Edwards et al., 1994a).

In the pH range of 7-9, both the corrosion rate and the degree of tuberculation of iron distribution systems generally increase with increasing pH (Larson and Skold, 1958; Stumm, 1960; Hatch, 1969; Pisigan and Singley, 1987). Iron levels, however, were usually reported to decrease with increasing pH (Karalekas et al., 1983; Kashinkunti et al., 1999; Broo et al., 2001; Sarin et al., 2003). In a pipe loop system constructed from 90- to100-year-old unlined cast iron pipes taken from a Boston distribution system, iron concentrations were found to steadily decrease when the pH was raised from 7.6 to 9.5 (Sarin et al., 2003). Similarly, when iron was measured in the distribution system following a pH increase from 6.7 to 8.5, a consistent downward trend in iron concentrations was found over 2 years (Karalekas et al., 1983). These observations are consistent with the fact that the solubility of iron-based corrosion by-products decreases with increasing pH.

Water with low pH, low alkalinity and low calcium is particularly aggressive towards cement materials. The water quality problems that may occur are linked to the chemistry of the cement. Lime from the cement releases calcium ions and hydroxyl ions into the drinking water. This, in turn, may result in a substantial pH increase, depending on the buffering capacity of the water (Leroy et al., 1996). Pilot-scale tests were conducted to simulate low-flow conditions of newly lined cement mortar pipes carrying low-alkalinity water (Douglas et al., 1996). In the water with an initial pH of 7.2, alkalinity of 14 mg/L as calcium carbonate and calcium at 13 mg/L as calcium carbonate, measures of pH as high as 12.5 were found.

Similarly, in the water with an initial pH of 7.8, alkalinity of 71 mg/L as calcium carbonate and calcium at 39 mg/L as calcium carbonate, measures of pH as high as 12 were found. The most significant pH increases were found during the 1st week of the experiment, and pH decreased slowly with aging of the lining. In a series of field and test rig trials to determine the impact of in situ cement mortar lining on water quality,

Understanding Amperometric Titration

It appears that DPD colorimetric determination and amperometric titration as described in Standard Methods are the procedures most commonly used for routine measurement of total chlorine. Few studies have been conducted to evaluate these or other total residual chlorine measurement techniques. Bender5 studied approximately 10 test procedures and found that results using the DPD colorimetric procedure were consistently higher than those using amperometric titration. Brooks and Seegert6 described an amperometric titration procedure employing a recording polargraph and microburette, which was reported to be accurate and free from interference. The reliability of the DPD colorimetric method for free chlorine has been increasingly questioned in recent years. The suitability of that procedure for accurate total chlorine determinations appears to the authors to be questionable, as well. Amperometric titration as described in Standard Methods cannot be used to measure total chlorine concentrations less than about 0.05 mg/L, which is at least an order of magnitude greater than levels of concern in natural waters for potential toxicity to aquatic organisms. A reliable, simple procedure for low-level total chlorine determinations is clearly needed.

Analytical Procedure
Section 409C of Standard Methods includes a General Discussion section on amperometric titration for the determination of chlorine in aqueous solutions. That discussion is applicable to the procedure used by the authors. Also included in Standard Methods is a section concerning the titration apparatus. Basically, the titration equipment consists of a buret capable of accurately delivering 0.01 mL of titrant, a sample cup, and a stirring device in which is housed a platinum electrode and a KCl reference electrode. Several companies manufacture amperometric titrators that fit this general description. The experience of the senior author is that some of the commercial titrators are less suitable than others, primarily because of the small surface area of some of the electrodes employed. A Wallace and Tiernan amperometric titrator was used by the authors in developing and applying the procedure described below.

Reagents
a. Chlorine-free water. Only distilled or demineralized water that is free of chlorine should be used in preparing reagents. Chlorine-free water may be prepared by passing distilled or demineralized water through a suitable activated carbon filter adsorption column. The water may be tested for the presence of chlorine by titrating a sample as described in the Procedure section. Any deflection in the meter upon the addition of PAO titrant indicates the presence of chlorine or other oxidants that would interfere in the titration procedure.
b. Standard phenylarsine oxide (PAO), 0.00564 N. See Standard Methods Section 409B, paragraph 3a.
Standardization – Dilute 50.00 mL of freshly prepared 0.0002256 N potassium biniodate to 200 mL in chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section. If the standard PAO is 0.00564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.
c. Phenylarsine oxide titrant, 0.000564 N. Dilute 10.00 mL of 0.00564 N PAO to 100.0 mL in chlorine-free water.
Standardization – Dilute 5.00 mL of 0.0002256 N potassium biniodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section below. If the PAO titrant is 0.000564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.
d. Potassium biniodate, 0.0002256 N. Dissolve 0.7332 g reagent grade $KH(IO_3)2$ in 500 mL chlorine-free water and dilute to 1.00 L. Dilute 10.00 mL of that solution to 100.0 mL with chlorine-free water. That solution is used for the standardization of the PAO and should be freshly prepared.
e. Acetate buffer solution, pH 4. See Standard Methods1 Section 409B, paragraph 3e.

f. Potassium iodide, (KI), reagent grade crystals.

Procedure

a. Titrant selection. Normally a 200-mL sample is used in titration. Each 0.1 mL of 0.000564 N PAO corresponds to 0.01 mg/L in a 200-mL sample. The titrant normality should be selected such that no more than about 4 mL of titrant will be required to reach the equivalence point. Thus, if the chlorine concentration in the majority of the samples to be titrated is less than about 0.4 mg/L, use 0.000564 N PAO as the titrant. If only samples containing chlorine concentrations in excess of 0.4 mg/L are to be analyzed, use 0.00564 N PAO as the titrant. If samples containing concentrations of chlorine in excess of about 0.4 mg/L are to be titrated only occasionally and the volume of 0.000564 N PAO required for titration is found to be excessive, a suitable subsample may be used and diluted to 200 mL with chlorine-free water.

b. Titration procedure (total residual chlorine). Prior to beginning the titration, rinse the buret with PAO titrant by filling it completely and allowing the titrant to run into an empty sample cup. Repeating this operation three or four times will ensure that the correct titrant concentration reaches the sample cup. Remove the sample cup and rinse with distilled water and with the sample to be titrated. Add 200 mL of the sample to the sample cup. Add approximately 1.5 g (± 0.2 g) crystalline KI and allow to dissolve, using the agitator on the titrator for mixing.

The exact amount of KI added is not critical, but the analyst should weigh 1.5 g of this reagent periodically to become familiar with the approximate amount required. Add 1 mL of acetate buffer and allow the microammeter on the titrator to reach a stable reading; the titration should be started within about 30 seconds following the addition of the KI to the sample.

Full-scale deflection on the microammeter is 100 units. The meter should be initially adjusted to read between 90 and 100 units. Record the initial reading prior to the addition of titrant. Titrate by adding suitable volumes of titrant and recording the titrant volume added and the resultant current reading. At least three (and preferably five to ten) readings of current and titrant volume added should be obtained prior to passing the equivalence point; then add excess titrant to ensure that there is no further meter deflection. Record the final meter reading. If, during the titration, the meter reading falls to near or below 10 units, record the low reading, re-adjust the meter to read between 90 and 100 units, record the high reading, and continue the titration. This approach allows calculation of the total meter deflection, which is used in determining the equivalence point.

The equivalence point is determined by plotting the total meter deflection as a function of titrant volume added. It is important that the total meter deflection be used in preparing this plot. A straight line is drawn through the first few points in the plot and a second straight line is drawn parallel to the abscissa and corresponding to the final total deflection in the meter reading. The equivalence point is determined by the intersection of those two lines. When 0.000564 N PAO is used as the titrant, the chlorine concentration is 0.1-times the titrant volume at the equivalence point. This plotting procedure is also outlined in the ASTM Water Manual8 under procedures ASTM D1253 (Tests for Residual Chlorine in Water) and ASTM D1427 (Tests for Residual Chlorine in Waste Water).

c. Sample storage and handling. Chlorine measurements should be made as soon after sample collection as possible. Samples to be analyzed for chlorine should be stored in the dark and packed on ice if they must be held for more than a few minutes before analysis. Chlorine compounds are highly reactive and may be rapidly lost from samples due to the effects of volatilization, phototransformation, and chlorine demand. Storage of samples on ice and in the dark between sampling and analysis will help minimize the rate of dissipation. It is important to estimate the changes that occur in chlorine content in the subject water between sample collection and analysis.

This can be accomplished by performing a “time-lag” test. To perform a time-lag test, a single large (approximately 2-L) sample of the water being analyzed is collected. The chlorine

concentration in that sample is determined six to ten times over a period of one to three hours, depending on the normal sample holding time. The measured concentrations are then plotted as a function of time, normally on semilog paper. In most cases, the decrease in chlorine concentration over time can be described by first-order reaction kinetics.

The original chlorine content in any sample can be computed given the measured concentration and the holding time. A time-lag study should be performed on a regular basis for each type of water being analyzed because of variability in water compositions. The sample set used for the study should be handled in the same way as other samples (i.e., the samples should be kept cold and in the dark). Even when time-lag studies are made a part of the routine analytical procedure, it is important that the delay between sample collection and chlorine analysis be held to a minimum.

Sodium Hypochlorite
Sodium Hypochlorite, or bleach, is produced by adding elemental chlorine to sodium hydroxide. Typically, hypochlorite solutions contain from 5 to 15% chlorine, and are shipped by truck in one- to 5,000- gallon containers.

Advantages
- ✓ Solution is less hazardous and easier to handle than elemental chlorine
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Limited shelf-life
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water
- ✓ Corrosive to some materials and more difficult to store than most solution chemicals
- ✓ Higher chemical costs than elemental chlorine

Calcium Hypochlorite
- ✓ Calcium hypochlorite is another chlorinating chemical used primarily in smaller applications. It is a white, dry solid containing approximately 65% chlorine, and is commercially available in granular and tablet forms.

Advantages
- ✓ More stable than sodium hypochlorite, allowing longer storage
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Dry chemical requires more handling than sodium hypochlorite
- ✓ Precipitated solids formed in solution complicate chemical feeding
- ✓ Higher chemical costs than elemental chlorine
- ✓ Fire or explosive hazard if handled improperly
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water

Onsite Hypochlorite Generation

In recent years some municipalities have installed on-site hypochlorite generators that produce weak hypochlorite solutions (~0.8%) using an electrolytic cell and a solution of salt water.

Advantages

- ✓ Minimal chemical storage and transport

Limitations

- ✓ More complex and requires a higher level of maintenance and technical expertise
- ✓ High capital cost
- ✓ Operating costs are often higher than for commercial hypochlorite
- ✓ Requires careful control of salt quality
- ✓ Weak solution requires high volume chemical feed and control
- ✓ Byproducts in generated hypochlorite may be difficult to monitor and control
- ✓ System backup may be more difficult and costly

Ozone

Ozone (O_3) is generated on-site at water treatment facilities by passing dry oxygen or air through a system of high voltage electrodes. Ozone is one of the strongest oxidants and disinfectants available. Its high reactivity and low solubility, however, make it difficult to apply and control. Contact chambers are fully contained and non-absorbed ozone must be destroyed prior to release to avoid corrosive and toxic conditions. Ozone is more often applied for oxidation rather than disinfection purposes.

Advantages

- ✓ Strongest oxidant/disinfectant available
- ✓ Produces no chlorinated THMs, HAAs
- ✓ Effective against Cryptosporidium at higher concentrations
- ✓ Used with Advanced Oxidation processes to oxidize refractory organic compounds

Limitations

- ✓ Process operation and maintenance requires a high level of technical competence
- ✓ Provides no protective residual
- ✓ Forms brominated byproducts (bromate, brominated organics)
- ✓ Forms nonhalogenated byproducts (ketenes, organic acids, aldehydes)
- ✓ Breaks down more complex organic matter; smaller compounds can enhance microbial re-growth in distribution systems and increase DBP formation during secondary disinfection processes.
- ✓ Higher operating and capital costs than chlorination
- ✓ Difficult to control and monitor particularly under variable load conditions

Ultraviolet Radiation

Ultraviolet (UV) radiation, generated by mercury arc lamps, is a non-chemical disinfectant. When UV radiation penetrates the cell wall of an organism, it damages genetic material, and prevents the cell from reproducing. Although it has a limited track record in drinking water applications, UV has been shown to effectively inactivate many pathogens while forming limited disinfection byproducts.

Advantages

- ✓ Effective at inactivating most viruses, spores and cysts
- ✓ No chemical generation, storage, or handling
- ✓ Effective against Cryptosporidium
- ✓ No known byproducts at levels of concern

Limitations

- ✓ No residual protection
- ✓ Low inactivation of some viruses (reoviruses and rotaviruses)

- ✓ Difficult to monitor efficacy
- ✓ Irradiated organisms can sometimes repair and reverse the destructive effects of UV through a process known as photo-reactivation
- ✓ May require additional treatment steps to maintain high-clarity water
- ✓ Does not provide oxidation, or taste and odor control
- ✓ High cost of adding backup/emergency capacity
- ✓ Mercury lamps may pose a potable water and environmental toxicity risk

Alternative Disinfectants

Up until the late 1970s, chlorine was virtually the only disinfectant used to treat drinking water. Chlorine was considered an almost ideal disinfectant, based on its proven characteristics:

- ✓ Effective against most known pathogens
- ✓ Provides a residual to prevent microbial re-growth and protect treated water throughout the distribution system
- ✓ Suitable for a broad range of water quality conditions
- ✓ Easily monitored and controlled

Test methods available for Residual Chlorine

Residual Chlorine can be measured using different methods. Iodometric and DPD colorimetric methods are the most common methods. Each method has its own set of reagents and concentration range.

Iodometric Method
Residual Chlorine by Iodometric has a minimum detectable concentration of 40ppb if 0.01N sodium thiosulfate is used. Prepare the sample for titration by adding 5mL of acetic acid and 1g of potassium iodide to the sample. Titrate the sample with 0.01N sodium thiosulfate. Concentrations below 1 mg/L should be measured by using either 0.00564N sodium thiosulfate or 0.00564N phenylarsine oxide.

DPD Colorimetric Method
Residual Chlorine can also be measured by the DPD Colorimetric method. This method has a minimum detectable concentration of 10ppb. In this method, the calibration is either made up from a chlorine solution or a potassium permanganate solution. The typical calibration range for this method is 0.05 to 4mg/L. The reagents used in this method are a phosphate buffer and N,N-diethyl-p-phenylenediamine indicator solution. The samples are mixed with the reagents and then read on a spectrophotometer at a wavelength of 515nm.

Chlorine in water solutions is not stable. As a result, its concentration in samples decreases rapidly. Exposure to sunlight or other strong light, air, or agitation will further reduce the quantity of chlorine present in solutions. Samples to be analyzed for chlorine cannot be stored or preserved. Tests must be started immediately after sampling. Therefore, samples taken for the chlorine residual test must be grab samples only and excessive agitation must be avoided.

It is not necessary to use special sampling devices or containers for the chlorine residual test. However, the sampling container should be capable of collecting samples from a representative sampling point following chlorine contact, and should be made of resistant materials that will not rust or corrode, and which can be easily cleaned.

NOTE: A long handled aluminum dipper attached to a wooden handle, or an equivalent device, is acceptable for collecting samples. Do not use coffee cans, bleach bottles, etc.

Preparation of Chemicals
At a minimum, hand and eye protection should be used when handling any of the chemicals mentioned in this section. Before working with any chemical, consult the appropriate Safety Data Sheet (formerly MSDS) (SDS) to determine if other safety precautions are necessary.

Chlorine Residual Reagents
Iodometric and Amperometric Methods:
I. Standard Phenylarsine Oxide (PAO) Solution, 0.00564 N

A. Prepare 0.3 N sodium hydroxide solution (NaOH) by dissolving 12.0 g NaOH in 800 mL distilled water and diluting to 1 liter.

B. Prepare a 6.0 N hydrochloric acid solution (HCl) by adding 108 mL concentrated HCl to 800 mL distilled water and diluting to 1 liter. (Caution: Concentrated HCl fumes can burn eyes and lungs—do not breathe fumes!)

C. Prepare an approximately 0.00564 N solution of PAO using the following procedures:

1. Dissolve approximately 0.8 g PAO powder in 150 mL of 0.3 N NaOH solution, and allow to settle.

2. Decant 110 mL into 800 mL distilled water and mix thoroughly.

3. Bring to pH 6 to 7 with 6N HCl and dilute to 950 mL with distilled water. (Caution: PAO is poisonous. Wash thoroughly after use and do not ingest.)

D. Standardization

1. Accurately measure 5 to 10 mL freshly standardized 0.0282 N iodine solution into a flask and add 1 mL potassium iodide solution (50g KI dissolved and diluted to 1 L with freshly boiled and cooled distilled water.

2. Titrate with PAO solution, using starch solution as an indicator, until blue disappears.

3. Normality (N) of PAO = (mL iodine solution x 0.0282)/mL PAO titrated.

4. Adjust PAO to 0.00564 N and recheck.

II. Standard Sodium Thiosulfate Solution, 0.00564 N

A. Prepare a 0.1 N sodium thiosulfate solution by dissolving 25 g $Na_2S_2O_3$ $5H_2O$ in 1000 mL of freshly boiled distilled water. Store reagent for at least 2 weeks to allow oxidation of any bisulfite ion present. Add a few mL of chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize by one of the following methods:

1. Iodate Method

a. Dissolve 3.249 g anhydrous primary standard quality potassium bi-iodate ($KH(IO_3)2$) or 3.567 g potassium iodate (KIO_3) dried at 103 +/-2°C for 1 hour in distilled water and dilute to 1000 mL to yield a 0.1000 N iodate solution. Store in a glass stoppered bottle.

b. Add, with constant stirring, 1 mL concentrated sulfuric acid (H_2SO_4), 10 mL 0.1000 N iodate solution, and 1 g potassium iodide (KI) to 80 mL distilled water. Titrate immediately with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color of the liberated iodine is almost discharged. Add 1 mL starch indicator solution and continue titration until the blue color disappears.

c. The normality (N) of the sodium thiosulfate is calculated as follows: N of $Na_2S_2O_3$ = 1/mL $Na_2S_2O_3$ for titration

2. Dichromate Method

A. Dissolve 4.904 g anhydrous primary standard grade potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1000 mL to yield a 0.1000 N dichromate solution. Store in a glass stoppered bottle.

B. For maximum stability of the standard 0.00564 N sodium thiosulfate solution, prepare by diluting an aged 0.1N $Na_2S_2O_3$ standard solution with freshly boiled distilled water. Add 10 mg Mercuric iodide and 4 g of sodium borate per liter of solution. Standardize daily using 0.00564 N potassium dichromate or iodate solution.

III. Standard Iodine Solution (I_2), 0.1 N

A. Dissolve 40 g potassium iodide (KI) in 25 mL chlorine-demand-free water.

B. Add 13 g resublimed iodine (I_2) and stir until dissolved.

C. Transfer to a 1 liter volumetric flask and dilute to the mark.

D. Standardization

1. Volumetrically measure 40 to 50 mL 0.1 N arsenite solution into a flask.

2. Titrate with 0.1 N iodine solution using starch solution as an indicator.

3. Just before end-point is reached, add a few drops of hydrochloric acid solution to liberate sufficient carbon dioxide (CO_2) to saturate the solution.

4. Titrate until blue color first appears and remains.

5. Normality (N) of iodine = (mL of arsenite solution used x 0.1)/mL of iodine titrated

IV. Standard Iodine Titrant (I_2), 0.0282 N

A. Dissolve 25 g KI in a bottle of distilled water in a 1L volumetric flask.

B. Add the correct amount of the exactly standardized 0.1 N iodine solution to yield a 0.0282 N solution.

C. Dilute to one liter with chlorine-demand-free water.

D. Store iodine solutions in amber bottles or in the dark, and protect from exposure to direct sunlight. Do not use rubber stoppers; keep iodine from all contact with rubber.

E. Check titrant normality daily against 0.00564 N PAO or sodium thiosulfate solution. A procedure for calculating a correction factor for this titrant is given in Appendix C.

V. Standard Potassium Iodate Titrant (KIO3), 0.00564 N

A. Dissolve 201.2 mg primary standard grade potassium iodate (KIO_3), dried for 1 hour at 103°C, or 183.3 mg primary standard grade anhydrous potassium bi-iodate ($KH(IO_3)2V$) in distilled water.

B. Dilute to 1 liter volumetrically.

C. Store in glass bottles in the dark and protect from exposure to direct sunlight.

VI. Potassium Iodide Solution (KI), 5% W/V

A. Dissolve 50 g KI in freshly boiled and cooled distilled water and dilute to 1 liter.

B. Store in a brown glass-stoppered bottle in the dark, preferably at 4°C.

C. Discard when solution becomes yellow.

VII. Acetate Buffer Solution, pH 4.0

A. Dissolve 146 g anhydrous sodium acetate ($NaC_2H_3O_2$ $3H_2O$) in 400 mL distilled water.

B. CAREFULLY add 458 mL concentrated (glacial) acetic acid.

C. Dilute to 1 liter with chlorine-demand-free water.

VIII. Standard Arsenite Solution (As_2O_3), 0.1N

A. Accurately weigh a dried, cooled stoppered weighing bottle.

NOTE: Use forceps or tongs—do not handle weighing bottle with fingers.

B. In weighing bottle, weigh out approximately 4.95 g arsenic trioxide (As_2O_3).

C. Transfer without loss to a 1 liter volumetric flask

NOTE: Do not attempt to brush out remaining arsenic trioxide).

D. Reweigh bottle and record weight of arsenic trioxide transferred.

E. Add enough distilled water to moisten the arsenic trioxide.

F. Add 15 g sodium hydroxide (NaOH) and 100 mL distilled water.

G. Swirl flask gently until As_2O_3 is dissolved.

H. Dilute to 250 mL and saturate the solution with carbon dioxide (CO_2) by bubbling CO_2 gas through the solution for a few minutes.

NOTE: This converts the sodium hydroxide (NaOH) to sodium bicarbonate ($NaHCO_3$).

I. Dilute to the 1 liter mark, stopper, and mix thoroughly.

J. This solution has an almost indefinite shelf life.

CAUTION: This solution is highly poisonous and is a suspected cancer causing agent: handle carefully!

IX. Starch Indicator

A. Weigh out 5 g soluble or potato starch.

B. Add enough distilled water to make a thin paste.

C. Pour into 1 liter boiling distilled water, stir and let settle overnight.

D. Transfer clear supernatant into a storage container and preserve by adding 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide per liter of starch solution.

E. Some commercial starch substitutes or powder indicators are acceptable.

X. Phosphoric Acid solution (H_3PO_4), 1 + 9

A. Carefully add 100 mL of phosphoric acid (H_3PO_4), 85%, to 900 mL of freshly boiled distilled water.

B. Caution should be used when handling this solution, as it can be corrosive.

XI. Phosphoric Acid—Sulfamic Acid Solution

A. Dissolve 20 g sulfamic acid (NH_2SO_3H) in 1 liter of 1 + 9 phosphoric acid (H_3PO_4).

DPD Titrimetric Method

I. Phosphate Buffer Solution

A. Dissolve 24 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) in 400 to 500 mL distilled water.

B. Add 46 g anhydrous potassium dihydrogen phosphate (KH_2PO_4).

C. Dissolve 800 mg disodium ethylenediaminetetraacetate dihydrate (EDTA) in a separate container.

NOTE: This chemical is also known as (ethylenediamine) tetraacetic acid sodium salt.

D. Combine the 2 solutions and dilute to 1 liter.

E. Add 20 mg mercuric chloride to prevent mold growth.

F. Caution: Mercuric chloride is toxic. Take care to avoid ingestion.

II. DPD Indicator Solution

A. Add 8 mL of a 1 + 3 sulfuric acid solution (H_2SO_4) into 500 mL distilled water. Prepare by mixing one part concentrated H_2SO_4 to 3 parts distilled water. (For example, 5 mL H_2SO_4 to 15 mL distilled water.)

B. Add 200 mg EDTA (disodium ethylenediaminetetraacetate dihydrate).

C. Add 1 g DPD Oxalate (N, N-Diethyl-p-phenylenediamine oxalate).

D. Dilute to 1 liter and store in a brown glass-stoppered bottle and discard when discolored.

CAUTION: The DPD oxalate is poisonous, handle carefully!

III. Standard Ferrous Ammonium Sulfate (FAS) Titrant, 0.00282 N

A. Add 1 mL of 1 + 3 sulfuric acid solution (H2SO4) to 500 mL of freshly boiled and cooled distilled water. Prepare by adding one part concentrated H2SO4 to 3 parts distilled water.

B. Dissolve 1.106 g ferrous ammonium sulfate ($Fe(NH_4)2(SO_4)2\ 6H_2O$)

C. Dilute to 1 liter.

D. This standard can be used for 1 month before replacement.

E. Standardize weekly using the following procedure:

1. Measure 100 mL of FAS standard solution into an Erlenmeyer flask.

2. Add 10 mL of 1 + 5 sulfuric acid. Prepare by adding one part concentrated H_2SO_4 to 5 parts distilled water.

3. Add 5 mL concentrated phosphoric acid.

4. Add 2 mL 0.1% barium diphenylamine sulfonate indicator. Prepare by dissolving 0.1 g ($C_6H_5NHC_6H_4$-4-SO_3) Ba in 100 mL distilled water.

5. Titrate with 0.100N potassium dichromate (see iodometric and amperometric section for preparation directions) to a violet end-point that persists for 30 seconds.

DPD Colorimetric Method

I. Phosphate Buffer Solution

(see DPD Titrimetric Method chemicals)

II. DPD Indicator Solution

(see DPD Titrimetric Method chemicals)

III. Potassium Permanganate Stack Solution

A. Dissolve 891 mg potassium permanganate ($KMnO_4$) in distilled water and dilute to 1000 mL.

IV. Potassium Permanganate Standard Solution

A. Dilute 10 mL of stock solution to 100 mL in a volumetric flask.

B. 1 mL of the standard solution diluted to 100 mL with distilled water will be equivalent to 1.0 mg/L chlorine residual in a DPD reaction.

C. Prepare standard solutions by diluting appropriate volumes to 100 mL with distilled water.

If a direct concentration readout colorimeter is used, the DPD and buffer reagents should be prepared or ordered in accordance with the instrument manufacturer's instructions. If the Hach DR100 colorimeter is used, the prepared DPD powder pillows used with the Hach direct reading colorimeters may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

Orion Model 97-70 Electrode Method

With the exception of the 1 ppm potassium iodate standard and the chlorine water (100 ppm), all of the reagents required for this method can be purchased from Orion Research at the following address:

Orion Research Incorporated
840 Memorial Drive
Cambridge, Massachusetts 02139

I. Prepare a 1 mg/L iodate standard by volumetrically diluting 1 mL of the 100 ppm iodate standard to 100 mL with distilled water.

II. Prepare the chlorine water (approximately 100 ppm) by diluting 1 mL hypochlorite solution (household chlorine bleach) to 500 mL with distilled water.

Hach Model CN-66 Test Kit Method
The DPD indicator powder pillows used in the Hach Model CN-66 Test Kit may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

More recently, drinking water providers have faced an array of new challenges, including:

- ✓ Treating resistant pathogens such as Giardia and Cryptosporidium
- ✓ Minimizing disinfection byproducts
- ✓ New environmental and safety regulations
- ✓ Strengthening security at treatment facilities

To meet these new challenges, water system managers must design unique disinfection approaches to match each system's characteristics and source water quality. While chlorination remains the most commonly used disinfection method by far, water systems may use alternative disinfectants, including chloramines, chlorine dioxide, ozone, and ultraviolet radiation. No single disinfection method is right for all circumstances, and in fact, water systems may use a variety of methods to meet overall disinfection goals at the treatment plant, and to provide residual protection throughout the distribution system.
In response to new regulations, emerging science on microbial contaminants, as well as safety and security concerns related to treatment chemicals, water system managers will continue to evaluate chlorine and other disinfection methods.

Despite these challenges, a number of factors indicate that drinking water chlorination will remain a corner-stone of waterborne disease prevention.

- ✓ Disinfection is unquestionably the most important step in drinking water treatment, and chlorine's wide range of benefits cannot be provided by any other single disinfectant.
- ✓ It is uncertain that alternative disinfectants reduce potential DBP risks significantly (IPCS 2000). All chemical disinfectants produce byproducts. Generally, the best approach to control disinfection byproducts is to remove natural organic precursors prior to disinfection (EPA 2001). To comply with the forthcoming Long Term 2 Enhanced Surface Water Treatment Rule, some systems with high levels of Cryptosporidium in their source water may choose to adopt alternative disinfection methods (e.g., chlorine dioxide, ozone, or UV). However, most water systems are expected to meet disinfection requirements without changing treatment technologies.
- ✓ The U.S. EPA's forthcoming Groundwater Rule, as well as efforts to strengthen Canadian drinking water standards following the E coli. outbreak in Walkerton, ON will likely increase the use of chlorination for ground water systems.
- ✓ Only chlorine-based disinfectants provide residual protection, an important part of the multi-barrier approach to preventing waterborne disease.
- ✓ World leaders increasingly recognize safe drinking water as a critical building block of sustainable development (see Sidebar). Chlorination can provide cost-effective disinfection for remote rural villages and large cities alike, helping to bring safe water to those in need.

Microscopic Waterborne Agents
It is easy to take for granted the safety of modern municipal drinking water, but prior to widespread filtration and chlorination, contaminated drinking water presented a significant public health risk. The microscopic waterborne agents of cholera, typhoid fever, dysentery and hepatitis A killed thousands of U.S. residents annually before disinfection methods were employed routinely, starting about a century ago. Although these pathogens are defeated regularly now by technologies such as chlorination, they should be thought of as ever-ready to stage a come-back given conditions of inadequate or no disinfection.

Understanding Bacteria
Bacteria are microorganisms often composed of single cells shaped like rods, spheres or spiral structures. Prior to widespread chlorination of drinking water, bacteria like Vibrio cholerae, Salmonella typhii and several species of Shigella routinely inflicted serious diseases such as cholera, typhoid fever and bacillary dysentery, respectively. As recently as 2000, a drinking water

outbreak of E. coli in Walkerton, Ontario sickened 2,300 residents and killed seven when operators failed to properly disinfect the municipal water supply.
While developed nations have largely conquered water-borne bacterial pathogens through the use of chlorine and other disinfectants, the developing world still grapples with these public health enemies

Understanding Viruses
Viruses are infectious agents that can reproduce only within living host cells. Shaped like rods, spheres or filaments, viruses are so small that they pass through filters that retain bacteria. Enteric viruses, such as hepatitis A, Norwalk virus and rotavirus are excreted in the feces of infected individuals and may contaminate water intended for drinking. Enteric viruses infect the gastrointestinal or respiratory tracts, and are capable of causing a wide range of illness, including diarrhea, fever, hepatitis, paralysis, meningitis and heart disease (American Water Works Association, 1999).

Understanding Protozoan Parasites
Protozoan parasites are single-celled microorganisms that feed on bacteria found in multicellular organisms, such as animals and humans. Several species of protozoan parasites are transmitted through water in dormant, resistant forms, known as cysts and oocysts. According to the World Health Organization, Cryptosporidium parvum oocysts and Giardia lamblia cysts are introduced to waters all over the world by fecal pollution. The same durable form that permits them to persist in surface waters makes these microorganisms resistant to normal drinking water chlorination (WHO, 2002b). Water systems that filter raw water may successfully remove protozoan parasites.

Emerging Pathogens
An emerging pathogen is one that gains attention because it is one of the following:
- a newly recognized disease-causing organism
- a known organism that starts to cause disease
- an organism whose transmission has increased

Understanding Oxidizing Agents
Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.
- ✓ Sodium hypochlorite is very commonly used. Common household bleach is a sodium hypochlorite solution and is used in the home to disinfect drains, toilets, and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound—not pure chlorine—that is being used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills. Commercial solutions with higher concentrations contain substantial amounts of sodium hydroxide for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions are strongly basic as a result.
- ✓ Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- ✓ Electrolyzed water or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- ✓ Chloramine is often used in drinking water treatment.
- ✓ Chloramine-T is antibacterial even after the chlorine has been spent, since the parent compound is a sulfonamide antibiotic.

- ✓ Chlorine dioxide is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- ✓ Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
- ✓ Hydrogen peroxide vapor is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and consequently the OSHA permissible exposure limit is 1 ppm (29 CFR 1910.1000 Table Z-1) calculated as an eight hour time weighted average and the NIOSH immediately dangerous to life and health limit is 75 ppm. Therefore, engineering controls, personal protective equipment, gas monitoring etc. should be employed where high concentrations of hydrogen peroxide are used in the workplace. Vaporized hydrogen peroxide is one of the chemicals approved for decontamination of anthrax spores from contaminated buildings, such as occurred during the 2001 anthrax attacks in the U.S. It has also been shown to be effective in removing exotic animal viruses, such as avian influenza and Newcastle disease from equipment and surfaces.
- ✓ The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide and is produced by Virox Technologies Inc. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes.[19] The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment.
- ✓ Iodine is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, tincture of iodine is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known.
- ✓ Ozone is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly, however, so that tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only.
- ✓ Peracetic acid is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase and peroxidase, the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40°C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues.
- ✓ Performic acid is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide.
- ✓ Potassium permanganate ($KMnO_4$) is a purplish-black crystalline powder that colors everything it touches, through a strong oxidizing action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community

swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.

Understanding Waterborne Viruses

More than 100 types of human pathogenic viruses may be present in fecal-contaminated waters (Havelaar and others, 1993). Treatment processes and watershed management strategies designed on the basis of bacteriological criteria do not necessarily protect against viral infection because viruses are generally more persistent in the environment and are not removed as completely by treatment. In addition, because of their smaller size, viruses (0.023 to 0.080 µm) are transported further in ground water than bacteria (0.5 to 3µ m) or protozoan pathogens (4 to 15 µm) (Abbaszadegan and others, 1998). Because of the importance of viruses as a major public health concern, new methods for detection of enteric viruses and the search for indicators of viral contamination continue.

The current method for culturing enteric viruses under the ICR (U.S. Environmental Protection Agency, 1996c) is recognized as being difficult to implement; therefore, the ICR does not preclude the use of additional methods for research purposes. In addition, cell-culture methods are not available or suitable
for all viruses of public health concern. One method, reverse-transcriptase-polymerase chain reaction (RT-PCR), a gene-probe method that amplifies and recognizes the nucleic acids of target viruses, has been adequately validated by the USEPA (G. Shay Fout, U.S. Environmental Protection Agency, written
commun., 1997) and is becoming widely used for environmental monitoring of enteric viruses. The RTPCR method, however, does not determine the infectivity of the virus, and it is technically demanding, time consuming, and costly for routine use.

Because monitoring of enteric viruses is recognized as being difficult and time consuming, some researchers advocate the use of coliphage as indicator viruses for fecal contamination (Sobsey and others, 1995). Coliphages are bacteriophages that infect and replicate in coliform bacteria. The two main groups of coliphages that are considered as candidates for viral indicators are somatic and F-specific coliphages.

Somatic coliphages infect coliform bacteria by attachment to the outer cell membrane or cell wall. They are widely distributed in both fecal-contaminated and uncontaminated waters; therefore, they may not be reliable indicators of fecal contamination (Sobsey and others, 1995). F-specific coliphages attach only to the F-pilus of coliforms that carry the F+ plasmid; F-pili are made only by bacteria grown at higher temperatures.

Hence, F-specific coliphages found in environmental samples presumably come from warm-blooded animals or sewage (Handzel and others 1993). Although somatic and F-specific coliphages are not consistently found in feces, they are found in high numbers in sewage and are thought to be reliable indicators of the sewage contamination of waters (International Association of Water Pollution Research and Control, 1991). Coliphage is also recognized to be representative of the survival and transport of viruses in the environment. To date, however, coliphage has not been found to correlate with the presence of pathogenic viruses.

Sampling Procedures

Streamwater Sample Collection

When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers used in by the majority of samplers can be used to collect streamwater samples for bacterial and viral indicators, Cryptosporidium, and Giardia providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.
• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.
• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite
by collecting subsamples at vertical locations in the cross section without overfilling the bottle.
• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).
• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for E. coli and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for C. perfringens and coliphage analysis.

Cryptosporidium and Giardia Analysis
For Cryptosporidium and Giardia analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for Cryptosporidium and Giardia. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.
• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for Cryptosporidium analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.).The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Ground-Water Sample Collection
Collecting
Ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps.

Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible.

Disinfection of Water and Wastewater
The disinfection of potable water and wastewater provides a degree of protection from contact with pathogenic organisms including those causing cholera, polio, typhoid, hepatitis and a number of other bacterial, viral and parasitic diseases. Disinfection is a process where a significant percentage of pathogenic organisms are killed or controlled. As an individual pathogenic organism can be difficult to detect in a large volume of water or wastewater, disinfection efficacy is most often measured using "indicator organisms" that coexist in high quantities where pathogens are present. The most common indicator organism used in the evaluation of drinking water is Total Coliform (TC), unless there is a reason to focus on a specific pathogen.

The most common indicator organism for wastewater evaluation is fecal coliform but there has been discussion regarding the use of Escherichia coli (E. coli) or Total Coliform. As domestic wastewater contains approximately 1,000 times more indicator organisms than typical surface water, understanding wastewater disinfection will make it easier to understand water disinfection.

Chlorine gas is primarily a respiratory irritant and concentrations in air above one ppm can usually be detected by most persons. Chlorine causes varying degrees of irritation of the skin, mucus membranes, and the respiratory system, depending on the concentration and the duration of exposure. Severe exposure can cause death, but the severe irritating effect makes it unlikely that anyone would remain in the chlorine-containing atmosphere unless trapped or unconscious.

Liquid chlorine may cause skin and eye burns upon contact with these tissues. Chlorine produces no known cumulative or chronic effect, and complete recovery usually can be expected to occur shortly following mild, short term exposure. An eight-hour time-weighted exposure of one ppm and a one-hour weighted exposure are the current federal Occupational Safety and Health Administration (OSHA) standards.

Understanding Bacteriophage
Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high. This mechanism is not identical to that of temperate phage going dormant and is usually temporary.

In contrast, the lysogenic cycle does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of E. coli.

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome in a phenomenon called lysogenic conversion. An eminent example is the conversion of a harmless strain of Vibrio cholerae by a phage into a highly virulent one, which causes cholera.

Attachment and Penetration

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Myovirus bacteriophages use a hypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possibly with the help of ATP present in the tail, injecting genetic material through the bacterial membrane. Podoviruses lack an elongated tail sheath similar to that of a myovirus, so they instead use their small, tooth-like tail fibers to enzymatically degrade a portion of the cell membrane before inserting their genetic material.

Virions

A virion is a complete functional virus that has the capacity to infect living tissue. This means that it includes the genetic material, the capsid, the enveloppe and the membrane proteins that allow the virus to bind to its host and enter it. A virus will not have an enveloppe within a cell. If the cell was burst artificially, then these virus particles cannot be called virion because they will lack certain proteins that will make them infectious even though the genetic material is present. Not all viruses have enveloppes, but most viruses have certain proteins that are necessary to permit them to enter the host cell.

Biomolecules found in virions: genetic material, either DNA or RNA, single or double stranded, nucleoprotein capsid, maybe an enveloppe usually receptor proteins or enzymes that permit binding or entry into the host. A viroid is a plant pathogen consisting of a circular piece of RNA without a protein coat.

Understanding Disinfection

Wastewater Disinfection
There are a number of chemicals and processes that will disinfect wastewater, but none are universally applicable. Most septic tanks discharge into various types of subsurface wastewater infiltration systems (SWIS), such as tile fields or leach fields. These applications rely on the formation of a biomat at the gravel-soil interface where "biodegradation and filtration combine to limit the travel of pathogens." Aerobic treatment processes reduce pathogens, but not enough to qualify as a disinfection process. "Chlorination/dechlorination has been the most widely used disinfection technology in the U.S.; ozonation and UV light are emerging technologies." Each of these three methods have different considerations for the disinfection of wastewater.

Water Disinfection
Disinfection is usually the final stage in the water treatment process in order to limit the effects of organic material, suspended solids and other contaminants. Like the disinfection of wastewater, the primary methods used for the disinfection of water in very small (25-500 people) and small (501-3,300 people) treatment systems are ozone, ultraviolet irradiation (UV) and chlorine. There are numerous alternative disinfection processes that have been less widely used in small and very small water treatment systems, including chlorine dioxide, potassium permanganate, chloramines and peroxone (ozone/hydrogen peroxide).

Surface waters have been the focal point of water disinfection regulations since their inception, as groundwaters (like wells) have been historically considered to be free of microbiological contamination. Current data indicates this to not be true. Amendments to the Safe Drinking Water Act in 1996 mandate the development of regulations to require disinfection of groundwater "as necessary." While these regulations will apply to very small systems serving twenty-five people at least 60 days out of the year, the rules will not apply to private wells. However, the EPA recommends that wells be tested at least once per year and disinfected as necessary. While these proposed regulations have not yet been finalized, they will likely include; testing by each state, identification of contaminated water supplies, corrective action requiring disinfection and compliance monitoring. The rules are currently scheduled to be implemented in July 2003.

Residual Disinfection
The EPA requires a residual level of disinfection of water in pipelines to prevent microbial re-growth and help protect treated water throughout the distribution system. EPA”s maximum residual disinfection levels (MRDLs) are 4 mg/l for chlorine, 4 mg/l for chloramines and 0.8 mg/l for chlorine dioxide. Although chlorine levels are usually significantly lower in tap water, EPA believes that levels as high as the MRDLs pose no risk of adverse health effects, allowing for an adequate margin of safety (U.S. EPA, 1998a).

Chlorate Ion
The chlorate anion has the formula ClO-3. In this case, the chlorine atom is in the +5 oxidation state. "Chlorate" can also refer to chemical compounds containing this anion; chlorates are the salts of chloric acid. "Chlorate", when followed by a roman numeral in parentheses, e.g. chlorate (VII), refers to a particular oxyanion of chlorine. As predicted by VSEPR, chlorate anions have trigonal pyramidal structures.

Chlorates are powerful oxidizers and should be kept away from organics or easily oxidized materials. Mixtures of chlorate salts with virtually any combustible material (sugar, sawdust, charcoal, organic solvents, metals, etc.) will readily deflagrate. Chlorates were once widely used in pyrotechnics for this reason, though their use has fallen due to their instability. Most pyrotechnic applications which formerly used chlorates in the past now use the more stable perchlorates instead

Examples of chlorates include

- ✓ potassium chlorate, $KClO_3$
- ✓ sodium chlorate, $NaClO_3$
- ✓ magnesium chlorate, $Mg(ClO_3)2$

Chloride Ion

The chloride ion is formed when the element chlorine, a halogen, gains an electron to form an anion (negatively-charged ion) Cl-. The salts of hydrochloric acid contain chloride ions and can also be called chlorides. The chloride ion, and its salts such as sodium chloride, are very soluble in water. It is an essential electrolyte located in all body fluids responsible for maintaining acid/base balance, transmitting nerve impulses and regulating fluid in and out of cells.

The word chloride can also form part of the name of chemical compounds in which one or more chlorine atoms are covalently bonded. For example, methyl chloride, more commonly called chloromethane, (CH_3Cl) is an organic covalently bonded compound, which does not contain a chloride ion.

Chloride is used to form salts that can preserve food such as sodium chloride. Other salts such as calcium chloride, magnesium chloride, potassium chloride have varied uses ranging from medical treatments to cement formation.

An example is table salt, which is sodium chloride with the chemical formula NaCl. In water, it dissociates into Na+ and Cl- ions.

Examples of inorganic covalently bonded chlorides that are used as reactants are:

- ✓ Phosphorus trichloride, phosphorus pentachloride, and thionyl chloride, all three of which reactive chlorinating reagents that have been used in a laboratory.
- ✓ Disulfur dichloride (S_2Cl_2), used for vulcanization of rubber.

A chloride ion is also the prosthetic group present in the amylase enzyme. Another example is calcium chloride with the chemical formula $CaCl_2$. Calcium chloride is a salt that is marketed in pellet form for removing dampness from rooms. Calcium chloride is also used for maintaining unpaved roads and for sanite fortifying roadbases for new construction. In addition, Calcium chloride is widely used as a deicer since it is effective in lowering the melting point when applied to ice.

In the petroleum industry, the chlorides are a closely monitored constituent of the mud system. An increase of the chlorides in the mud system may be an indication of drilling into a high-pressure saltwater formation. Its increase can also indicate the poor quality of a target sand. Chloride is also a useful and reliable chemical indicator of river / groundwater fecal contamination, as chloride is a non-reactive solute and ubiquitous to sewage & potable water. Many water regulating companies around the world utilize chloride to check the contamination levels of the rivers and potable water sources.

Chlorite Ion

The chlorite ion is ClO_2-. A chlorite (compound) is a compound that contains this group, with chlorine in oxidation state +3. Chlorites are also known as salts of chlorous acid. Chlorine can assume oxidation states of -1, +1, +3, +5, or +7 within the corresponding anions Cl-, ClO-, ClO_2-, ClO_3-, or ClO_4-, known commonly and respectively as chloride, hypochlorite, chlorite, chlorate, and perchlorate. An additional oxidation state of +4 is seen in the neutral compound chlorine dioxide ClO_2, which has a similar structure to chlorite ClO_2- (oxidation state +3) and the cation chloryl (ClO_2+) (oxidation state +5).

Chlorine Dioxide

Chlorine dioxide is a chemical compound with the formula ClO_2. This yellowish-green gas crystallizes as bright orange crystals at -59 °C. As one of several oxides of chlorine, it is a potent and useful oxidizing agent used in water treatment and in bleaching. The molecule ClO2 has an odd number of valence electrons and it is therefore a paramagnetic radical. Its electronic structure has long baffled chemists because none of the possible Lewis structures are very satisfactory. In 1933 L.O. Brockway proposed a structure that involved a three-electron bond.

Chemist Linus Pauling further developed this idea and arrived at two resonance structures involving a double bond on one side and a single bond plus three-electron bond on the other. In Pauling's view the latter combination should represent a bond that is slightly weaker than the double bond. In molecular orbital theory this idea is commonplace if the third electron is placed in an anti-bonding orbital. Later work has confirmed that the HOMO is indeed an incompletely-filled orbital.

Chlorine dioxide is a highly endothermic compound that can decompose extremely violently when separated from diluting substances. As a result, preparation methods that involve producing solutions of it without going through a gas phase stage are often preferred. Arranging handling in a safe manner is essential.

In the laboratory, ClO_2 is prepared by oxidation of sodium chlorite:

$$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$$

Over 95% of the chlorine dioxide produced in the world today is made from sodium chlorate and is used for pulp bleaching. It is produced with high efficiency by reducing sodium chlorate in a strong acid solution with a suitable reducing agent such as methanol, hydrogen peroxide, hydrochloric acid or sulfur dioxide. Modern technologies are based on methanol or hydrogen peroxide, as these chemistries allows the best economy and do not co-produce elemental chlorine. The overall reaction can be written;

Chlorate + Acid + reducing agent - Chlorine Dioxide + By-products

The reaction of sodium chlorate with hydrochloric acid in a single reactor is believed to proceed via the following pathway:

$$HClO_3 + HCl - HClO_2 + HOCl$$

$$HClO_3 + HClO_2 - 2\ ClO_2 + Cl_2 + 2\ H_2O$$

$$HOCl + HCl - Cl_2 + H_2O$$

The commercially more important production route uses methanol as the reducing agent and sulfuric acid for the acidity. Two advantages by not using the chloride-based processes are that there is no formation of elemental chlorine, and that sodium sulfate, a valuable chemical for the pulp mill, is a side-product. These methanol-based processes provide high efficiency and can be made very safe.

A much smaller, but important, market for chlorine dioxide is for use as a disinfectant. Since 1999 a growing proportion of the chlorine dioxide made globally for water treatment and other small-scale applications has been made using the chlorate, hydrogen peroxide and sulfuric acid method, which can produce a chlorine-free product at high efficiency.

Traditionally, chlorine dioxide for disinfection applications has been made by one of three methods using sodium chlorite or the sodium chlorite - hypochlorite method:

$2\ NaClO_2 + 2\ HCl + NaOCl - 2\ ClO_2 + 3\ NaCl + H_2O$

or the sodium chlorite - hydrochloric acid method:

$5\ NaClO_2 + 4\ HCl - 5\ NaCl + 4\ ClO_2 + 2\ H_2O$

All three sodium chlorite chemistries can produce chlorine dioxide with high chlorite conversion yield, but unlike the other processes the chlorite-HCl method produces completely chlorine-free chlorine dioxide but suffers from the requirement of 25% more chlorite to produce an equivalent amount of chlorine dioxide. Alternatively, hydrogen peroxide may efficiently be used also in small scale applications.

Very pure chlorine dioxide can also be produced by electrolysis of a chlorite solution:

$2\ NaClO_2 + 2\ H_2O - 2\ ClO_2 + 2\ NaOH + H_2$

High purity chlorine dioxide gas (7.7% in air or nitrogen) can be produced by the Gas: Solid method, which reacts dilute chlorine gas with solid sodium chlorite.

$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$

These processes and several slight variations have been reviewed.

Haloacetic Acids
Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$). The inductive effect caused by the electronegative halogens often result in the higher acidity of these compounds by stabilizing the negative charge of the conjugate base.

Contaminants in Drinking Water
Haloacetic acids (HAAs) are a common undesirable by-product of drinking water chlorination. Exposure to such disinfection by-products in drinking water has been associated with a number of health outcomes by epidemiological studies, although the putative agent in such studies has not been identified.

In water, HAAs are stable, with the five most common being:

- ✓ monochloroacetic acid (MCA) $ClCH_2COOH$;
- ✓ dichloroacetic acid (DCA) $Cl_2CHCOOH$;
- ✓ trichloroacetic acid (TCA) Cl_3CCOOH;
- ✓ monobromoacetic acid (MBA) $BrCH_2COOH$;
- ✓ dibromoacetic acid (DBA) $Br_2CHCOOH$.

Collectively, these are referred to as the HAA5. HAAs can be formed by chlorination, ozonation or chloramination of water with formation promoted by slightly acidic water, high organic matter content and elevated temperature. Chlorine from the water disinfection process can react with organic matter and small amounts of bromide present in water to produce various HAAs. A study published in August 2006 found that total levels of HAAs in drinking water were not affected by storage or boiling, but that filtration was effective in decreasing levels.

Hypochlorites

Hypochlorites are calcium or sodium salts of hypochlorous acid and are supplied either dry or in liquid form (as, for instance, in commercial bleach). The same residuals are obtained as with gas chlorine, but the effect on the pH of the treated water is different. Hypochlorite compounds contain an excess of alkali and tend to raise the pH of the water. Calcium hypochlorite tablets are the predominant form in use in the United States for swimming pools. Sodium hypochlorite is the only liquid hypochlorite disinfectant in current use. There are several grades and proprietary forms available. Pound-for-pound of available chlorine, hypochlorite compounds have oxidizing powers equal to gas chlorine and can be employed for the same purposes in water treatment. Gas chlorination requires a larger initial investment for feed equipment than what is needed for hypochlorite compounds.

Calcium hypochlorite materials used in the water industry are chemically different from those materials variously marketed for many years as bleaching powder, chloride of lime, or chlorinated lime. Materials now in common use are high-test calcium hypochlorites containing about 70 percent available chlorine and marketed under several trade names.

High-test calcium hypochlorites are white corrosive solids that give off a strong chlorine odor. Granular powdered or tablet forms are commercially available and all are readily soluble in water.

Sodium hypochlorite is sold only as a liquid and is normally referred to as liquid bleach. It is generally available in concentrations of 5 to 15 percent available chlorine. These solutions are clear, light yellow, strongly alkaline, and corrosive in addition to having a strong chlorine smell.

High-test hypochlorites, though highly active, are relatively stable throughout production, packaging, distribution, and storage. Storage at 86° F. for a year may reduce the available chlorine by about 10 percent. Storing at lower temperatures reduces the loss. All sodium-hypochlorite solutions are unstable to some degree and deteriorate more rapidly than the dry compounds. Most producers recommend a shelf life of 60 to 90 days. Because light and heat accelerate decomposition, containers should be stored in a dry, cool, and dark area.

Disinfection Byproducts
Disinfection byproducts are formed when disinfectants used in water treatment plants react with bromide and/or natural organic matter (i.e., decaying vegetation) present in the source water. Different disinfectants produce different types or amounts of disinfection byproducts. Disinfection byproducts for which regulations have been established have been identified in drinking water, including trihalomethanes, haloacetic acids, bromate, and chlorite.

Trihalomethanes (THM)
Trihalomethanes (THM) are a group of four chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The trihalomethanes are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate total trihalomethanes (TTHM) at a maximum allowable annual average level of 80 parts per billion. This standard will replace the current standard of a maximum allowable annual average level of 100 parts per billion in December 2001 for large surface water public water systems. The standard will become effective for the first time in December 2003 for small surface water and all ground water systems.

Haloacetic Acids (HAA5)
Haloacetic Acids (HAA5) are a group of chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The regulated haloacetic acids, known as HAA5, are: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate HAA5 at 60 parts per billion annual

average. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Bromate is a chemical that is formed when ozone used to disinfect drinking water reacts with naturally occurring bromide found in source water. EPA has established the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate bromate at annual average of 10 parts per billion in drinking water. This standard will become effective for large public water systems by December 2001 and for small surface water and all ground public water systems in December 2003.

Chlorite
Chlorite is a byproduct formed when chlorine dioxide is used to disinfect water. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate chlorite at a monthly average level of 1 part per million in drinking water. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Chloroform
Chloroform, typically the most prevalent THM measured in chlorinated water, is probably the most thoroughly studied disinfection byproduct. Toxicological studies have shown that high levels of chloroform can cause cancer in laboratory animals. Extensive research conducted since the early 1990s provides a clearer picture of what this means for humans exposed to far lower levels through drinking water.

One study (Larson et al. 1994a) conducted by the Centers for Health Research (CIIT) observed that a very large dose of chloroform, when given to mice once per day into the stomach (a procedure known as gavage), produced liver damage and eventually cancer. In a second CIIT cancer study (Larson et al., 1994b), mice were given the same daily dose of chloroform through the animals' drinking water. This time, no cancer was produced. Follow-up research showed that the daily gavage doses overwhelmed the capability of the liver to detoxify the chloroform, causing liver damage, cell death and regenerative cell growth, thereby increasing risks for cell mutation and cancer in exposed organs. When chloroform was given through drinking water, however, the liver could continually detoxify the chloroform as the mice sipped the water throughout the day. Without the initial liver toxicity, there was no cancer in the liver, kidney or other exposed organs (Butterworth et al., 1998).

In its most recent risk assessment, EPA considered the wealth of available information on chloroform, including the important work done at CIIT. EPA concludes that exposure to chloroform below the threshold level that causes cell damage is unlikely to increase the risk of cancer. While chloroform is likely to be carcinogenic at a high enough dose, exposures below a certain dose range are unlikely to pose any cancer risk to humans (US EPA, 2002a). For drinking water meeting EPA standards, chloroform is unlikely to be a health concern.

Sodium Chlorate
Sodium chlorate is a chemical compound with the chemical formula ($NaClO_3$). When pure, it is a white crystalline powder that is readily soluble in water. It is hygroscopic. It decomposes above 250 °C to release oxygen and leave sodium chloride. Industrially, sodium chlorate is synthesized from the electrolysis of a hot sodium chloride solution in a mixed electrode tank:

$$NaCl + 3H_2O - NaClO_3 + 3H_2$$

It can also be synthesized by passing chlorine gas into a hot sodium hydroxide solution. It is then purified by crystallization.

Chemical Oxygen Generation

Chemical oxygen generators, such as those in commercial aircraft, provide emergency oxygen to passengers to protect them from drops in cabin pressure by catalytic decomposition of sodium chlorate. The catalyst is normally iron powder. Barium peroxide (BaO_2) is used to absorb the chlorine which is a minor product in the decomposition. Iron powder is mixed with sodium chlorate and ignited by a charge which is activated by pulling on the emergency mask. The reaction produces more oxygen than is required for combustion. Similarly, the Solidox welding system used pellets of sodium chlorate mixed with combustible fibers to generate oxygen.

Toxicity in Humans
Due to its oxidative nature, sodium chlorate can be very toxic if ingested. The oxidative effect on hemoglobin leads to methaemoglobin formation, which is followed by denaturation of the globin protein and a cross-linking of erythrocyte membrane proteins with resultant damage to the membrane enzymes.

This leads to increased permeability of the membrane, and severe hemolysis. The denaturation of hemoglobin overwhelms the capacity of the G6PD metabolic pathway. In addition, this enzyme is directly denatured by chlorate reducing its activity. Therapy with ascorbic acid and methylene blue are frequently used in the treatment of methemoglobinemia. However, since methylene blue requires the presence of NADPH that requires normal functioning of G6PD system, it is less effective than in other conditions characterized by hemoglobin oxidation.

Acute severe hemolysis results, with multi-organ failure, including DIC and renal failure. In addition there is a direct toxicity to the proximal renal tubule. The treatment will consist of exchange transfusion, peritoneal dialysis or hemodialysis.

Developmental and Reproductive Effects
Several epidemiology studies have reported a possible association between disinfection byproducts and adverse reproductive outcomes, including spontaneous abortion (miscarriage). One study of women in several California communities (Waller et al. 1998) found a stronger association with bromodichloromethane (BDCM) than with other byproducts. Because the available studies have significant limitations, EPA and the American Water Works Association Research Foundation are sponsoring a new epidemiology study to replicate the 1998 Waller study.

When the Waller study was published, the available toxicology data on reproductive and developmental effects of some DBPs was quite limited. It was recognized that BDCM, in particular, should be thoroughly studied for a potential causal relationship to reproductive and developmental toxicity. The Research Foundation for Health and Environmental Effects, a tax-exempt foundation established by the Chlorine Chemistry Division of the American Chemistry Council, sponsored a set of animal studies (Christian et al. 2001, 2002) including two developmental toxicity studies on BDCM, a reproductive toxicity study on BDCM, and a reproductive toxicity study on dibromoacetic acid (DBA). The studies, published in the International Journal of Toxicology, found no adverse effects from BDCM and DBA at dose levels thousands of times higher than what humans are exposed to through drinking water. The studies were designed to comply with stringent EPA guidelines, and each study was independently monitored and peer reviewed.

Formulations
Sodium chlorate comes in dust, spray and granule formulations. There is a risk of fire and explosion in dry mixtures with other substances, especially organic materials, and other herbicides, sulfur, phosphorus, powdered metals, strong acids. In particular, when mixed with sugar, it has explosive properties. If accidentally mixed with one of these substances it should not be stored in human dwellings. Marketed formulations contain a fire retardant, but this has little effect if deliberately ignited. Most commercially available chlorate weedkillers contain approximately 53% sodium chlorate with the balance being a fire depressant such as sodium metaborate or ammonium phosphates.

Sodium Chlorite
Sodium chlorite, like many oxidizing agents, should be protected from inadvertent contamination by organic materials to avoid the formation of an explosive mixture. The chemical explodes on percussive impact, and will ignite if combined with a strong reducing agent.

Toxicity
Sodium chlorite is a strong oxidant and can therefore be expected to cause clinical symptoms similar to the well-known sodium chlorate: methemoglobinemia, hemolysis, renal failure.[14] A dose of 10-15 grams of sodium chlorate can be lethal. Methemoglobemia had been demonstrated in rats and cats, and recent studies by the EMEA have confirmed that the clinical symptomatology is very similar to the one caused by sodium chlorate in the rat, mouse, rabbit, and the green monkey. There is only one human case in the medical literature of chlorite poisoning. It seems to confirm that the toxicity is equal to sodium chlorate. From the analogy with sodium chlorate, even small amounts of about 1 gram can be expected to cause nausea, vomiting and even life-threatening hemolysis in Glucose-6-Phosphate Dehydrogenase deficient persons. The EPA has set a maximum contaminant level of 1 milligram of chlorite per liter (1 mg/L) in drinking water.

Manufacture
The free acid, chlorous acid, $HClO_2$, is only stable at low concentrations. Since it cannot be concentrated, it is not a commercial product. However, the corresponding sodium salt, sodium chlorite, $NaClO_2$ is stable and inexpensive enough to be commercially available. The corresponding salts of heavy metals (Ag+, Hg+, Tl+, Pb2+, and also Cu2+ and NH_4+) decompose explosively with heat or shock. Sodium chlorite is derived indirectly from sodium chlorate, $NaClO_3$. First, the explosive (only at concentrations greater than 10% in atmosphere) chlorine dioxide, ClO_2 is produced by reducing sodium chlorate in a strong acid solution with a suitable reducing agent (for example, sodium sulfite, sulfur dioxide, or hydrochloric acid). The chlorine dioxide is then absorbed into an alkaline solution and reduced with hydrogen peroxide (H_2O_2), yielding sodium chlorite.

Stachybotrys
Stachybotrys is a genus of molds, or asexually-reproducing, filamentous fungi. Closely related to the genus Memnoniella, most Stachybotrys species inhabit materials rich in cellulose. The genus has a widespread distribution, and contains about 50 species. The most infamous species, S. chartarum (also known as S. atra) and S. chlorohalonata are known as "black mold" or "toxic black mold" in the U.S. and are frequently associated with poor indoor air quality that arises after fungal growth on water-damaged building materials

Symptoms of Stachybotrys Exposure in Humans
Exposure to the mycotoxins present in Stachybotrys chartarum or Stachybotrys atra can have a wide range of effects. Depending on the length of exposure and volume of spores inhaled or ingested, symptoms can manifest as chronic fatigue or headaches, fever, irritation to the eyes, mucous membranes of the mouth, nose and throat, sneezing, rashes, and chronic coughing. In severe cases of exposure or cases exacerbated by allergic reaction, symptoms can be extreme including nausea, vomiting, and bleeding in the lungs and nose.

Understanding Commonly Used Water Disinfectants
Almost all U.S. systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants. In addition to controlling disease-causing organisms, chlorination offers a number of benefits including:

- Reduces many disagreeable tastes and odors;
- Eliminates slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks;
- Removes chemical compounds that have unpleasant tastes and hinder disinfection; and
- Helps remove iron and manganese from raw water.

As importantly, only chlorine-based chemicals provide “residual disinfectant” levels that prevent microbial re-growth and help protect treated water throughout the distribution system.

The Risks of Waterborne Disease
Where adequate water treatment is not readily available, the impact on public health can be devastating. Worldwide, about 1.2 billion people lack access to safe drinking water, and twice that many lack adequate sanitation. As a result, the World Health Organization estimates that 3.4 million people, mostly children, die every year from water-related diseases.

Even where water treatment is widely practiced, constant vigilance is required to guard against waterborne disease outbreaks. Well-known pathogens such as E. coli are easily controlled with chlorination, but can cause deadly outbreaks given conditions of inadequate or no disinfection. A striking example occurred in May 2000 in the Canadian town of Walkerton, Ontario. Seven people died and more than 2,300 became ill after E. coli and other bacteria infected the town’s water supply. A report published by the Ontario Ministry of the Attorney General concludes that, even after the well was contaminated, the Walkerton disaster could have been prevented if the required chlorine residuals had been maintained.
Some emerging pathogens such as Cryptosporidium are resistant to chlorination and can appear even in high quality water supplies. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April 1993. More than 100 deaths are attributed to this outbreak. New regulations from the U.S. Environmental Protection Agency (EPA) will require water systems to monitor Cryptosporidium and adopt a range of treatment options based on source water Cryptosporidium concentrations. Most water systems are expected to meet EPA requirements while continuing to use chlorination.

The Benefits of Chlorine
Potent Germicide
Chlorine disinfectants can reduce the level of many disease-causing microorganisms in drinking water to almost immeasurable levels. Chlorine is added to drinking water to destroy pathogenic (disease-causing) organisms. It can be applied in several forms: elemental chlorine (chlorine gas), sodium hypochlorite solution (bleach) and dry calcium hypochlorite.

When applied to water, each of these forms “free chlorine” (see Sidebar: How Chlorine Kills Pathogens). One pound of elemental chlorine provides approximately as much free available chlorine as one gallon of sodium hypochlorite (12.5% solution) or approximately 1.5 pounds of calcium hypochlorite (65% strength). While any of these forms of chlorine can effectively disinfect drinking water, each has distinct advantages and limitations for particular applications. Almost all water systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants.

Taste and Odor Control
Chlorine disinfectants reduce many disagreeable tastes and odors. Chlorine oxidizes many naturally occurring substances such as foul-smelling algae secretions, sulfides and odors from decaying vegetation.

Biological Growth Control
Chlorine disinfectants eliminate slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks.

Chemical Control
Chlorine disinfectants destroy hydrogen sulfide (which has a rotten egg odor) and remove ammonia and other nitrogenous compounds that have unpleasant tastes and hinder disinfection. They also help to remove iron and manganese from raw water.

Water Treatment

Every day, approximately 170,000 (U.S. EPA, 2002) public water systems treat and convey billions of gallons of water through approximately 880,000 miles (Kirmeyer, 1994) of distribution system piping to U.S. homes, farms and businesses. Broadly speaking, water is treated to render it suitable for human use and consumption. While the primary goal is to produce a biologically (disinfected) and chemically safe product, other objectives also must be met, including: no objectionable taste or odor; low levels of color and turbidity (cloudiness); and chemical stability (non-corrosive and non-scaling). Individual facilities customize treatment to address the particular natural and manmade contamination characteristic of their raw water.

Surface water usually presents a greater treatment challenge than groundwater, which is naturally filtered as it percolates through sediments. Surface water is laden with organic and mineral particulate matter, and may harbor protozoan parasites such as Cryptosporidium parvum and Giardia lamblia.

Water Distribution
In storage and distribution, drinking water must be kept safe from microbial contamination. Frequently, slippery films of bacteria, known as biofilms, develop on the inside walls of pipes and storage containers. Among disinfection techniques, chlorination is unique in that a pre-determined chlorine concentration may be designed to remain in treated water as a measure of protection against harmful microbes encountered after leaving the treatment facility. In the event of a significant intrusion of pathogens resulting, for example, from a broken water main, the level of the average “chlorine residual” will be insufficient to disinfect contaminated water. In such cases, it is the monitoring of the sudden drop in the chlorine residual that provides the critical indication to water system operators that there is a source of contamination in the system.

The Challenge of Disinfection Byproducts
While protecting against microbial contamination is the top priority, water systems must also control disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with natural organic matter in water. In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. EPA set the first regulatory limits for THMs in 1979. While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formation are available and should be adopted where possible.

Chemical Safety (IPCS 2000) Strongly Cautions:
The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts. Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic material prior to disinfection.

Chlorine and Water System Security
The prospect of a terrorist attack has forced all water systems, large and small, to re-evaluate and upgrade existing security measures. Since September 11th, 2001, water system managers have taken unprecedented steps to protect against possible attacks such as chemical or biological contamination of the water supply, disruption of water treatment or distribution, and intentional release of treatment chemicals.

With passage of the Public Health Security and Bioterrorism Response Act of 2002, Congress required community water systems to assess their vulnerability to a terrorist attack and other intentional acts. As part of these vulnerability assessments, systems assess the transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). Water systems using elemental chlorine, in particular, must determine whether existing protection

systems are adequate. If not, they must consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Disinfection is crucial to water system security, providing the “front line” of defense against biological contamination. However, conventional treatment barriers in no way guarantee safety from biological attacks. Additional research and funding are needed to improve prevention, detection and responses to potential threats.

The Future of Chlorine Disinfection
Despite a range of new challenges, drinking water chlorination will remain a cornerstone of waterborne disease prevention. Chlorine’s wide array of benefits cannot be provided by any other single disinfectant. While alternative disinfectants (including chlorine dioxide, ozone, and ultraviolet radiation) are available, all disinfection methods have unique benefits, limitations, and costs. Water system managers must consider these factors, and design a disinfection approach to match each system’s characteristics and source water quality.

Understanding Disinfection Byproducts (DBPS)

Chlorine and other chemical disinfectants have been widely used by public water systems (along with filtration) to protect the public from microbial pathogens in drinking water. DBPs are formed when certain disinfectants react with DBP precursors (organic and inorganic materials) in source waters. In most cases, natural organic matter (NOM) is an important factor that affects the levels of DBPs that form (NOM is usually measured as TOC). The levels of DBPs in drinking water can vary significantly from one point in a distribution system to another, as many continue to form in the distribution system. DBP levels are generally higher in surface water systems because surface water usually contains higher DBP precursor levels and requires stronger disinfection.

Updating the Safe Drinking Water Act Regulations
EPA has regulated DBPs in drinking water since 1979. The first DBP standards limited THM levels to 100 parts per billion (ppb) for systems serving more the 10,000 people. In the 1996 Safe Drinking Water Act (SDWA) reauthorization, Congress called for EPA to revise its standards for disinfectants and DBPs in two stages. The revised regulations are designed to reduce potential DBP risks, while ensuring that drinking water is protected from microbial contamination.

Stage 1 DBP Rule
In December 1998 USEPA issued the Stage 1 Disinfectants and Disinfection Byproducts (Stage 1 DBP) rule. The regulations are based on an agreement between members of a Federal Advisory Committee that included representatives from water utilities, the Chlorine Chemistry Division of the American Chemistry Council, public health officials, environmentalists and other stakeholder groups. This diverse group of experts developed a consensus set of recommendations to cost-effectively reduce DBP levels, without compromising protection from microbial contaminants.

The Stage 1 DBP rule mandates a process called enhanced coagulation to remove natural organic matter, reducing the potential for DBPs to form. The rule also sets enforceable Maximum Contaminant Levels (MCLs) for total trihalomethanes at 80 ppb and the sum of five Haloacetic Acids (HAAs) at 60 ppb. These MCLs are based on system-wide running annual averages, meaning that concentrations may be higher at certain times and at certain points in the system, as long as the system-wide average for the year is below the MCL. In developing the Stage 1 DBP rule, EPA was very cautious about encouraging the use of alternative disinfectants. The Agency recognized that alternative disinfectants might reduce THMs and HAAs, but produce other, less understood, byproducts. The Agency also avoided making recommendations that would encourage utilities to reduce the level of disinfection currently being practiced.

Large water systems (those serving more than 10,000 persons) were required to comply with the Stage 1 DBP rule by December 2001. Systems serving fewer than 10,000 persons must comply by December 2003.

Stage 2 DBP Rule
As the Stage 1 rule is coming into full force, EPA is completing work on its Stage 2 DBP rule. The Stage 2 rule is being developed simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) in order to address the risk trade-offs between pathogen control and exposure to DBPs. The LT2 rule deals primarily with controlling Cryptosporidium and other resistant pathogens discussed in Chapter 3. Again, the EPA sought recommendations from an advisory group, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee.

As outlined in the advisory committee's September 2000 Agreement in Principle, the MCLs for THMs and five HAAs will remain 80 ppb and 60 ppb respectively, based on each utility's system-wide running annual averages. However, the Stage 2 rule will also limit DPB levels at specific locations within distribution systems. When fully implemented, these locational running annual average limits will mean that no part of the distribution system will be allowed to exceed the MCLs for these substances.

Total Trihalomethanes
Trihalomethanes (THMs) are chemical compounds in which three of the four hydrogen atoms of methane (CH4) are replaced by halogen atoms. Many trihalomethanes find uses in industry as solvents or refrigerants. THMs are also environmental pollutants, and many are considered carcinogenic. Trihalomethanes with all the same halogen atoms are called haloforms. Trihalomethanes are formed as a by-product predominantly when chlorine is used to disinfect water for drinking. They represent one group of chemicals generally referred to as disinfection by-products. They result from the reaction of chlorine and/or bromine with organic matter present in the water being treated. The THMs produced have been associated through epidemiological studies with some adverse health effects. Many governments set limits on the amount permissible in drinking water. However, trihalomethanes are only one group of many hundreds of possible disinfection by-products—the vast majority of which are not monitored—and it has not yet been clearly demonstrated which of these are the most plausible candidate for causation of these health effects. In the United States, the EPA limits the total concentration of the four chief constituents (chloroform, bromoform, bromodichloromethane, and dibromochloromethane), referred to as total trihalomethanes (TTHM), to 80 parts per billion in treated water.

THM Treatment
THM levels tend to increase with pH, temperature, time, and the level of "precursors" present. Precursors are organic material which reacts with chlorine to form THM's. One way to decrease THM's is to eliminate or reduce chlorination before the filters and to reduce precursors. There are more precursors present before filtration, so we want to reduce or eliminate the time chlorine is in contact with this water. If some oxidation before filtration is required, an alternative disinfectant like potassium permanganate or peroxide could be considered. Note that this may not be an option if prechlorination is necessary to achieve required CT values.

The EPA has indicated that the best available technology for THM control at treatment plants is removal of precursors through "enhanced coagulation". Enhanced coagulation refers to the process of optimizing the filtration process to maximize removal of precursors. Removal is improved by decreasing pH (to levels as low as 4 or 5), increasing the feed rate of coagulants, and possibly using ferric coagulants instead of alum.

Understanding Cryptosporidiosis
Cryptosporidium is an emerging parasitic protozoan pathogen because its transmission has increased dramatically over the past two decades. Evidence suggests it is newly spread in increasingly popular day-care centers and possibly in widely distributed water supplies, public pools and institutions such as hospitals and extended-care facilities for the elderly. Recognized in humans largely since 1982 and the start of the AIDS epidemic, Cryptosporidium is able to cause potentially life-threatening disease in the growing number of immunocompromised patients. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history,

affecting over 400,000 people in Milwaukee in April, 1993. More than 100 deaths are attributed to this outbreak. Cryptosporidium remains a major threat to the U.S. water supply (Ibid.).

The EPA is developing new drinking water regulations to reduce Cryptosporidium and other resistant parasitic pathogens. Key provisions of the Long Term 2 Enhanced Surface Water Treatment Rule include source water monitoring for Cryptosporidium; inactivation by all unfiltered systems; and additional treatment for filtered systems based on source water Cryptosporidium concentrations. EPA will provide a range of treatment options to achieve the inactivation requirements. Systems with high concentrations of Cryptosporidium in their source water may adopt alternative disinfection methods (e.g., ozone, UV, or chlorine dioxide). However, most water systems are expected to meet EPA requirements while continuing to use chlorination. Regardless of the primary disinfection method used, water systems must continue to maintain residual levels of chlorine-based disinfectants in their distribution systems.

Understanding Giardia lamblia

Giardia lamblia, discovered approximately 20 years ago, is another emerging waterborne pathogen. This parasitic microorganism can be transmitted to humans through drinking water that might otherwise be considered pristine. In the past, remote water sources that were not affected by human activity were thought to be pure, warranting minimal treatment. However, it is known now that all warm-blooded animals may carry Giardia and that beaver are prime vectors for its transmission to water supplies.

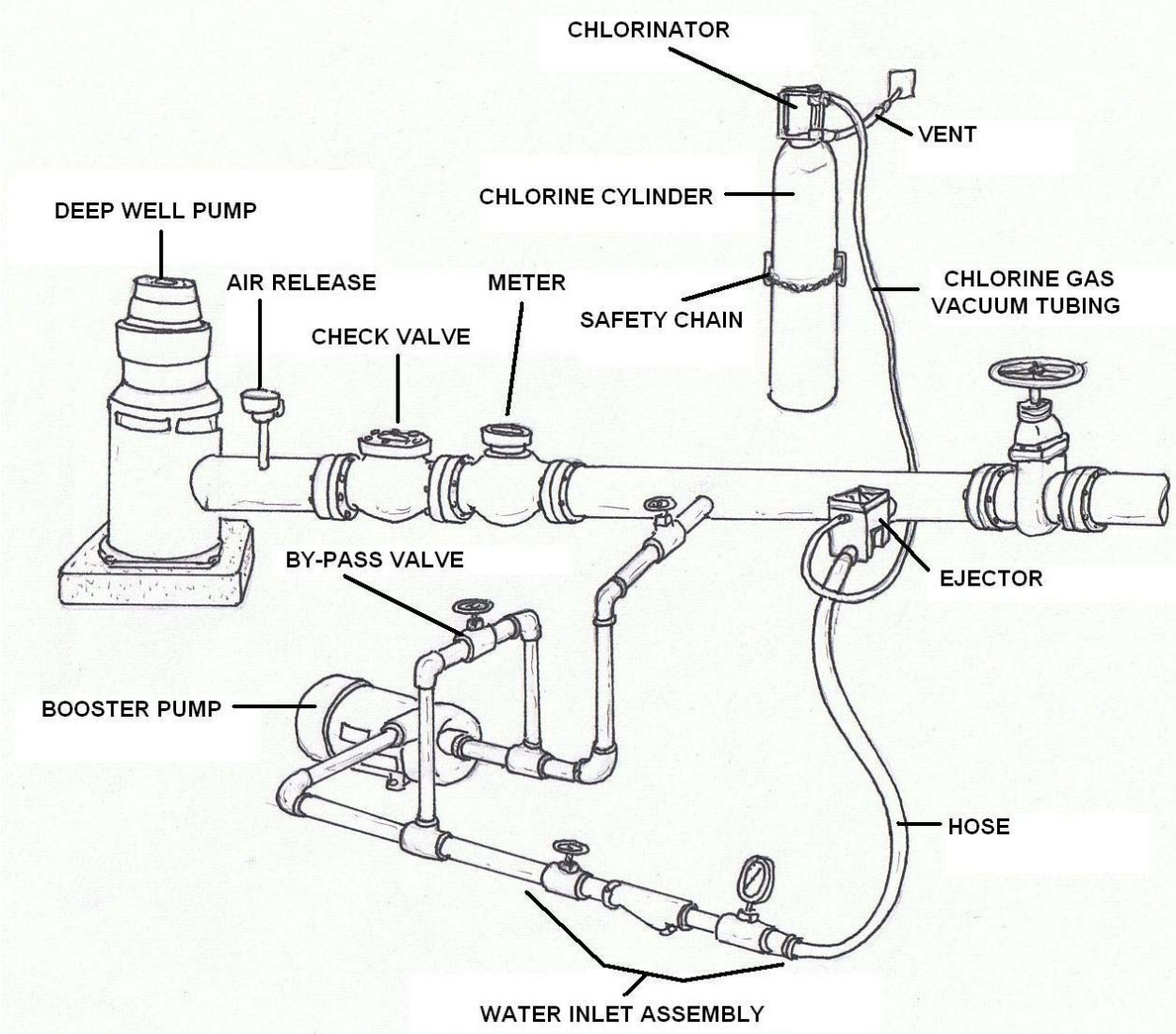
There is a distinct pattern to the emergence of new pathogens. First, there is a general recognition of the effects of the pathogen in highly susceptible populations such as children, cancer patients and the immunocompromised. Next, practitioners begin to recognize the disease and its causative agent in their own patients, with varied accuracy. At this point, some may doubt the proposed agent is the causative agent, or insist that the disease is restricted to certain types of patients. Finally, a single or series of large outbreaks result in improved attention to preventive efforts. From the 1960's to the 1980's this sequence of events culminated in the recognition of Giardia lamblia as a cause of gastroenteritis (Lindquist, 1999).

Understanding Waterborne Diseases

Detection and investigation of waterborne disease outbreaks is the primary responsibility of local, state and territorial public health departments, with voluntary reporting to the CDC. The CDC and the U.S. Environmental Protection Agency (EPA) collaborate to track waterborne disease outbreaks of both microbial and chemical origins. Data on drinking water and recreational water outbreaks and contamination events have been collected and summarized since 1971.

While useful, statistics derived from surveillance systems do not reflect the true incidence of waterborne disease outbreaks because many people who fall ill from such diseases do not consult medical professionals. For those who do seek medical attention, attending physicians and laboratory and hospital personnel are required to report diagnosed cases of waterborne illness to state health departments. Further reporting of these illness cases by state health departments to the CDC is voluntary, and statistically more likely to occur for large outbreaks than small ones.

Despite these limitations, surveillance data may be used to evaluate the relative degrees of risk associated with different types of source water and systems, problems in current technologies and operating conditions, and the adequacy of current regulations. (Craun, Nwachuku, Calderon, and Craun, 2002).


CHLORINATOR
VENT
CHLORINE CYLINDER
DEEP WELL PUMP
AIR RELEASE
METER
CHLORINE GAS
VACUUM TUBING
SAFETY CHAIN
CHECK VALVE
BY-PASS VALVE
EJECTOR
BOOSTER PUMP
HOSE
WATER INLET ASSEMBLY

Understanding Bacteriological Monitoring

26 waterborne-disease outbreaks have been documented each year in the United States over the past 25 years (Kramer and others, 1996). The persistence of outbreaks over time indicates that more progress is needed to meet the “drinkable and swimmable” goals of Federal water-quality legislation. Although significant improvements in drinking water and wastewater treatment have been achieved, waterborne disease outbreaks indicate that certain types and sources of waterborne pathogens (disease-causing organisms) are still a threat to human health in the United States (Craun, 1992). In particular, waterborne disease outbreaks caused by Escherichia coli O157.:H7 were reported more frequently in 1995-96 than in previous years, and during that same period, Cryptosporidium and Giardia caused large outbreaks associated with recreational water quality (Levy and others, 1998).

Microbiological examination of water is used to determine the sanitary quality of water and the public health risk from waterborne disease. Although microbiological monitoring of finished waters is well established, microbiological monitoring of source waters and recreational waters is considered by some to be fragmented, incomplete, or virtually nonexistent in many parts of the Nation (Rose and others, 1999). Data to characterize the microbiological quality of source waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. For example, monitoring programs vary widely at the local level for recreational waters, and the result is the inconsistent use of indicator organisms across the United States (U.S. Environmental Protection Agency, 1999a).

There is a need to identify human and animal factors associated with contamination of different source and recreational waters and to understand the processes that affect microbiological water quality. Concepts about the relation between the occurrence and distribution of microbiological contaminants and a range of environmental factors such as climate, hydrology, land use, and human and animal population densities need to be tested in areas that represent the national water-use patterns for public and domestic supply and for recreational uses.

Understanding Bacteriological Monitoring
Understanding Bacteria Sampling

Waterborne bacterial pathogens in the United States include species in the genera Salmonella, Shigella, Vibrio, Campylobacter, Yersinia, and pathogenic strains of E. coli. Because bacterial pathogens generally appear intermittently in low concentrations in the environment and because methods of culturing are difficult, fecal-indicator bacteria are used to indicate the possible presence of pathogens. The most widely used bacterial indicators include total coliforms, fecal coliforms, E. coli, fecal streptococci, enterococci, and Clostridium perfringens (C. perfringens). A good indicator organism should be applicable in all types of water; unable to reproduce in ambient waters; be harmless to man and other animals; lend itself to easy, quantitative testing procedures; be of warm-blooded animal origin; correlate with fecal contamination; and be present in waters in greater numbers than and survive as long as or longer than pathogens.

The historical definition of the total-coliform group has been based on the method used for detection (lactose fermentation) rather than on systematic bacteriology (American Public Health Association and others, 1998). Total coliforms are defined as aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation at $35^{O}C$ within 48 hours (Britton and Greeson,1989). Elevated temperature tests identify those genera of total coliform bacteria that belong in the more specific fecal-coliform group. Fecal coliforms are total coliforms capable of producing gas from lactose at $44.5^{O}C$.

Escherichia coli is a species of the fecal-coliform group. Total coliforms include several genera that are found in the human intestine; however, some genera are also found in soils, on vegetation, and in industrial wastes. This multiplicity of sources makes the sanitary significance of total coliforms difficult to establish (Palmer and others, 1984).

They are used as a rough measure of source-water quality and as a screen for fecal contamination. In addition, speciation of the total-coliform group may provide information on treatment effectiveness and the source of colonization of a distribution system or well (American Public Health Association, 1998, p. 9-1). The fecal-coliform indicator used to assess fecal contamination of water has been faulted because of nonfecal sources of at least one member of the fecal coliform group. For example, thermotolerant Klebsiella species have been observed in pulp- and papermill effluents, textile-processing-plant effluent, cotton-mill wastewaters, and sugar-beet wastes, in the absence of fecal contamination (U.S. Environmental Protection Agency, 1986a).

Alternatively, E. coli is a natural inhabitant of the gastrointestinal tract of warm-blooded animals and is direct evidence of fecal contamination from warm-blooded animals. The fecal streptococci are a group of fecal-indicator bacteria that include a variety of species and strains that are all gram positive cocci. Although the normal habitat of fecal streptococci is the gastrointestinal tract of warm-blooded animals, some species are not exclusive to animals (American Public Health Association, 1998, p. 9-74). In fact, studies on the distribution of fecal streptococci in water indicate that at least one strain commonly found in environmental samples is ubiquitous and can exist for extended periods in soil and water (Geldreich, 1976). Fecal streptococci, therefore, have limited value as an indicator of fecal contamination in environmental samples. The enterococci group is a subgroup of the fecal streptococci, and it is considered a more specific indicator of fecal contamination.

The enterococci are differentiated from other streptococci by their ability to grow in 6.5 percent chloride, at pH 9.6, and at elevated temperatures. The enterococci method is valuable for determining the extent of fecal contamination of recreational surface waters, especially marine waters (American Public Health Association, 1998, p. 9-75).

In addition, because enterococccci cells are a different shape and have different survival rates than members of the coliform group, enterococci may be useful in assessing transport of fecal contamination in ground water. Clostridium perfringens is present in large numbers in human and animals wastes, and its spores are more resistant to disinfection and environmental stresses than is E. coli. Clostridium perfringens has been suggested as a conservative tracer of past fecal contamination and as an indicator for chlorinated water in distribution systems (Bisson and Cabelli, 1980).

Clostridium perfringens, however, is probably not an appropriate indicator for most recreational waters because spores in the sediment are resuspended into the water column from swimmer or wave disturbances (Bisson and Cabelli, 1980). One exception is that C. perfringens may be a reliable indicator of streamwater quality in tropical climates, where warm water temperatures support the growth and reproduction of E. coli and aerobic conditions preclude the growth and sporulation of C. perfringens (Fujioka and Shizumura, 1985).

Clostridium perfringens has also been found to be a sensitive indicator of microorganisms entering streams from point sources but not a reliable indicator of nonpoint sources (Sorenson and others, 1989). Detection of C. perfringens in water has been proposed as an indicator of the presence and density of pathogenic viruses and possibly other stress resistant microorganisms (U.S. Environmental Protection Agency, 1996c).

Protozoan Pathogens

The principal protozoan pathogens that affect the public health acceptability of waters in the United States are Giardia lamblia (Giardia) and Cryptosporidium parvum (Cryptosporidium). These organisms are widely distributed in the aquatic environment and have been implicated in several recent outbreaks of waterborne disease, including a well-publicized outbreak of cryptosporidiosis in Milwaukee, Wisconsin (Rose and others, 1997). Both Giardia and Cryptosporidium produce environmentally resistant forms (called cysts and oocysts), which allow for the extended survival of the parasites in water and treated water.

Because cysts and oocysts are more resistant to disinfection and survive longer in the environment than bacterial indicators, fecal-indicator bacteria are not adequate indicators for Giardia and Cryptosporidium in source waters. The presence of protozoan pathogens in water, therefore, must be verified by identification of the pathogens themselves. The USEPA-required method for detection of Giardia and Cryptosporidium in source and drinking water under the ICR involves nominal porosity filtration and indirect fluorescent antibody procedures (U.S. Environmental Protection Agency, 1996c). The ICR method has been criticized for being difficult to implement, being characterized by poor recovery of target organisms, and yielding highly variable results (U.S. Environmental Protection Agency, 1996b). As a result, the USEPA supported the development of Method 1622 for Cryptosporidium (U.S. Environmental Protection Agency, 1998b), and Method 1623 for Giardia and Cryptosporidium (U.S. Environmental Protection Agency, 1999c). Method 1622 was validated through an interlaboratory study and revised as a final, valid method in January 1999.

Understanding Routine Coliform Sampling
Streamwater sample collection
When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers can be used to collect streamwater samples for bacterial and viral indicators, *Cryptosporidium*, and *Giardia* providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.

• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.

• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).

• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for *E. coli* and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for *C. perfringens* and coliphage analysis.

Method 1623
For *Cryptosporidium* and *Giardia* analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*.

Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.

• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for *Cryptosporidium* analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.). The sampling equipment is obtained from the analyzing laboratory; for example, the USGS Ohio District Laboratory has modified the sampling apparatus (G. Shay Fout, U.S. Environmental Protection Agency, 1997) into a self-contained box with easy-to-use control valves. The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Groundwater Sample Collection
Collecting ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps. Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible. Because this is operationally prohibitive for private domestic wells, a tap that yields water directly from the well and before entering the holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.
• Document the stage of the distribution system from which water was collected and details about the distribution system, including the type of tank and condition of the tank and pipes. In addition, if the well can easily be opened for inspection, document the condition of the well, including the sanitary seal (if any) and the amount of debris in the well. Any information on the location of the well, including proximity to septic systems or feedlots, should also be documented in the field at the time of sampling.

For wells without in-place pumps, samples should be obtained by use of the following methods
(in descending order from most to least desirable):
(1) a peristaltic or vacuum pump with autoclavable silicon tubing, (2) a sterile bailer, (3) a chlorine-disinfected pump and tubing, or (4) a detergent-cleaned pump and tubing. Pre-sampling activities, such as purging, must be carried out in such a way as to avoid contaminating the well. All equipment must be properly cleaned and sterilized between sites, using a Liquinox wash and a thorough tap water or deionized-water rinse. If using this last method, collect additional field blanks to evaluate the effectiveness of the cleaning procedure. Refer to Myers and Sylvester (1997) for a detailed discussion of ground-water sampling for microbiological analysis.

Because ground water is less prone to microbiological contamination than surface water, larger volumes of ground water are needed than of surface water.

• For regular sampling, collect 3 L of ground water for bacterial and viral indicators.

• Process the sample for total coliforms, *E. coli*, and enterococci using 200-mL sample volumes for each analysis; send the remainder (at least 2.5 L) to the laboratory for coliphage analysis. In the laboratory, coliphage analysis is done using 1 L for somatic and 1 L for F-specific coliphage.

• For enteric virus analysis by RT-PCR and cell culture, use the same sampler for ground-water samples as for streamwater samples; pump 2,000 L of ground water through the sampling apparatus and 1MDS filter.

Sample Preservation and Storage

Holding times for samples before processing are 6 hours for total coliforms, *E. coli*, and enterococci and 24 hours for *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and the 1MDS filters for enteric viruses by RTPCR and cell culture.

• After collection, immediately store the sample on ice.

• Be sure to keep the sample out of direct sunlight, because ultraviolet rays kill microorganisms.

• Add sodium thiosulfate to sample bottles for bacterial and viral indicators if the water collected contains residual chlorine. (Samples may have residual chlorine if the sampling site is downstream from a wastewater-treatment plant that chlorinates its effluents). Add ethylene diaminetetracetic acid to sample bottles when water is suspected to contain trace elements such as copper, nickel, and zinc at concentrations greater than 1 mg/L (Britton and Greeson, 1989, p. 5-6; U.S. Environmental Protection Agency, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). (Sodium thiosulfate or ethylene diaminetetracetic acid are not added to containers for *Cryptosporidium* and *Giardia)*.

Analytical Methods

Field Analysis

Analysis of water samples for total coliforms, *E. coli,* and enterococci, are done by use of membrane filtration (MF) or most-probable number (MPN) methods. Because membrane filtration is easier to use and provides a more precise quantification of bacteria than MPN, MF is recommended for most analyses. Refer to Myers and Sylvester (1997) for complete MF procedures.

Different MF methods are used for quantification of bacteria in ground-water and streamwater samples.

• For examining streamwater samples for *E. coli*, use the USEPA-recommended mTEC agar method (Environmental Protection Agency, 1986b).

• For examining ground-water samples for total coliforms and *E. coli*, use the MI method (Brenner and others, 1993).

• For enterococci, use the mEI method (U.S. Environmental Protection Agency, 1997).

• For streamwater, plate sufficient sample volumes in order to obtain at least one plate in the ideal count range. For ground water, a 200-mL sample volume is usually sufficient.

Testing of new microbiological monitoring methods and comparing the recoveries of new methods to the USEPA-approved method can be done by use of the NAWQA network. For ground-water samples, for example, one may include a commercially available MPN kit, Colilert (Idexx Laboratories, Westbrook, Maine), for simultaneous detection of total coliforms and *Escherichia coli*. For streamwater sampling, one may include a single-step modified Mtec medium with 5-bromo-6-chloro-3-indolyl'β-d-glucuronide (Bennett Smith, USEPA, Cincinnati, Ohio, oral commun., 1997); this method was developed to replace the mTEC method. Other new methods can be added to the monitoring program for field testing as they are developed.

Laboratory Analysis

Samples need to be kept on ice and shipped to a central laboratory for analysis of coliphage, *C. perfringens*, *Cryptosporidium, Giardia*, and enteric viruses by the current analytical methods. The single-agar layer (SAL), direct plating method with induction of β-galactosidase (Ijzerman and Hagedorn, 1992) is recommended for detection of somatic and F-specific coliphage in streamwater samples. In this method, 100-mL sample volumes are mixed with an agar medium, *E. coli* host culture, chemicals that induce the β-galactosidase enzyme, and appropriate antibiotics. The mixtures are poured into four 150- x 15-mm plates and incubated at 35°C.

Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is dark blue is visible within each plaque. Viral plaques are easily identified and enumerated by the distinct blue circle. Because of contamination by naturally occurring bacteria in streamwater samples, antibiotic- resistant host-culture strains, *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for somatic and F-specific coliphage, respectively. Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water. Because the SAL method is impractical for sample volumes above 100 mL, an alternative method should be used for ground-water sample analysis.

One example, currently being tested by USEPA, is a two-step enrichment presence-absence method (U.S. Environmental Protection Agency, 1999e). Samples for enumeration of *C. perfringens* are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c). Standard MF techniques are used, and the plates are incubated anaerobically for 24 hours at 44.5°C. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as *C. perfringens*. In the laboratory, *C. perfringens* analyses are done on 100-, 30-,and 10-mL volumes of streamwater. In the case of a high-flow or high-turbidity streamwater sample, lower sample volumes may be plated.

Method 1623 (U.S. Environmental Protection Agency, 1999c) is recommended for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. The oocysts are concentrated on a capsule filter from a 10-L water sample, eluted from the capsule filter with buffer, and concentrated by centrifugation. Immunomagnetic separation (IMS) is used to separate the oocysts from other particulates in the sample. In IMS, the oocysts are magnetized by attachment of magnetic beads conjugated to an antibody and then are separated from sediment and debris by means of a magnet.

Fluorescently labeled antibodies and vital dye are used to make the final microscopic identification of oocysts and cysts. The reverse-transcriptase, polymerase chain reaction (RT-PCR) and cell-culture methods are recommended for detection of enteric viruses in water samples (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). To prepare samples for RT-PCR and cell culture, attached viruses are eluted from a 1MDS filter with beef extract (pH 9.5), concentrated using celite (pH 4.0), and eluted with sodium phosphate (pH 9.5).

For RT-PCR analysis, viruses are isolated from the eluate by ultracentrifugation through a sucrose gradient, and trace contaminants are removed by extraction with a solvent mixture. During these steps, the 10-L streamwater sample (or 2,000-L ground-water sample) is concentrated down to 40 µL. An aliquot of the concentrate is used for RT-PCR, wherein any target viral RNA is converted to DNA and amplified by use of an enzymatic process. The RT-PCR products are analyzed by agarose gel electrophoresis and confirmed by hybridization. The enteric viruses detected by use of this method include enterovirus, hepatitis-A, rotavirus, reovirus, and calicivirus.

For cell-culture analysis, the sample eluate is added to a monlayer of a continuous cell line derived from African green monkey kidney cells (U.S. Environmental Protection Agency, 1996c). Each cell culture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE
is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water.

QA/QC Activities and Measures

QA/QC activities and measures to take to reduce contamination.

• Use a sterilization indicator, such as autoclave tape, in preparing sample bottles and other equipment
for collection of microbiological samples to determine whether adequate temperatures and pressures have been attained during autoclaving.

• Prepare a separate set of sterile equipment for microbiological sampling at each site.

• Before processing samples in the field vehicle, wipe down the area with a disinfectant (such as isopropyl alcohol) to ensure a sterile working surface.

• Monitor the incubators daily to ensure temperatures are appropriate for the methods used.

For bacteria samples, membrane-filtration (MF) equipment and MF procedure blanks are used to estimate analytical bias.

Field personnel should do the following:

• Prepare an MF equipment blank, a 50- to 100-mL aliquot of sterile buffered water plated before the
sample—for every sample by field personnel for total coliform, *E. coli*, and enterococci analyses to
determine the sterility of equipment and supplies.

• Prepare a MF procedure blank, a 50- to 100-mL aliquot of sterile buffered water plated after the sample— for every fourth sample to measure the effectiveness of the analyst's rinsing technique or
presence of incidental contamination of the buffered water.

If contamination from a MF equipment or procedure blank is found, results are suspect and are qualified
or not reported. Proper and consistent procedures for counting and identifying target colonies will be followed, as described in Myers and Sylvester (1997).

• After counting, turn the plate 180° and ensure the second count is within 5 percent of the first count.

Have a second analyst check calculations of bacterial concentrations in water for errors.

For coliphage, *Cryptosporidium, Giardia*, and enteric virus samples, equipment and field blanks are
used to determine sampling and analytical bias. Equipment blanks for these analyses are different from the MF equipment blanks for bacterial analysis. An equipment blank is a blank solution (sterile buffered water) subjected to the same aspects of sample collection, processing, storage, transportation, and laboratory handling as an environmental sample, but it is processed in an office or laboratory. Field blanks are the same as equipment blanks except that they are generated under actual field conditions.

• For enteric virus analysis, collect one equipment blank after collection of the first sample to ensure
that equipment cleaning and sterilization techniques are adequate.

• For coliphage, *Cryptosporidium, Giardia*, and enteric virus analyses, collect field blanks periodically.

At a minimum, the number of field blanks should equal 5 percent of the total number of samples collected. Five percent of samples collected for bacterial and viral indicators (total coliforms, *E. coli*, enterococci, *C. perfringens*, and coliphage) should be nested replicate samples to estimate

sampling and analytical variability. For streamwater samples, concurrent replicates to estimate sampling variability are collected by alternating subsamples in each vertical between two collection bottles. For ground-water samples, sequential replicates are collected one after another into separate sterile bottles. Concurrent and sequential replicates are then analyzed in duplicate (split replicates) to estimate analytical variability.
• Because of the expense associated with collection and analysis of samples for pathogens (*Cryptosporidium* and enteric viruses), collect only one replicate sample per year at a site wherein detection of pathogens was found in an earlier sample.
To assess analytical bias of the sampling and analytical method, 2 to 5 percent of the samples collected
for enteric virus should be field matrix spikes.
• Run all but 10 L of ground water through the 1 MDS filter and collect the remaining 10 L in a carboy. In the laboratory, the poliovirus vaccine will be added to the 10 L and then passed through the same 1MDS filter. Analysis will be done by use of the cell-culture and RT-PCR methods. • All cell-culture positive samples are serotyped to identify or discount laboratory contamination. Because of the variability in the performance of Method 1623 for recovery of *Cryptosporidium* and *Giardia*, each sample will be collected in duplicate—one will be a regular sample and the other a matrix spike. The laboratory will add a known quantity of cysts and oocysts to the matrix spike to determine recovery efficiency, as described in USEPA (1999c).

Quality Assurance and Quality Control in the Laboratory
The following criteria may be used to evaluate each production analytical laboratory: (1) appropriate, approved, and published methods, (2) documented standard operating procedures, (3) approved quality-assurance plan, (4) types and amount of quality-control data fully documented and technical defensible, (5) participation in the standard reference sample project (6) scientific capability of personnel, and (7) appropriate laboratory equipment.

The microbiology laboratories must follow good laboratory practices—cleanliness, safety practices, procedures for media preparation, specifications for reagent water quality—as set forth by American Public Health Association (1998) and Britton and Greeson (1989). Some specific guidelines are listed in the following paragraphs.

Reference cultures are used by the central laboratory to evaluate the performance of the test procedures, including media and reagents. Pure cultures of *E. coli, Enterobacter aerogenes,* and *Streptococcus faecalis* (American Type Culture Collection, Rockville, Md.) are used to ensure that MF culture media and buffered water are performing adequately. A pure culture of *C. perfringens*, isolated from a sewage sample and verified by standard procedures, is used to evaluate the test procedure and each lot of media and reagents.

Because contamination of samples from coliphage during the analytical procedure is highly probable (Francy and others, 2000), a negative control of host and sterile buffered water is run concurrently with each batch of samples. In addition, to ensure that the method is being executed properly, a positive-control sewage sample is run with each batch of samples. A laminar flow safety hood is recommended for processing the samples for coliphage analysis. Alternatively, a separate coliphage room may be established to discourage laboratory contamination during the analytical process. An ultraviolet light is installed and operated for 8 hours every night in the safety hood or coliphage room to reduce contamination.

The laboratory should follow the QA/QC guidelines in Method 1623 (U.S. Environmental Protection Agency, 1999c) for *Cryptosporidium* and *Giardia* and in the cell-culture and RT-PCR analysis for enteric viruses (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c).

Understanding Coliforms

Coliforms are bacteria that are always present in the digestive tracts of animals, including humans, and are found in their wastes. They are also found in plant and soil material. A fecal coliform (sometimes faecal coliform) is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium. Fecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactose within 48 hours at 44 ± 0.5°C.

Coliform bacteria include genera that originate in feces (e.g. Escherichia) as well as genera not of fecal origin (e.g. Enterobacter, Klebsiella, Citrobacter). The assay is intended to be an indicator of fecal contamination; more specifically of E. coli which is an indicator microorganism for other pathogens that may be present in feces. Presence of fecal coliforms in water may not be directly harmful, and does not necessarily indicate the presence of feces.

"Indicator" Organisms
Water pollution caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens (disease causing organisms). Frequently, concentrations of pathogens from fecal contamination are small, and the number of different possible pathogens is large. As a result, it is not practical to test for pathogens in every water sample collected. Instead, the presence of pathogens is determined with indirect evidence by testing for an "indicator" organism such as coliform bacteria. Coliforms come from the same sources as pathogenic organisms. Coliforms are relatively easy to identify, are usually present in larger numbers than more dangerous pathogens, and respond to the environment, wastewater treatment, and water treatment similarly to many pathogens. As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present.

Total Coliforms, Fecal Coliforms, and E. Coli
The most basic test for bacterial contamination of a water supply is the test for total coliform bacteria. Total coliform counts give a general indication of the sanitary condition of a water supply.
A. Total coliforms include bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste.
B. Fecal coliforms are the group of the total coliforms that are considered to be present specifically in the gut and feces of warm-blooded animals. Because the origins of fecal coliforms are more specific than the origins of the more general total coliform group of bacteria, fecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms.
C. Escherichia coli (E. coli) is the major species in the fecal coliform group. Of the five general groups of bacteria that comprise the total coliforms, only E. coli is generally not found growing and reproducing in the environment. Consequently, E. coli is considered to be the species of coliform bacteria that is the best indicator of fecal pollution and the possible presence of pathogens.

Are Coliform Bacteria Harmful?
Most coliform bacteria do not cause disease. However, some rare strains of E. coli, particularly the strain 0157:H7, can cause serious illness. Recent outbreaks of disease caused by E. coli 0157:H7 have generated much public concern about this organism. E. coli 0157:H7 has been found in cattle, chickens, pigs, and sheep. Most of the reported human cases have been due to eating under cooked hamburger. Cases of E. coli 0157:H7 caused by contaminated drinking water supplies are rare.

Coliform Testing
Testing for bacteria is the only reliable way to know if your water is safe. You cannot tell by the look, taste, or smell of the water if disease-causing organisms are in it. The New York State

Department of Health recommends that well owners test their water for coliform bacteria at least once a year. If you have experienced bacteria problems in the past, it is recommended that you test your well more
frequently.

When Should I Test?
Late spring or early summer are the best times to test your well, since coliform contamination is most likely to show up during wet weather. Whether your test results are positive or negative, understand that the sample you collected is just a "snapshot" of your well's water quality. The more samples you have tested, the more confident you can be about the quality of the water you are drinking.

What do the Results Mean?
If coliform bacteria are present in your drinking water, your risk of contracting a water-borne illness is increased. Although total coliforms can come from sources other than fecal matter, a positive total coliform sample should be considered an indication of pollution in your well. Positive fecal coliform results, especially positive E. Coli results, should be considered indication of fecal pollution in your well.

What should be done if Coliform Bacteria are detected in a Well?
When coliforms have been detected, repairs or modifications of the water system may be required. Boiling the water is advised until disinfection and retesting can confirm that contamination has been eliminated. A defective well is often the cause when coliform bacteria are found in well water.

What Kinds of Defects can Allow Contamination?
- a missing or defective well cap - seals around wires, pipes, and where the cap meets the casing may be cracked, letting in contaminants
- contaminant seepage through the well casing - cracks or holes in the well casing allow water that has not been filtered through the soil to enter the well. This seepage is common in the wells made of concrete, clay tile, or brick
- contaminant seeping along the outside of the well casing - many older wells were not sealed with grout when they were constructed
- well flooding - a common problem for wellheads located below the ground in frost pits that frequently flood during wet weather.

Long-Term Options for Dealing with Bacterial Contamination of a Well
- Connecting to the regional public water system, if possible
- Inspecting wells for defects and repairing them if possible
- Constructing a new well
- Installing continuous disinfection equipment
- Using bottled water for drinking and food preparation

Understanding Positive or Coliform Present Results
The Total Coliform Bacteria test is the standard microbiological test of the sanitary quality of drinking water. The EPA has stated that good drinking water should not contain any Coliform bacteria.

There are primarily 18 different bacteria which make up the group known as “Coliforms”. In most cases, Coliform bacteria are not harmful. However, if these bacteria are found in your water supply, this indicates that other disease causing bacteria may enter through the same pathway and be present in your drinking water.

If Coliform bacteria are found, the water supply is considered a potential health hazard and is classified as “UNSAFE” for human consumption. This test DOES NOT indicate if the water is chemically safe to drink.

E. COLI – One of the Coliform Bacteria

E. Coli is one of the approximate 18 members of Coliform group. The standard Coliform Bacteria test tells the difference between coliforms of fecal origin, specifically E. coli (from the intestines of warm-blooded animals) and coliforms from other sources. Drinking water contaminated with E. coli is considered an “EXTREME HEALTH HAZARD”.

The test results are reported as “PRESENT” or “ABSENT”

“ABSENT” – Good news, it means that Coliform bacteria were NOT in the test sample. In other words it “PASSED” the test.

“PRESENT” – Means that Coliform Bacteria were found in the sample and it “FAILED” the test. If Coliforms were found in the sample then E.coli (as a member of the Coliform Group) is also reported as
“Present” or “Absent”.

Coliforms “Present” and E. coli “Absent” – Very Unsafe – Potential Health Hazard.

Coliforms “Present” and E. coli “Present” – “EXTREME HEALTH HAZARD”.

If your water system fails, the first step is usually to perform a “batch chlorination” on your drinking water supply. NRC will send written instructions for this procedure if your test results indicate a failure. After the chlorination procedure has been performed, use your water supply for a minimum of 5-7 days and retest. Until you have a test which shows that the bacteria are ABSENT, exercise caution with this system. It is recommended that you test for Coliform bacteria at least every six months.

Understanding Maximum Contaminant Levels (MCLs)
National Primary Drinking Water Regulations (NPDWRs or primary standards) are legally enforceable standards that apply to public water systems. Primary standards protect public health by limiting the levels of contaminants in drinking water.

Emerging Pathogens
Although many of the established waterborne pathogens have been controlled by sanitation measures and water treatment processes, new diseases continue to be identified, and new discoveries present a better understanding of existing chronic diseases. Many of these discoveries raise questions about possible waterborne transmission. The bacteria that now need to be considered in this developing area are Helicobacter pylori, Mycobacterium species, Burkholderia pseudomallei and Francisella tularensis.

Helicobacter Pylori
Although spiral-shaped organisms have been observed in the stomachs of humans for many years, it was not until 1982 that a Campylobacter-like organism was isolated from patients with gastritis and a causative relationship between a new species, Helicobacter pylori, and gastric disease realized (Warren and Marshall 1983). H. pylori is a pathogen of global proportions and is

generally accepted as the cause of most gastric and peptic ulcers. These diseases may lead to gastric adenocarcinoma.

H. pylori occurs worldwide in developing and developed countries. Where low degrees of hygiene and socioeconomic problems exist, infection rates may approach 100%. In developed countries, infection rates are probably between 30 and 60%. Transmission from person to person is not fully understood, mainly because of the difficulty in culturing the organism and identifying it outside the body. Epidemiological studies show the cluster phenomenon of H. pylori infection in families. It is suggested that infected mothers may play a key role in transmission within families (Rothenbacher et al. 1999).

H. pylori has been identified in faeces, and it is assumed that transmission is therefore oral–oral or faecal–oral. The organism has not been isolated from the environment or from drinking water, and waterborne transmission remains a possibility that should be investigated. The epidemiology, however, points to person-to-person transmission in early life.

Mycobacterium

The mycobacteria are a group of slow-growing organisms. The most important is Mycobacterium tuberculosis, the causative organism of tuberculosis, which takes about 4–6 weeks to grow in the diagnostic laboratory. M. tuberculosis is not a waterborne pathogen; there are, however, a number of Mycobacterium species that occur in the environment and can cause disease in humans. Mycobacterium avium and its related species cause an infection of cervical lymph nodes; it occurs in the environment and is most probably accompanied by ingestion or inhalation. M. avium can grow in water to which no additional nutrients have been added; although water treatment processes of coagulation and filtration appear to reduce the numbers, it is not affected by chlorine levels of 1 mg/ml. It is therefore not surprising that these organisms can regrow and colonize domestic water systems. Once ingested, M. avium can colonize the pharynx without causing any disease. The number of cases reported was very low, but patients with HIV/AIDS are very susceptible.

Another species, Mycobacterium xenopi, has been reported as the waterborne cause of spinal infections following a look-back exercise on over 3000 patients who had undergone discectomy operations some years beforehand (Astagneau et al. 2001).

Mycobacterium paratuberculosis causes Johne's disease in cattle. It is a chronic wasting disease with considerable economic consequences. The organism is extremely difficult to culture; when it does grow, it is very slow and dependent on an exogenous source of mycobactin, which is an iron chelating agent produced by all other mycobacteria. Transmission is by either direct or indirect contact with infected animals and occurs mainly through the faecal–oral route. Organisms are ingested in large numbers by young animals when they feed in troughs that have been contaminated by faeces of shedding animals
(Chiodini et al. 1984).

M. paratuberculosis has recently been suggested as a cause of Crohn's disease, a non-specific chronic transmural inflammatory disease of humans that affects the intestinal tract, commonly the ileum. The disease is chronic, debilitating and of a relapsing nature; the symptoms experienced include diarrhea with blood in the stools and abdominal pain. Complications include obstruction, fistulation and abscesses. There have been many bacteria implicated over the years, but no definite etiological agent has been found. It is thought that immunological mechanisms may play an important role.

Molecular techniques have been developed for the diagnosis of M. paratuberculosis infections and applied to human surgically resected tissues. M. paratuberculosis was detected in approximately 30% of samples, but the sets of results from different laboratories have been conflicting. Some studies were unable to detect the organism; in other studies, the organism was detected in a smaller percentage of healthy subjects. In addition, a few Crohn's disease patients

have shown clinical remission when treated with anti-tuberculosis drugs. There is therefore much more work to be done to acquire a better understanding. M. paratuberculosis may be present in surface water contaminated by cattle faeces. Routine testing for indicator organisms would detect faecal pollution, and normal water treatment processes of coagulation and filtration are likely to remove mycobacteria. It is unlikely that drinking-water is a major source of M. paratuberculosis, and its association with Crohn's disease is still under investigation.

Burkholderia Pseudomallei
Burkholderia pseudomallei is the cause of melioidosis, an acute pneumonia often followed by systemic infection with later presentations of abscesses. The organism is widespread in the environment and was originally described in Rangoon in patients compromised by severe poverty who had presumably inhaled the organism in dust when sleeping on the ground. It occurs commonly in southeast Asia and has been detected in service personnel repatriated from those areas in the past. It was also investigated as a biological weapon by several nations, to be released as an aerosol and cause pneumonia infection in those exposed. A recent study in Bologna, Italy, detected B. pseudomallei in 7% of 85 samples of drinking-water collected from public and private buildings. The mean count was 578 cfu/100 ml. The occurrence of the organism was found to correlate with the HPC at 22 and 36 °C (Zanetti et al. 2000).

Francisella Tularensis
Tularaemia is a zoonosis caused by a highly infective and virulent organism Francisella tularensis, which occurs throughout the northern hemisphere but has never been isolated within the United Kingdom. It occurs in a wide range of animal reservoir posts and can be isolated from the environment in water and mud. It is transmitted to humans who come in close contact with the animal reservoir, arthropods that feed on them or debris and dust associated with them. It can also be transmitted through the ingestion of contaminated water. Human epidemics sometimes occur and are associated with epizootics in the animal

Bacteria of potential health concern 75 populations, evidenced by die-offs. There are several presentations of tularaemia in humans, depending on the route of exposure. Ingestion usually results in
oropharyngeal tularaemia, with fever, pharyngitis and cervical lymphadenitis. Other forms include ulcero-glandular, pleuropneumonic and typhoidal. Following the recent war in Kosovo, over 900 suspected cases of tularaemia were identified and 327 cases confirmed serologically. The epidemiological investigation pointed to rodent-contaminated wells, and rodent carcasses found in some wells tested positive for F. tularensis (Reintjes et al. 2002). In a waterborne outbreak reported from Spain, 19 cases who had contact with river-caught crayfish were identified (Anda et al. 2001). Attempts to isolate F. tularensis from water were unsuccessful. Drinking-water was not involved. F. tularensis is notoriously difficult to culture, requiring a source of cysteine. F. tularensis was investigated and developed as a biological weapon; the infectious dose was found to be extremely low — 10 organisms.

Examples of classical biological warfare agents

Agent	**Disease**
Bacillus anthracis	Anthrax
Brucella species	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Francisella tularensis	Tularaemia
Yersinia pestis	Plague

Rickettsia species	Typhus
Coxiella burnetii	Q fever
Clostridium botulinum toxin	Botulism
Staphylococcus aureus enterotoxin B	Staphylococcal food poisoning
Smallpox virus	Smallpox

The remaining bacteria of concern are either heterotrophs that might have a role in disease or emerging pathogens that do have a role in disease and could possibly be waterborne. It is important that these organisms and diseases are kept under surveillance in order to confirm or refute the suggested associations. Many of the organisms are difficult to grow, and there is no validated trigger of when to look for them.

The HPC does not measure all organisms present, of which many will be non-culturable but viable, and indeed several of the organisms of concern described above would not grow on HPC media. The HPC, however, does give an indication of change in the flora of drinking-water, and the HPC should be evaluated as a trigger for further investigation.

Many new molecular techniques for the detection of pathogens and putative pathogens in water are being described (Waage et al. 1999a,b,c; Lightfoot et al. 2001). DNA chips that have the capacity to detect up to 44 pathogens on one single chip are being developed.

These tests are very expensive when compared with the routine monitoring tests carried out in the water industry and in public health monitoring. The HPC should be evaluated as the signal of changing events in a drinking-water supply to trigger the utilization of these new molecular tests to detect the new bacteria of concern and any associated virulence genes.

Understanding Heterotrophic Plate Count
The analytical methods promulgated under the authority of Section 304(h) of the Clean Water Act are sometimes referred to as the "304(h)" or "Part 136" methods. The methods measure chemical and biological pollutants in media, such as wastewater, ambient water, sediment, and biosolids (sewage sludge). These various CWA methods are tested in a variety of labs and matrices. In addition to Part 136 methods, some approved methods are published or incorporated by reference at 40 CFR Parts 401-503, approved industry-specific methods.

In addition to general purposes methods, EPA has approved special purpose analytical methods. These methods were developed to work in samples or for pollutants specific to certain industrial categories. For example, methods specific to the Pharmaceutical Manufacturing and Pesticide Chemicals industrial categories are listed in Tables IF and IG, respectively, at 40 CFR Part 136.

Methods specific to other industrial categories are listed or incorporated by reference into the regulations at 40 CFR Parts 401-503. These include methods specific to the Pulp, Paper, and Paperboard category (40 CFR Part 430), and specific to Use or Disposal of Sewage Sludge (biosolids) (at 40 CFR Part 503.) Industry-specific methods that are approved for compliance monitoring in the industry for which they are designated may be used for general use, if the same method is listed in Tables IA to IE, or IH at 40 CFR 136.3.

Understanding Total Coliforms
The Total Coliform Rule was published in 1989 and became effective in 1990. The rule set both health goals (MCLGs) and legal limits (MCLs) for the presence of total coliform in drinking water. The rule also detailed the type and frequency of testing that water systems must undertake. In 2003, EPA announced its intent to revise the Total Coliform Rule.

2010 Proposed Revisions to the Total Coliform Rule
The Environmental Protection Agency (EPA) is proposing revisions to the 1989 Total Coliform Rule (TCR), a national primary drinking water regulation (NPDWR). The purpose of the TCR is to protect public health by ensuring the integrity of the drinking water distribution system and monitoring for the presence of microbial contamination. EPA anticipates greater public health protection under the proposed revised requirements, which are based on recommendations by a federal advisory committee.

The proposed revisions to the TCR will:

- require public water systems that are vulnerable to microbial contamination to identify and fix problems, and

- establish criteria for systems to qualify for and stay on reduced monitoring, thereby providing incentives for improved water system operation.

The proposed RTCR maintains and strengthens the objectives of the current TCR and is consistent with the recommendations in the AIP. The objectives are: (1) To evaluate the effectiveness of treatment, (2) to determine the integrity of the distribution system, and (3) to signal the possible presence of fecal contamination. The proposed revision better addresses these objectives by requiring systems that may be vulnerable to fecal contamination (as indicated by their monitoring results) to do an assessment, to identify whether any sanitary defect(s) is (are) present, and to correct the defects.

Therefore, the Agency anticipates greater public health protection under the proposed RTCR compared to the current TCR because of its more preventive approach to identifying and fixing problems that affect or may affect public health.

The following is an overview of the key provisions of the proposed RTCR:

- MCLG and MCL for E. coli and coliform treatment technique for protection against potential fecal contamination. The proposed RTCR establishes a maximum contaminant level goal (MCLG) and maximum contaminant level (MCL) for E. coli. It takes a preventive approach to protecting public health by establishing a coliform treatment technique for protection against potential fecal contamination. The treatment technique uses both total coliforms and E. coli monitoring results to start an evaluation process that, where necessary, will require the PWS to conduct follow-up corrective action that could prevent future incidences of contamination and exposure to fecal contamination and/or waterborne pathogens.

- Monitoring. As with the current TCR, PWSs will continue to monitor for total coliforms and E. coli according to a sample siting plan and schedule specific to the system.

Sample siting plans under the proposed RTCR must continue to be representative of the water throughout the distribution system. Under the proposed RTCR, systems have the flexibility to propose repeat sample locations that best verify and determine the extent of potential contamination of the distribution system rather than having to sample within five connections upstream and downstream of the total coliform-positive sample location. In lieu of proposing new repeat sample locations, the systems may stay with the default used under the current TCR of five connections upstream and downstream of the total coliform-positive sample location.

As with the current TCR, the proposed RTCR allows reduced monitoring for some small ground water systems. The proposed RTCR is expected to improve public health protection compared to the current TCR by requiring small ground water systems that are on or wish to conduct reduced monitoring to meet certain eligibility criteria. Examples of the criteria include a sanitary survey

showing that the system is free of sanitary defects, a clean TCR compliance history for 12 months, and a recurring annual site visit by the State and/or a voluntary Level 2 assessment for systems on annual monitoring.

For small ground water systems, the proposed RTCR requires increased monitoring for high-risk systems that meet certain criteria such as unacceptable compliance history under the RTCR. The proposed RTCR specifies conditions under which systems will no longer be eligible for reduced monitoring and be required to return to routine monitoring or to monitor at an increased frequency.

The proposed RTCR requires systems on a quarterly or annual monitoring frequency (applicable only to ground water systems serving 1,000 or fewer people) to conduct additional routine monitoring the month following one or more total coliform-positive samples. Under the proposed RTCR, systems must collect at least three routine samples during the next month, unless the State waives the additional routine monitoring. This is a reduction in the required number of additional routine samples from the current TCR, which requires at least five routine samples in the month following a total coliform-positive sample for all systems serving 4,100 or fewer people.

The current TCR requires all systems serving 1,000 or fewer people to collect at least four repeat samples while PWSs serving 1,000 people or greater to collect three repeat samples. The proposed rule requires three repeat samples after a routine total coliform-positive sample, regardless of the system type and size.

Seasonal Systems
The proposed RTCR establishes monitoring requirements for seasonal systems for the first time. Seasonal systems represent a special case in that the shutdown and start-up of these water systems present additional opportunities for contamination to enter or spread through the distribution system. Seasonal systems must demonstrate completion of a State-approved start-up procedure. In addition, they must designate the time period(s) for monitoring based on site-specific considerations (such as during periods of highest demand or highest vulnerability to contamination) in their State-approved sample siting plan. See section III.A.3 of this preamble for a detailed discussion of seasonal systems.

Assessment and Corrective Action
As part of a treatment technique, all PWSs are required to assess their systems when monitoring results show that the system may be vulnerable to contamination. Systems must conduct a simple self-assessment (Level 1) or a more detailed assessment (Level 2) depending on the severity and frequency of contamination. The system is responsible for correcting any sanitary defect(s) found through either a Level 1 or Level 2 assessment.

Violations and Public Notification
The proposed RTCR establishes an E. coli MCL violation, a treatment technique violation, a monitoring violation, and a reporting violation. Public notification is required for each type of violation, with the type of notification dependent on the degree of potential public health concern. This is consistent with EPA's current public notification requirements under 40 CFR part 141 subpart Q. The proposed RTCR also modifies the public notification and Consumer Confidence Report language to reflect the construct of the proposed rule. See sections III.A.6 and III.A.7 of this preamble for detailed discussions of violations and public notification under the proposed RTCR.

Transition to the RTCR
The proposed RTCR allows all systems to transition to the new rule at their current TCR monitoring frequency, including systems on reduced monitoring under the current TCR. States will then evaluate the monitoring frequency during each sanitary survey conducted after the compliance effective date of the RTCR. This process reduces State burden by not requiring the

State to determine appropriate monitoring frequency at the same time as when the State is trying to adopt primacy, develop policies, and train their own staff and the PWSs in the State.

The provisions of the proposed RTCR are contained in the new 40 CFR part 141 subpart Y, superseding 40 CFR 141.21 beginning three years following the publication of the final revised rule.

A. Proposed Rule Provisions and Rationale
1. Terms used in the proposed RTCR
a. Provisions. i. Clean compliance history. For the purposes of the proposed RTCR, EPA is proposing to define "clean compliance history" as a record of no maximum contaminant level (MCL) violations under 40 CFR 141.63; no monitoring violations under 40 CFR 141.21 or subpart Y; and no coliform treatment technique trigger exceedances or coliform treatment technique violations under subpart Y.
ii. Sanitary defect. EPA is proposing to define "sanitary defect" as a "defect that could provide a pathway of entry for microbial contamination into the distribution system or that is indicative of a failure or imminent failure in a barrier that is already in place" (USEPA 2008c).
iii. Seasonal systems. EPA is proposing to define a seasonal system as a non-community water system that is operated in three or fewer calendar quarters per calendar year.
b. EPA's rationale. i. Clean compliance history. EPA is proposing a definition of "clean compliance history" because without a definition, the use of the phrase could result in multiple interpretations. Clean compliance history is one of the criteria a system must meet to be eligible for reduced monitoring. The advisory committee recommended this definition (USEPA 2008c, AIP p. 10).
ii. Sanitary defect. The advisory committee recommended the definition of sanitary defect. The proposed RTCR takes a more preventive approach to protect public health by establishing a framework for the assessment of public water systems to identify sanitary defects and to correct them as appropriate. The first part of the proposed definition of a "sanitary defect" focuses on problems in the distribution system that may provide a pathway for contaminants to enter the distribution system and its implication for potential exposure to both microbial and chemical contaminants. The second part of the definition also recognizes the importance of having barriers in place to prevent the entry of microbial contaminants into the distribution system. Indications of failure or imminent failure of these barriers are defects that require corrective action.

Sanitary Defect
Sanitary defect is a term specific to the proposed RTCR assessment and corrective action provisions. Sanitary defects are not intended to be linked directly to "significant deficiencies" under the Interim Enhanced Surface Water Treatment Rule (IESWTR) (USEPA 1998b, 63 FR 69389, December 16, 1998) and Ground Water Rule (GWR) (USEPA 2006c, 71 FR 65574, November 8, 2006), although some problems could meet either definition. Nothing in this proposed rule is intended to limit the existing authorities of States under other regulations. The following is a list of examples of sanitary defects and defects in the distribution system coliform monitoring practices.

Examples of sanitary defects:
- ✓ Cross connection and backflow issues such as a required backflow prevention device not in place or not operating properly; or an unprotected cross connection found. Operator issues such as failure to follow standard operating procedures (SOPs) that protect distribution system integrity and sanitary conditions.

- ✓ Distribution system issues such as inadequate inspection and maintenance of the distribution system; loss of distribution system integrity such as main breaks; failure to maintain adequate pressure; improper flushing operations; improper construction of new, replaced, or renovated lines; inadequate disinfection during and after repair/replacement activities; or inability to maintain required residual throughout the distribution system.

- ✓ Storage issues such as overflow, vents, hatches, and other penetrations not properly configured, screened, or sealed; inadequate maintenance of storage facilities; or inadequate disinfection during and after repair/replacement activities

- ✓ Disinfection issues such as inability to maintain required residual throughout the distribution system.

iii. Seasonal systems. Seasonal systems fall under the broader category of non-community water systems (NCWS) and therefore are subject to provisions applicable to that category of systems. However, seasonal systems have unique characteristics and timetables that make them particularly susceptible to contamination. Seasonal systems represent a special case in that the shut down and start-up of the water system present opportunities for contamination to enter or spread through the distribution system. For example, loss of pressure after a system's shut down can lead to intrusion of contaminants. Microbial growth prior to start-up can result in biofilm formation, which can lead to the accumulation of contaminants. These systems are also more susceptible to contamination due to changes in the conditions of the source water (such as variable contaminant loading due to increased septic tank or septic field use), the seasonal nature of the demand, and the stress that the system experiences. As a result, the Agency is establishing a definition for seasonal systems and setting forth provisions that mitigate the risk associated with the unique characteristics of this type of system. The advisory committee recommended that such provisions pertain to seasonal systems.

c. Request for comment. EPA requests comment on the proposed definitions and whether they work within the construct of the proposed RTCR. Specifically, EPA requests comment on the proposed definition of seasonal systems. The advisory committee recommended that seasonal systems be identified and be subject to additional regulatory requirements because the shutdown and startup of the system presents opportunities for contaminants to enter or spread through the distribution system. These results are possible in any system that shuts down and does not maintain adequate pressure throughout the distribution system. The AIP describes a seasonal system as “one which operates less than four calendar quarters per year” (USEPA 2008c). EPA has interpreted this to mean that a seasonal system is one which is shut down for at least one full calendar quarter (i.e., it operates in three or fewer calendar quarters). EPA requests comment on whether this proposed definition of “seasonal system” is adequate to address the concern that motivated the advisory committee's recommendation and is consistent with its intent. For example, a system that operated from March to October would operate in all four calendar quarters and would not be considered a seasonal system, but would be subject to the same possibility of distribution system intrusion as a seasonal system that operated April to November (i.e., in only three calendar quarters).

Should EPA modify the definition to address this issue? If so, how should the definition be modified? Should systems that close for some specified period (e.g., 30 days, 60 days, 90 days) be subject to seasonal system requirements? What should that specified period be?

Systems that operate intermittently (e.g., only on weekends or only when a camp is open) may also be subject to distribution system contamination due to lack of adequate pressure. Should this be addressed? If so, how should it be addressed—through regulation, guidance, or some other approach? Is there a specific shutdown time that should be considered for intermittent systems in developing the approach and determining which systems should be included?

In addition to the public health benefits associated with these requirements, EPA is aware of the burden that States will have in determining which systems must comply and in tracking compliance. Therefore, EPA requests comment on ways to reduce State burden and facilitate implementation of seasonal system provisions.

2. MCLG and MCL for E. coli, and Coliform Treatment Technique

a. Provisions. The current TCR established a maximum contaminant level goal (MCLG) of zero for total coliforms (including fecal coliforms and E. coli) and an MCL for total coliforms. EPA is proposing in the RTCR to eliminate the MCLG for total coliforms (including fecal coliforms) and the MCL for total coliforms. Under the proposed RTCR, EPA establishes an MCLG of zero and an MCL for E. coli and a treatment technique for coliform. The proposed MCL for E. coli is based on the monitoring results for total coliforms and E. coli.

A system is in compliance with the E. coli MCL unless any of the following conditions occur:

- A system has an E. coli positive repeat sample following a total coliform-positive routine sample; or
- A routine sample is E. coli-positive and one of its associated repeat samples is total coliform-positive; or
- A system fails to test for E. coli when any repeat sample tests positive for total coliforms; or
- A system fails to take all required repeat samples following a routine sample that is positive for E. coli.

The proposed MCL is similar to the criteria that define the conditions (if exceeded) when a Tier 1 acute MCL violation occurs under the current TCR but with two modifications. First, the proposed MCL excludes fecal coliforms. Second, the proposed MCL also includes an additional condition by which a system violates the MCL, namely failing to collect all repeat samples following an initial E. coli-positive sample. Although not explicitly stated, as a logical consequence of the second condition, a system also violates the MCL when an E. coli- positive routine sample is followed by an E. coli-positive repeat sample because E. coli are a subset of total coliforms. EPA is also proposing a coliform treatment technique, which uses total coliforms and E. coli as indicators of a possible breach in the distribution system that could lead to fecal contamination.

b. EPA's rationale. i. Inclusion of MCLG for E. coli and removal of MCLG for total coliforms (including fecal coliforms). EPA is proposing in the RTCR to include an MCLG of zero for E. coli and to remove the current MCLG of zero for total coliforms (including fecal coliforms). This is because E. coli is a more specific indicator of fecal contamination and potential harmful pathogens in drinking water than are total coliforms (including fecal coliforms). Many of the organisms detected by total coliform and fecal coliform methods are not of fecal origin and do not have any direct public health implication. See also the discussion of fecal coliforms in section III.A.9 of this preamble. New information has become available since promulgation of the current TCR in 1989 that indicates that measurement of fecal coliforms sometimes detects organisms that may not have any connection to fecal contamination (Edberg et al. 2000). An MCLG of zero for E. coli is more appropriate than an MCLG of zero for total coliforms (including fecal coliforms) since E. coli is a more specific indicator of the presence of fecal contamination.

Total coliforms (including fecal coliforms) do not in and of themselves pose a public health risk, but they may indicate the presence of a pathway by which fecal contamination can occur. Therefore, the removal of the MCLG for total coliforms (including fecal coliforms) would prevent possible public confusion as a result of attributing greater public health significance to the presence of total coliforms than is warranted. EPA believes that the removal of the MCLG for total coliforms, along with the other proposed changes discussed in the succeeding paragraphs, leads to a rule that is more protective of public health, and is less confusing to the public. The proposed MCLG of zero for E. coli and the removal of the MCLG for total coliforms (including fecal coliforms) are also consistent with the recommendation made by the advisory committee in the AIP.

ii. Inclusion of MCL for E. coli and removal of MCLs for total coliforms and fecal coliforms. EPA is proposing to include in the RTCR an MCL for E. coli because approved analytical methods continue to be available to measure the presence of E. coli in water samples, i.e., the presence of E. coli is technologically feasible to ascertain. Violation of the proposed MCL for E. coli signifies fecal contamination occurrence and a possible high risk of exposure to pathogens. EPA is proposing to eliminate the MCLs for total coliforms and fecal coliforms because under the proposal there is no longer an MCLG for either total coliforms or fecal coliforms, for the reasons explained earlier. The proposed MCL for E. coli is consistent with the recommendation made by the advisory committee in the AIP.

iii. Coliform treatment technique. The 1996 SDWA amendments authorize EPA to promulgate a treatment technique in lieu of an MCL if EPA determines that "it is not economically or technologically feasible to ascertain the level of the contaminant" (SDWA 1412(b)(7)(A)). While it is technologically feasible to ascertain levels of E. coli (i.e., analytical methods continue to be available to measure the presence of E. coli in water samples), because of the intermittent nature of fecal contamination, it is not economically feasible to ascertain the level of E. coli occurrence below which the water may be deemed safe. This is because it is not economically feasible to monitor E. coli with sufficient frequency to ensure such safety.

Because total coliform bacteria are part of the soil ecosystem, positive samples are indicators of fecal contaminant entry into drinking water via a pathway from the soil. EPA is proposing a coliform treatment technique, supplemental to directly measuring E. coli, to provide additional protection against fecal contamination. Under the proposed coliform treatment technique, as specified in the AIP, total coliform-positive samples, in the absence of E. coli, are still indicators of an E. coli or other fecal contaminant pathway.

A PWS that exceeds a specified frequency of total coliform occurrence must conduct a Level 1 or Level
2 assessment to determine if any sanitary defect(s) exist(s) and, if found, to correct the defect(s). In addition, under the proposed treatment technique requirements, a PWS that incurs an E. coli MCL violation must conduct a Level 2 assessment and take remedial action if any sanitary defects are found.

The treatment technique requirements as proposed enhance public health protection beyond the E. coli MCL for the following reasons:

- ✓ The assessment and corrective action provisions of the treatment technique when the MCL for E. coli is exceeded require PWSs to investigate the potential causes of the fecal contamination and require timely remedial action if any sanitary defects are found. Under the current TCR, there are no requirements for investigation and corrective action after an MCL exceedance. Without such a find-and-fix provision, the pathway for contamination may not be identified and eliminated as sampling alone may not be adequate to identify intermittent sources of fecal contamination. The assessment and corrective action provisions of the proposed rule increase the likelihood of finding and correcting any sanitary defect and reduce the chance of recurrence of fecal contamination in the future.

- ✓ Using total coliforms in addition to E. coli as an indicator to prompt assessment and corrective action increases the sensitivity for identifying potential pathways for contamination. As discussed in section II.D.2 of this preamble, the presence of total coliforms indicates the potential existence of a pathway through which fecal contamination could follow. The absence (versus the presence) of total coliforms in the distribution system indicates a reduced likelihood that fecal contamination and/or waterborne pathogens are occurring in the distribution system. Analyses from EPA's

2005 Six-Year Review 2 data (USEPA 2006b; USEPA 2010e) (see section VI.B of this preamble for details on the Six-Year Review 2 data) and from the proposed RTCR Economic Analysis (EA) occurrence modeling show that total coliform presence in drinking water is approximately 20 to 40 times higher than E. coli occurrence in drinking water (see chapter 4 of the Proposed RTCR EA (USEPA 2010a)). Similarly, under the current TCR, non-acute MCL (also referred to as monthly MCL) violations (informed by total coliform occurrence) occur roughly 10 times more often than acute MCL violations (informed by total coliform and E. coli occurrence, essentially equivalent to the occurrence that triggers an E. coli MCL violation under this proposed rule). Thus, including monitoring of total coliforms, as well as E. coli, as part of a treatment technique to indicate when systems must find and fix any sanitary defects, substantially increases the likelihood of identifying such defects.

- ✓ The proposed treatment technique was supported by the advisory committee and is consistent with the recommendations in the AIP.

c. Request for comment. EPA requests comment on its proposal to eliminate the MCLG and MCL provisions for total coliforms and fecal coliforms and to include an MCLG and MCL for E. coli and coliform treatment technique provisions based on monitoring for total coliforms and E. coli. EPA also requests comment on its proposed definition of the E. coli MCL.

3. Monitoring

a. Provisions. As with the current TCR, the proposed RTCR requires all PWSs to collect and test samples for total coliforms and E. coli according to a sample siting plan and schedule specific to the system. Under the proposed RTCR, all PWSs are still required to take repeat samples within 24 hours of learning of any routine monitoring sample that is total coliform-positive. PWSs must comply with the repeat monitoring requirements and E. coli analytical requirement, discussed in detail in section III.A.4 of this preamble. All samples taken for proposed RTCR compliance (routine and repeat) may occur at a customer's premises, dedicated sampling station, or other designated compliance sampling location.

Under the proposed RTCR, system sample siting plans must include routine and repeat sample sites and any sampling points necessary to meet the Ground Water Rule (GWR) requirements. The sample siting plan is subject to State review and revision. The PWS may propose repeat monitoring locations that are expected to be representative of a pathway for contamination into the distribution system (for example, near a storage tank). Instead of identifying set repeat sampling locations (i.e., within five service connections upstream and downstream of the original sampling location that tested total coliform-positive), systems may elect to specify criteria for selecting their repeat sampling locations on a situational basis in a standard operating procedure (SOP), which is part of the sample siting plan. Upon State review, the PWS must demonstrate to the State's satisfaction that the sample siting plan remains representative of the water quality in the distribution system. The State may modify the SOP as needed. To address access issues, small systems must specify in their sampling plans where the two additional samples will be taken. The State may determine that monitoring at the entry point to the distribution system (especially for undisinfected ground water systems) is effective to differentiate between potential source water and distribution problems.

Under the proposed RTCR, PWSs may take more than the minimum required number of routine samples and include the results in calculating whether the total coliform treatment technique trigger for conducting an assessment has been exceeded only if the samples are taken in accordance with the sample siting plan and are representative of water throughout the distribution system.

EPA is not proposing to make substantive changes to the current TCR requirements for (1) special purpose samples, and (2) invalidation of total coliform samples. EPA is proposing a minor modification to the provision for special purpose samples by changing "total coliform MCL" to "coliform treatment technique trigger."

The following are the proposed monitoring requirements for different categories of systems.
i. Ground water NCWSs serving ? 1,000 people. (a). Routine monitoring. The proposed RTCR requires ground water NCWS serving 1,000 or fewer people to routinely monitor each quarter for total coliforms and E. coli. Seasonal systems under this category must routinely monitor every month (seasonal systems are discussed later in this section).
(b). Transition to the RTCR. The proposed RTCR requires all ground water NCWSs serving 1,000 or fewer people, including seasonal systems, to continue with their TCR monitoring schedules as of the compliance date of the RTCR, unless or until any of the conditions for increased monitoring discussed later on in this section are triggered on or after the compliance date or unless otherwise directed by the State, including through the special monitoring evaluation conducted under a sanitary survey. In addition, systems on annual monitoring, including seasonal systems, must have an initial annual site visit by the State within one year of the compliance date (or an annual voluntary Level 2 assessment by a party approved by the State) and an annual site visit each year thereafter to remain on annual monitoring.

This rule proposes that after the compliance date of the final RTCR, during each sanitary survey the State (which would be either EPA or a State that has received primacy for this rule) must perform a special monitoring evaluation to review the status of the water system, including the distribution system, to determine whether the system is on an appropriate monitoring schedule and modify the monitoring schedule as necessary. States must evaluate system factors such as the pertinent water quality and compliance history, the establishment and maintenance of contamination barriers, and other appropriate protections and validate the appropriateness of the water system's existing monitoring schedule and modify as necessary. For seasonal systems on quarterly or annual monitoring, this evaluation must also include review of the approved sample siting plan which designates the time period(s) for monitoring based on site-specific considerations (such as during periods of highest demand or highest vulnerability to contamination). The system must collect compliance samples during these time periods.
(c). Reduced monitoring. The State has the discretion to reduce the monitoring frequency for well-operated ground water NCWSs from the quarterly routine monitoring to no less than annual monitoring, if the water system can demonstrate that it meets the criteria for reduced monitoring provided in this section.

To be eligible to qualify for and remain on annual monitoring after the compliance date, a ground water NCWS serving 1,000 or fewer people must meet all of the following criteria:

- The most recent sanitary survey shows the system is free of sanitary defects, has a protected water source and meets approved construction standards;

- The system must have a clean compliance history (no MCL violations or monitoring violations under the current TCR and/or proposed RTCR, no Level 1 or Level 2 trigger exceedances or treatment technique violations under the proposed RTCR) for a minimum of 12 months. (For a more detailed discussion on Level 1 and Level 2 triggers, see section III.A.5 of this preamble); and

- An initial site visit by the State within the last 12 months to qualify for reduced annual monitoring, and recurring annually to stay on reduced annual monitoring; and correction of all identified sanitary defects. A voluntary Level 2 assessment by a party approved by the State may be substituted for the State annual site visit in any given year.

(d). Increased monitoring. Ground water NCWS serving 1,000 or fewer people on quarterly or annual monitoring that experience any of the following events must begin monthly monitoring the month following the event:

- ✓ The system triggers a Level 2 assessment or two Level 1 assessments in a rolling 12 month period;

- ✓ The system has an E. coli MCL violation;

- ✓ The system has a coliform treatment technique violation (for example, if the system fails to conduct a Level 1 assessment or correct for sanitary defects if required to do so); or

- ✓ The system on quarterly monitoring has two monitoring violations in a rolling 12-month period or system on annual monitoring has one monitoring violation.

The system must continue monthly monitoring until the requirements in this section for returning to quarterly or annual monitoring are met.

(e). Requirements for returning to quarterly monitoring. To be eligible to return to quarterly monitoring, ground water NCWSs serving 1,000 or fewer people must meet all of the following criteria:

* Within the last 12 months, the system must have a completed sanitary survey or a site visit by the State or a voluntary Level 2 assessment by a party approved by the State. The system is free of sanitary defects, and has a protected water source; and
* The system has a clean compliance history (no E. coli MCL violations, Level 1 or 2 triggers, coliform treatment technique violations or monitoring violations) for a minimum of 12 months.

(f). Requirements for returning to reduced annual monitoring. To be eligible to return to reduced annual monitoring after being placed on increased monitoring, the system must meet the criteria to return to routine quarterly monitoring plus the following criteria:

- ✓ An annual site visit (recurring) by the State and correction of all identified sanitary defects. An annual voluntary Level 2 assessment may be substituted for the State annual site visit in any given year; and

- ✓ The system must have in place or adopt one or more additional enhancements to the water system barriers to contamination as approved by the State. These measures could include but are not limited to the following:

—Cross connection control, as approved by the State;
—An operator certified by an appropriate State certification program, which may include regular visits by a circuit rider;
—Continuous disinfection entering the distribution system and a residual in the distribution system in accordance with criteria specified by the State; and
—Maintenance of at least a 4-log inactivation or removal of viruses each day of the month based on daily monitoring as specified in the GWR (with allowance for a 4-hour exception).
—Other equivalent enhancements to water system barriers as approved by the State.

(g). Seasonal systems. The proposed rule requires all seasonal systems to demonstrate completion of a State-approved start-up procedure on and after the compliance date of the final RTCR. Seasonal systems may continue with their TCR monitoring frequency after the compliance date of the final RTCR unless or until any of the conditions for increased monitoring discussed previously are triggered on or after the compliance date or as directed by the State. Under the proposed RTCR, seasonal systems are required to take routine samples monthly.

To be eligible for reduced monitoring after the compliance date, seasonal systems must meet the following criteria:

- ✓ The system must have an approved sample siting plan that designates the time period for monitoring based on site-specific considerations (e.g., during periods of highest demand

or highest vulnerability to contamination). The system must collect compliance samples during this time period; and

- ✓ To be eligible for reduced quarterly monitoring, the system must also meet all the reduced monitoring criteria discussed in section III.A.3.a.i.(e) of this preamble, Requirements for returning to quarterly monitoring.

- ✓ To be eligible for reduced annual monitoring, the system must also meet all the reduced monitoring criteria discussed in section III.A.3.a.i.(f) of this preamble, Requirements for returning to reduced annual monitoring.

(h). Additional routine monitoring. All systems collecting samples on a quarterly or annual frequency must conduct additional routine monitoring following a single total coliform-positive sample (with or without a Level 1 trigger event). The additional routine monitoring consists of three samples in the month following the total coliform-positive sample at routine monitoring locations identified in the sample siting plan. This is a change from the current TCR additional routine monitoring requirement of taking a total of five samples the month following a total coliform-positive sample for systems that take four or fewer samples per month.

In this proposal, consistent with the current TCR, the State may waive the additional routine monitoring requirement if:

- ➢ The State, or an agent approved by the State, performs a site visit before the end of the next month the system provides water to the public. Although a sanitary survey need not be performed, the site visit must be sufficiently detailed to allow the State to determine whether additional monitoring and/or any corrective action is needed. The State cannot approve an employee of the system to perform this site visit, even if the employee is an agent approved by the State to perform sanitary surveys.

- ➢ The State has determined why the sample was total coliform-positive and establishes that the system has corrected the problem or will correct the problem before the end of the next month the system serves water to the public. In this case, the State must document this decision to waive the following month's additional monitoring requirement in writing, have it approved and signed by the supervisor of the State official who recommends such a decision, and make this document available to the EPA and public. The written documentation must describe the specific cause of the total coliform-positive sample and what action the system has taken and/or will take to correct this problem.

The State may not waive the requirement to collect three additional routine samples the next month in which the system provides water to the public solely on the grounds that all repeat samples are total coliform-negative. If the State determines that the system has corrected the contamination problem before the system takes the set of repeat samples required in § 141.858, and all repeat samples were total coliform-negative, the State may waive the requirement for additional routine monitoring the next month.

All additional routine samples are included in determining compliance with the MCL and coliform treatment technique requirements.

ii. Ground water CWSs serving 1,000 people. (a). Routine monitoring. The proposed RTCR requires ground water CWSs serving 1,000 or fewer people to routinely monitor each month for total coliforms and E. coli.

The State may reduce the monitoring frequency for ground water CWS from the monthly routine monitoring to quarterly reduced monitoring if the water system can demonstrate that it meets the criteria for reduced monitoring provided later in this section
(b). Transition to the RTCR. All ground water CWSs serving 1,000 or fewer people continue with their current TCR monitoring schedules unless or until any of the increased monitoring requirements in this section occur or as directed by the State.

After the compliance date of the final RTCR, the State must determine whether the system is on an appropriate monitoring schedule by performing a special monitoring evaluation during each sanitary survey to review the status of the PWS, including the distribution system. The State must evaluate system factors such as the pertinent water quality and compliance history, the establishment and maintenance of barriers to contamination, and other appropriate protections to validate the water system's existing monitoring schedule or require more frequent monitoring.
(c). Reduced monitoring. The State has the flexibility to reduce the monitoring frequency for well-operated ground water CWS from the monthly routine monitoring to no less than quarterly monitoring if the water system can demonstrate that it meets the criteria for reduced monitoring provided in this section.

To be eligible for quarterly reduced monitoring, ground water CWSs serving 1,000 or fewer people on monthly monitoring after the compliance date must be in compliance with State-certified operator provisions and meet each of the following criteria:

- The most recent sanitary survey shows the system is free of sanitary defects (or has an approved plan and schedule to correct them), has a protected water source, and meets approved construction standards;
- The system must have a clean compliance history (no MCL violations or monitoring violations under the current TCR and/or proposed RTCR, no Level 1 or Level 2 trigger exceedances or treatment technique violations under the proposed RTCR) for a minimum of 12 months; and
- The system must meet at least one of the following criteria:

—An annual site visit by the State or a voluntary Level 2 assessment by a party approved by the State or meeting criteria established by the State and correction of all identified sanitary defects (or an approved plan and schedule to correct them), or
—A cross connection control program, as approved by the State,
—The system must maintain continuous disinfection entering the distribution system and a residual in the distribution system in accordance with criteria specified by the State,—The system must maintain at least a 4-log inactivation or removal of viruses each day of the month based on daily monitoring as specified in the GWR (with allowance for a 4-hour exception) (USEPA 2006c, 71 FR 65574, November 8, 2006); or
—Other equivalent enhancements to water systems as approved by the State.

(d). Return to routine monitoring requirements. When a system on quarterly monitoring experiences any of the following events the system must begin monthly monitoring:

- System triggers a Level 2 assessment or two Level 1 assessments in a rolling 12 month period;
- System has an E. coli MCL violation;
- System has a coliform treatment technique violation (e.g., fails to conduct a Level 1 or Level 2 assessment or to correct for a sanitary defect if required to do so); or
- System has two routine monitoring violations in a rolling 12-month period.

The system must continue monthly monitoring until all the reduced monitoring requirements discussed previously in this section are met. A system that loses its certified operator must also return to monthly monitoring the month following the loss.
(e). Additional routine monitoring. All systems collecting samples on a quarterly frequency must conduct additional routine monitoring following a single total coliform-positive sample (with or without a Level 1 trigger event). The additional routine monitoring consists of three samples in the month following the total coliform-positive sample at routine monitoring locations identified in the sample siting plan. The current TCR additional routine monitoring requirements consist of taking a total of five samples the month following a total coliform-positive sample for systems that take four or fewer samples per month.

In this proposal, consistent with the current TCR, the State may waive the additional routine monitoring requirement if:

- The State, or an agent approved by the State, performs a site visit before the end of the next month the system provides water to the public. Although a sanitary survey need not be performed, the site visit must be sufficiently detailed to allow the State to determine whether additional monitoring and/or any corrective action is needed. The State cannot approve an employee of the system to perform this site visit, even if the employee is an agent approved by the State to perform sanitary surveys.
- The State has determined why the sample was total coliform-positive and establishes that the system has corrected the problem or will correct the problem before the end of the next month the system serves water to the public. In this case, the State must document this decision to waive the following month's additional monitoring requirement in writing, have it approved and signed by the supervisor of the State official who recommends such a decision, and make this document available to the EPA and public. The written documentation must describe the specific cause of the total coliform-positive sample and what action the system has taken and/or will take to correct this problem.

The State may not waive the requirement to collect three additional routine samples the next month in which the system provides water to the public solely on the grounds that all repeat samples are total coliform-negative. If the State determines that the system has corrected the contamination problem before the system takes the set of repeat samples required in § 141.858, and all repeat samples were total coliform-negative, the State may waive the requirement for additional routine monitoring the next month.

All additional routine samples are included in determining compliance with the MCL and the coliform treatment technique requirements.

iii. Subpart H systems of this part serving 1,000 people. The monitoring requirements for subpart H systems of this part (PWSs supplied by surface water source or ground water source under the direct influence of surface water (GWUDI)) serving 1,000 or fewer people remain the same as under the current rule (see§ 141.856). These systems are not eligible for reduced monitoring. In addition, the proposed rule requires all seasonal systems, on and after the compliance date of the final RTCR, to demonstrate completion of a State-approved start-up procedure.
iv. PWSs serving 1,000 people. The monitoring requirements for PWSs serving more than 1,000 people remain the same as under the current TCR (see§ 141.857), with the exception of the applicable revisions to the repeat sampling locations provided in § 141.858 and additional routine monitoring provisions. Systems on monthly monitoring are not required to take additional routine samples the month following a total coliform-positive sample. These systems are not eligible for reduced monitoring. In addition, the proposed rule requires all seasonal systems, on and after the compliance date of the final RTCR, to demonstrate completion of a State-approved start-up procedure.

b. EPA's rationale. i. Sampling sites and monitoring plans. Consistent with current practice, the proposed RTCR requires systems to develop a sample siting plan that is representative of the

water throughout the distribution system. EPA is proposing to maintain the provision from the current TCR that indicates that sample siting plans are subject to State review and revision. The advisory committee recommended that States review and revise sample siting plans consistent with current practice and that the State develops and implements a process to ensure the adequacy of sample siting plans including a periodic review. The advisory committee also recommended that specific elements be included in the sampling plans such as the routine and repeat sample sites and sampling locations necessary to meet the requirements of the GWR. Alternative repeat monitoring locations (e.g., at storage tanks and entry points to the distribution system) are subject to State approval. The system must demonstrate to the State's satisfaction that these alternative monitoring locations are representative of the water quality in the distribution system.

By allowing systems to specify criteria for selecting their repeat sampling locations in their SOP instead of setting fixed repeat sampling locations, systems can provide a more flexible and more protective response. The system can focus the repeat samples at locations that will best verify and determine the extent of potential contamination of the distribution system based on specific situations. In addition, EPA is proposing to require State approval if a ground water system serving 1,000 or fewer people wants to use a single sample to meet both the repeat monitoring requirements of the RTCR and the source water monitoring requirements of the GWR. EPA is proposing to allow the use of dedicated sampling locations for the following reasons:

To reduce potential contamination of the taps. Utilities will have more control to prevent contamination of the tap by preventing its use by unauthorized persons and allowing no routine use of the tap except for sampling;

- To facilitate access to sampling taps. Currently systems may be constrained by where they sample, e.g., only at public buildings or in certain individual customer's houses.
- To improve sampling representation of the distribution system. Allowing dedicated sample taps in areas where systems have not been able to gain access will facilitate better sampling representation of the distribution system.
- ii. Ground water PWSs serving 1,000 people. (a). Routine monitoring. The advisory committee recommended that ground water NCWSs serving 1,000 or fewer people remain under a routine quarterly monitoring as provided in the current TCR. They believed that in conjunction with the assessment and corrective action requirements, public health protection would be maintained or improved without increasing sampling costs over current TCR requirements. The advisory committee also recognized that current sampling costs are not insignificant for small systems, and wanted to recognize the good performance of systems by allowing them to be able to continue to qualify for reduced monitoring, but under the more specific and rigorous criteria described previously. To continue to provide adequate health protection, systems on reduced monitoring must adhere to criteria that ensure that barriers are in place and are effective. Furthermore, systems with problems that may indicate poor system integrity, maintenance, or operations, or systems that fail to monitor, are triggered into monthly monitoring. This approach leverages the limited resources of these small ground water NCWSs and of States, so that systems with minimal problems can minimize their costs and States can focus their resources on systems needing the greatest attention, such as systems with problems or vulnerabilities.

The advisory committee thought it best to continue with existing routine monthly monitoring requirements for ground water CWSs serving 1,000 or fewer people in order to maintain the current levels of effort to identify potential problems. Since sanitary surveys are required under the GWR and these surveys provide substantial diagnostic value and corrective action response for problems identified, specifying higher routine monitoring frequency for these systems was not deemed necessary.

These systems may also qualify for reduced monitoring if they meet certain criteria.
(b). Transition to the RTCR. The advisory committee was concerned about the ability of the States and systems to adopt the new regulations and to make all the determinations that may be necessary to determine the appropriate monitoring frequency within three years of rule promulgation. Requiring significant changes in monitoring frequencies in a short period (i.e., without a transition period) could overwhelm State resources. The advisory committee recommended phasing in the requirements and using the sanitary survey process to facilitate a successful transition and implementation. The advisory committee, therefore, recommended that these systems continue with their current monitoring frequency during a transition period and that the State review the monitoring frequency to determine whether it is appropriate during each sanitary survey (USEPA 2008c, AIP p.9). This gives the systems the opportunity to address operation and maintenance issues to maintain existing monitoring frequency or qualify for reduced monitoring. Systems on reduced TCR monitoring stay on reduced monitoring during the transition period if they continue to meet the reduced monitoring criteria. During the special monitoring evaluation conducted as part of the periodic sanitary survey, the State will determine whether the individual systems are on the proper monitoring schedule.

(c) Reduced monitoring. The reduced monitoring requirements are intended to recognize that well-operated systems may be less vulnerable to contamination. Therefore, certain conditions are specified under which reduced monitoring could be allowed. These include a clean compliance history for a minimum of 12 months, and an annual visit from the State for systems taking one sample per year and correction of all identified sanitary defects. Ground water NCWSs serving 1,000 or fewer people, with a routine quarterly monitoring frequency, could qualify for reduced annual monitoring, while ground water CWSs serving 1,000 or fewer people, with a routine monthly monitoring frequency, could qualify for reduced quarterly monitoring.

For NCWSs on annual monitoring, the advisory committee believed that requiring a system to have an annual site visit or a Level 2 assessment provides at least an equivalent level of diagnosis of problems and vulnerabilities that might exist as compared to quarterly monitoring without an annual site visit. Several States have elected to conduct annual site visits while also doing annual monitoring for some NCWSs.

(d) Increased monitoring requirements for NCWSs. The advisory committee wanted to recognize that if certain vulnerabilities are identified in a system, the system should be required to conduct more frequent monitoring to identify and correct its problems and better protect public health. Other than sanitary surveys or other site visits, monitoring is the primary means to identify pathways for potential contamination. If the system is deemed more vulnerable to such pathways, as indicated by the increased monitoring criteria, it must conduct more monitoring.

(e) Requirements for returning to routine monitoring and reduced monitoring. The advisory committee believed that systems that address or correct vulnerabilities as indicated by a clean compliance history should be allowed to return to routine monitoring, and subsequently to reduced monitoring (for NCWS). This provision allows for reduced monitoring costs.

(f) Seasonal systems. The advisory committee recognized that seasonal systems have unique characteristics that make them more susceptible to contamination. These systems do not maintain pressure while not in operation, which can result in the intrusion of contaminants. During the time when a seasonal system is not in operation, septic tank drain fields or other pollution sources may accumulate that could affect the conditions or quality of the source water (especially for intermittent contaminants) that infrequent monitoring may not be able to capture. If monitoring is done only at the start-up, there may not be enough time for the system to reach equilibrium (i.e., there might not be enough time to recognize if microorganisms from a septic tank moved to the wellhead in seasonally operated systems). Therefore, the proposed rule requires seasonal systems to monitor routinely at a monthly frequency. Seasonal systems can qualify for reduced monitoring if they meet certain criteria. For a seasonal system to be allowed to monitor at a reduced frequency, the proposed rule requires the system to have an approved sample siting

plan that designates the time period for monitoring and takes into consideration site-specific conditions. A system on a reduced monitoring schedule (less than monthly) must collect samples when there is the greatest chance that contamination could be identified and, due to the variability in water demands, when systems could be most challenged.

(g) Additional routine monitoring. EPA is proposing to retain the requirement of taking additional routine samples the month following a total coliform-positive sample for systems on quarterly or annual monitoring. The advisory committee recognized both the benefits and the limitations of additional routine monitoring. Additional routine samples are meant to enhance the diagnostic ability and supplement the infrequent routine monitoring of systems on quarterly or annual monitoring. Without the provision of additional monitoring, systems on annual or quarterly monitoring with a total coliform-positive sample would not take any samples the following month. The advisory committee believed that additional samples collected the following month are appropriate to help recognize the problem if it still persists.
For systems required to take the additional routine samples the following month (i.e., systems on quarterly or annual monitoring), the proposed RTCR changes the requirement from taking a total of five routine samples to a requirement of just three routine samples. The advisory committee recognized that it is appropriate to drop from five to three samples the following month to reduce monitoring costs while still maintaining a substantial likelihood of identifying a problem if a problem persists. EPA recognizes that a reduction in the number of samples taken could also mean a reduction in the number of positive samples found. However, the reduction in the number of additional routine samples in conjunction with the new assessment and corrective action provisions of the proposed RTCR.

For systems taking at least one sample monthly, the advisory committee recommended no additional routine samples for these systems for the following reason. Taking no additional routine samples the following month substantially reduces monitoring costs. The assessment and corrective action provisions will give systems the ability to identify and prevent the occurrence of problems. EA modeling results show that although there is a decrease in the number of E. coli MCL violations found with the decrease in the number of additional routine samples taken (i.e., going from five samples to one during the month following a total coliform-positive), the assessment and corrective action provisions lead to more E. coli MCL violations being prevented compared to the current TCR.

In addition, whenever a total coliform-positive occurs during routine sampling, there is also a requirement to conduct repeat sampling to determine the extent of contamination or if potential pathways to contamination persist. For small systems serving 1,000 or fewer people on monthly monitoring, if a repeat sample is total coliform-positive, at least a Level 1 assessment will be triggered. If a sanitary defect(s) is (are) found, the system is required to correct the sanitary defect(s).

For systems on monthly monitoring, the assessment and corrective action provisions and the repeat sampling provisions mitigate the need for additional routine sampling for the following month.

iii. Subpart H systems of this part serving ? 1,000 people. EPA is not proposing to change the routine monitoring requirements for systems using surface water or GWUDI serving 1,000 or fewer people, which include not allowing reduced monitoring for these systems. Since systems using surface water or ground water under the influence of surface water tend to have much higher levels of contaminants in their source water, and in general have more complex operations than ground water systems, it is appropriate to allow reduced routine monitoring for ground water systems but not for subpart H systems of this part. The advisory committee recommended that no reduced routine monitoring provisions be allowed for subpart H systems of this part serving 1,000 or fewer people.
iv. Public water systems serving 1,000 people. EPA is proposing to eliminate the additional routine samples the month following a total coliform-positive sample for PWSs serving between

1,000 and 4,100 people for the same reasons discussed previously for small ground water systems monitoring monthly. PWSs serving more than 1,000 people are currently required to routinely monitor monthly (one to four samples per month depending on size) and continue to do so under the proposed RTCR.

c. Request for comment. EPA requests comment on the proposed monitoring requirements for PWSs. Specifically, EPA requests comment on the following questions: Are there other issues that EPA should consider in its approach to help systems transition to the RTCR? Should EPA develop guidance that would help States identify seasonal systems and implement the RTCR requirements (e.g., suggestions for startup procedures and identifying vulnerable time periods)? What start-up procedures or other provisions regarding seasonal systems would be appropriate for inclusion in such guidance? EPA also requests comment on whether seasonal systems should be required to comply with State-directed shut down procedures (in addition to start-up procedures).

EPA requests comment on the following additional questions: Should daily measurement of chlorine residual count toward the maximum residual disinfectant level (MRDL) monitoring and be one of the criteria for reduced monitoring? Should NTNCWSs be required to comply with the CWS requirements (as they are in other rules such as DBP rules) since NTNCWSs serve the same people over time and include populations that may be at greater risk (e.g., schools, hospitals, nursing homes)? Will the reduced, routine, and increased monitoring requirements for NCWSs shift the fixed State resources from CWS oversight to NCWS oversight in those States with large numbers of NCWSs? If so, what might be done to limit the impact? Should EPA develop guidance on how to develop a sample siting plan? Should sample siting plans require State approval.

EPA and the advisory committee did not identify any specific issues regarding consecutive systems in the proposed RTCR. EPA requests comment on whether there are such issues and how they should be addressed in the RTCR.

4. Repeat Samples

a. Provisions. Under the proposed RTCR, all systems must take at least three repeat samples for each routine total coliform-positive sample. This is a change from the current TCR requirements where systems serving 1,000 or fewer people must collect at least four repeat samples while the rest of the systems must collect three repeat samples. EPA is not changing the following provisions: The 24-hour limit within which the system must collect the repeat samples; the authority of the State to extend this limit on a case-by-case basis; and the non-waiver by the State of the requirement for a system to collect repeat samples.

In addition to taking repeat samples, systems must test each routine total coliform-positive sample for E. coli. They must also test any repeat total coliform-positive sample for E. coli. As with the current TCR, if E. coli is present, the system must notify the State the same day it learns of the positive result or by the end of the next business day at the latest. The proposed rule is not changing the provision that a State has the discretion to allow the system to forgo E. coli testing in cases where the system assumes that the total coliform-positive sample is E. coli-positive. If the State allows a system to forgo E. coli testing, the system must still notify the State and comply with the E. coli MCL requirements specified in § 141.858.

As with the current TCR, the system must collect at least one repeat sample from the sampling tap where the original total coliform-positive sample was taken. Unless different locations are specified in its sample siting plan, the system must also collect at least one repeat sample at a tap within five service connections upstream, and at least one repeat sample at a tap within five service connections downstream of the original sampling site. The State may waive the requirement to collect at least one repeat sample upstream or downstream of the original sampling site if the total coliform-positive sample is at the end of the distribution system, or one service connection away from the end of the distribution system.

Under the proposed rule, ground water systems (GWSs) required to conduct triggered source monitoring under the GWR must take ground water source samples in addition to the repeat samples. However, a ground water system serving 1,000 or fewer people may use a repeat sample collected from a ground water source to meet both the repeat monitoring requirements of the proposed RTCR and the source water monitoring requirements of the GWR, but only if the State approves the use of a single sample to meet both rule requirements (i.e., a dual purpose sample) and the use of E. coli as a fecal indicator for source water monitoring. If the sample is E. coli-positive, the system violates the E. coli MCL under the proposed RTCR and must also comply with the GWR requirements following a fecal indictor-positive sample. These provisions are consistent with the GWR.

If a system with a limited number of monitoring locations (such as a system with only one service connection or a campground with only one tap) takes more than one repeat sample at the triggered source water monitoring location, the system may reduce the number of additional source water samples by the number of repeat samples taken at that location that were not E. coli-positive. For example, if a system takes two dual purpose samples and one is E. coli- positive and the other is E. coli-negative, the system has an E. coli MCL violation under the proposed RTCR and is required to take four additional source water samples, rather than five, under the GWR (see 40 CFR 141.402(a)(3)). If the system takes more than one of these repeat samples at the triggered source water monitoring location and has more than one repeat sample that is E. coli-positive, then the system would have both an E. coli MCL violation under the proposed RTCR and a second fecal indicator-positive source sample under the GWR. The system would then need to also comply with the treatment technique requirements under 40 CFR 141.403.

Under the proposed rule, the system must collect all repeat samples on the same day consistent with current TCR requirements. The State may allow systems with a single service connection to collect the required set of repeat samples over a three-day period or to collect a larger volume repeat sample(s) in one or more sample containers of any size, as long as the total volume collected is at least 300 ml.
The proposed RTCR is not changing the requirement that systems collect an additional set of repeat samples for each total coliform-positive repeat sample. As with the original set of repeat samples, the system must collect the additional repeat samples within 24 hours of being notified of the positive result, unless the State extends the time limit. The system must repeat this process until either total coliforms are not detected in one complete set of repeat samples or the system determines that the coliform treatment technique trigger has been exceeded and notifies the State. After a trigger (see section III.A.5 of this preamble) is reached, the system is required to conduct only one round of repeat monitoring after each total coliform-positive or E. coli- positive routine sample. If a trigger is reached as a result of a repeat sample being total coliform- or E. coli-positive, no further repeat monitoring related to that sample is necessary.

The proposed RTCR is also not changing the current TCR provision that a subsequent routine sample, which is within five service connections of the initial routine sample and is collected after an initial routine sample but before the system learns the initial routine sample is total coliform-positive, may count as a repeat sample instead.

Results of all routine and repeat samples not invalidated by the State must be used to determine whether the coliform treatment technique trigger has been exceeded (see section III.A.5 of this preamble for a discussion of the coliform treatment technique triggers).

b. EPA's rationale. i. Why EPA is maintaining a provision for repeat sampling. As with the current TCR, the proposed RTCR requires systems to take repeat samples after a total coliform-positive sample. EPA believes that sampling immediately after an initial positive sample (i.e., conducting repeat sampling) increases the likelihood of identifying the source and/or nature of the possible contamination. Analysis conducted by EPA indicated that once a total coliform-positive is found, there is a much greater likelihood of finding another total coliform-positive within a short period of

time of the initial finding. Repeat sampling (when total coliform-positive) can indicate a current pathway for potential external contamination into the distribution system.

EPA used the Six-Year Review 2 (USEPA 2010e) data to support statistical modeling which produced estimates of average occurrence of routine total coliform-positive samples and repeat total coliform-positive samples and to characterize how occurrence varies from system to system. EPA's occurrence model assumes that, among similar systems, the positive rate for total coliforms in routine samples varies as a beta random variable. EPA used the Six-Year Review 2 data (USEPA 2010e) to estimate the parameters for the distribution of occurrences of routine and repeat total coliform-positive samples.

ii. Frequency of repeat samples. The advisory committee recommended that the current TCR requirement for systems serving more than 1,000 people to take three repeat samples subsequent to a routine total coliform-positive be retained. The advisory committee recommended that systems serving 1,000 or fewer people also be required to take three repeat samples rather than the four required under the current TCR. This view is supported by analysis of repeat sample records from the Six-Year Review 2 data (USEPA 2010e).

Repeat sampling helps utility operators to better understand the extent and duration of potential pathways of contamination into the distribution system. The Six-Year Review 2 data (USEPA 2010e) show that the average percentage of samples that are positive among repeat samples is much higher than that of routine samples, demonstrating that when operators are required to take a second look at their systems following the positive routine sample, they find, on average, a higher rate of coliform presence than during routine sampling. In other words, the high repeat total coliform-positive rate indicates the persistence of total coliforms at such locations in the distribution system.

Further analysis of the data shows that for all PWSs serving 1,000 or fewer people, two or more of the repeat samples are positive in 75 percent of those instances in which there are any positive repeat samples. For those 75 percent of instances, reducing the number of repeat samples from four to three would have no effect on the number of systems that would be triggered to conduct an assessment of the system under the proposed RTCR. In these cases, at least one of the remaining repeat samples would still be total coliform-positive, and only one positive repeat sample is required to trigger an assessment.

The data show that one repeat sample is positive in 25 percent of the instances in which any of the four repeat samples is positive. For these instances, EPA estimates that if only three repeat samples had been taken instead of four, three out of four (or 75 percent) of these positive samples would still have been encountered.

Although dropping the required number of repeat samples from four to three means that some fraction of triggers may be missed, the other provisions of the proposed RTCR compensate for that change and, taken as a whole, the provisions of the proposed RTCR provide for greater protection of public health. One such provision includes enhanced consequences for monitoring violations. For example, systems that do not take all of their repeat samples under the proposed RTCR are triggered to conduct a Level 1 assessment. This permits an increase in public health protection over the current TCR because PWSs are required to assess their systems when monitoring results show that the PWS may be vulnerable to contamination (indicated by exceeding the trigger). Moreover, because of the substantial cost of this potential consequence, systems would be more likely to take all of their required repeat samples in the first place.

It is important to point out that the majority of systems in this category are ground water systems treating to less than 4-log inactivation for viruses (see Exhibit 4.1 of the Proposed RTCR EA (USEPA 2010a)). Because of the triggered source monitoring provision under the GWR, these systems are required to collect a fecal indicator sample from the source water following a total coliform-positive sample in the distribution system in addition to the repeat samples. Under the

existing GWR and TCR, systems taking four repeat samples are permitted to take the fourth repeat sample at the source water if they measure for E. coli as the fecal indicator and if they have State approval. Under the proposed RTCR, systems would continue to take this source water sample to comply with the GWR in addition to the required repeat samples in the distribution system to comply with the TCR.

A positive sample at the source that is not also considered a repeat sample would not trigger an assessment under the proposed RTCR, but it would provide diagnostic value to the system in addition to triggering additional requirements under the GWR (i.e., corrective action or five additional source water fecal indicator samples).

As under the existing GWR and current TCR, with State approval, ground water systems serving 1,000 or fewer people may use the sample taken at the location required for triggered source monitoring to also count toward the repeat monitoring requirements of the proposed RTCR. In this case, the State must also approve the use of E. coli as the fecal indicator under the GWR, and the system would comply with both GWR and the proposed RTCR when a total coliform-positive or E. coli-positive sample occurs. The advisory committee recommended this flexibility to reduce the burden on small ground water systems that in most cases will have a very limited distribution system and only one source, consistent with the GWR.

iii. Location of repeat samples. The advisory committee believed that requiring repeat samples to be taken within five service connections up and downstream of the original total coliform-positive location can be difficult for systems to implement within the required 24 hours for a repeat sample because of issues such as access to the site. Therefore, the advisory committee recommended that systems be allowed to develop standard operating procedures (SOPs) as part of their sample siting plan to identify alternative monitoring sites and facilitate the identification of the source and extent of any problem. EPA is not requiring prior State approval for this provision since there is no reduction in monitoring and the SOP is expected to be used only by larger systems with the technical resources to justify alternative monitoring sites.

The advisory committee also recommended that ground water systems have the flexibility to propose repeat sampling locations that differentiate potential source water and distribution system contamination (e.g., by sampling at entry points to the distribution system). See section III.A.3 of this preamble for additional discussion on this topic. Consistent with its understanding of the intent of the TCRDSAC, EPA has proposed that systems be allowed to exercise this flexibility only with prior State approval. State approval is required because this constitutes a reduction in monitoring (no separate triggered source water samples). EPA believes that this reduction in monitoring is appropriate only if the State determines that the dual purpose sample provides public health protection equivalent to that provided by separate repeat and source water samples. EPA believes that many ground water systems serving 1,000 or fewer people, such as systems with extensive distribution systems, will not be able to show that this reduction in monitoring (i.e., a loss of repeat sample that is near the total coliform-positive routine sample site, but not near the source water sample site) will provide public health protection equivalent to separate samples. EPA believes that systems with limited or no distribution systems are the best candidate for approval.

c. Request for comment. EPA requests comment on the foregoing proposed repeat sampling requirements. Specifically, EPA requests comment on the proposal to allow samples taken at the ground water source to serve both as a triggered source sample under the GWR and as one of the repeat samples under the proposed RTCR. EPA is also requesting comment on whether systems should be allowed to use a dual purpose sample simply by including that in the sample siting plan, without prior State approval. Also, should systems using repeat monitoring sites more than five connections upstream or downstream from the routine total coliform-positive site be required to get prior State approval?

5. Treatment Technique Requirements

a. Provisions. i. Coliform treatment technique triggers. The non-acute MCL violation for total coliforms under the current TCR is effectively replaced by a coliform treatment technique involving monitoring for total coliforms under the proposed RTCR. Under the proposed treatment technique framework, the presence of total coliforms is used as an indicator of a potential pathway of contamination into the distribution system. As discussed in section III.A.2 of this preamble, the proposed RTCR eliminates the associated MCLG and MCL for total coliforms. The proposed revision specifies two levels of treatment technique triggers, Level 1 and Level 2, and their corresponding levels of response. Whether systems are required to conduct either a Level 1 or Level 2 assessment is based on the degree of potential pathway for contamination. The proposed rule further lays out an additional trigger for a Level 1 assessment and defines Level 2 triggers that require a more in-depth examination of the system and its monitoring and operational practices.

The system has exceeded the trigger immediately once any of the following conditions have been met:

Level 1 treatment technique triggers:

- ✓ For systems taking 40 or more samples per month, the PWS exceeds 5.0 percent total coliform-positive samples for the month; or
- ✓ For systems taking fewer than 40 samples per month, the PWS has two or more total coliform-positive samples in the same month; or
- ✓ The PWS fails to take every required repeat sample after any single routine total coliform-positive sample.

Level 2 treatment technique triggers:

- ➢ The PWS has an E. coli MCL violation (see section III.A.6 of this preamble for description of what constitutes an E. coli MCL violation); or
- ➢ The PWS has a second Level 1 treatment technique trigger within a rolling 12-month period, unless the first Level 1 treatment technique trigger was based on exceeding the allowable number of total coliform-positive samples, the State has determined a likely reason for the total coliform-positive samples that caused the initial Level 1 treatment technique trigger, and the State establishes that the system has fully corrected the problem.
- ➢ For PWSs with approved reduced annual monitoring, a Level 1 treatment technique trigger in two consecutive years.

ii. Assessment. EPA is proposing an assessment process in the RTCR to strengthen public health protection. Under the current TCR, a system is not required to perform an assessment following a monthly/non-acute MCL violation or an acute MCL violation. In contrast, the proposed RTCR requires systems to conduct assessments following the triggers specified above.

EPA is proposing two levels of assessment based on the associated treatment technique trigger: Level 1 assessment for a Level 1 treatment technique trigger and Level 2 assessment for a Level 2 treatment technique trigger.

At a minimum, both Level 1 and 2 assessments must include review and identification of the following elements:

- ➢ Inadequacies in sample sites, sampling protocol, and sample processing,
- ➢ Atypical events that may affect distributed water quality or indicate that distributed water quality was impaired,

- Changes in distribution system maintenance and operation that may affect distributed water quality, including water storage,

- Source and treatment considerations that bear on distributed water quality, where appropriate, and

- Existing water quality monitoring data.

EPA expects that States will tailor specific assessment elements to the size and type of the system and that each public water system in turn will tailor its assessment activities based on the characteristics of its distribution system.

Level 1 assessment:
A Level 1 assessment must be conducted when a PWS exceeds one or more of the Level 1 treatment technique triggers specified above. Under the proposed rule, this self-assessment shall consist of a basic examination of the source water, treatment, distribution system and relevant operational practices. The PWS might look at conditions that could have occurred prior to and caused the total coliform-positive sample. Example conditions include treatment process interruptions, loss of pressure, maintenance and operation activities, recent operational changes, etc. In addition, the PWS might check the conditions of the following elements: sample sites, distribution system, storage tanks, source water, etc.

The PWS must complete the Level 1 assessment as soon as practical after notification of its monitoring results or failure to take repeat samples. The PWS must submit the completed assessment form to the State for review within 30 days after determination that the PWS has exceeded the trigger. Failure to submit the completed assessment form within 30 days is a reporting violation. If the State determines that the assessment is insufficient, the State will consult with the PWS. If necessary after consultation, the PWS must submit a revised assessment to the State on an agreed upon schedule not to exceed 30 days from the date of the initial consultation.

The completed assessment form must include assessments conducted, all sanitary defects identified (or a statement that no sanitary defects were identified), corrective actions completed, and a timetable for any corrective actions not already completed. Upon completion and submission of the assessment form by the PWS to the State, the State shall determine if the system has identified the likely cause(s) for the Level 1 treatment technique trigger and establish whether the system has corrected the problem(s).
Level 2 assessment:
A Level 2 assessment must be conducted when a PWS exceeds one or more of the Level 2 treatment technique triggers specified previously.

A Level 2 assessment is a more comprehensive examination of the system, its monitoring and operational practices than the Level 1 assessment. The level of effort and resources committed to undertaking a Level 2 assessment will be commensurate with the more comprehensive investigation and review of available information, and engage additional parties and expertise relative to the Level 1 assessment. Level 2 assessments must be conducted by a party approved by the State: The State itself, a third party, or the PWS where the system has staff or management with the required certification or qualifications specified by the State. If the PWS or a third party conducts the Level 2 assessment, the PWS or third party must follow the State requirements for conducting the Level 2 assessment.

The PWS must complete the Level 2 assessment as soon as practical after notification that the PWS has exceeded a Level 2 treatment technique trigger. The PWS must submit the completed assessment form to the State for review within 30 days after determination that the PWS has exceeded the trigger. Failure to submit the completed assessment form after the PWS properly

conducts the assessment is a reporting violation. The State may direct expedited action or additional actions such as in the case of an E. coli MCL violation. If the State determines that the assessment is insufficient, the State will consult with the PWS. If necessary after consultation, the PWS must submit a revised assessment to the State on an agreed upon schedule not to exceed 30 days from the date of the initial consultation.

The completed assessment form must include assessments conducted, all sanitary defects (or a statement that no sanitary defects were identified), corrective actions completed, and a timetable for any corrective actions not already completed. Upon completion and submission of the assessment form by the PWS to the State, the State shall determine if the system has identified the likely cause(s) for the Level 2 treatment technique trigger and, if so, establish that the system has corrected the problem(s).

iii. Corrective action. The current TCR does not require systems that have MCL violations to perform corrective actions. Under this proposal, EPA is requiring PWSs to correct sanitary defects found through either a Level 1 or Level 2 assessment. Ideally, systems will be able to correct any sanitary defects found in the assessment within 30 days and report that correction on the assessment form. When the correction of sanitary defects is not completed by the time the PWS submits the completed assessment form to the State, the PWS must complete the corrective action(s) on a schedule determined by the State. This schedule may be developed in consultation with the PWS. The schedule must include when the corrective action will be completed and any necessary milestones and temporary public health protection measures. The PWS must notify the State when each scheduled corrective action is completed.

At any time during the assessment or corrective action phase, either the PWS or the State may request a consultation with the other entity to discuss and determine the appropriate actions to be taken. The system may consult with the State on all relevant steps that the system is considering to complete the corrective action, including the method of accomplishment, an appropriate timeframe, and other relevant information.

E. coli detection in the distribution system indicates a public health hazard and can result in an E. coli MCL violation. Under the proposed rule, when an E. coli MCL violation has occurred, the system must complete a Level 2 assessment and corrective action must be implemented as soon as practical. The Agency encourages systems to promptly find the source of E. coli and fix the problem before the completed assessment form is due to the State.

b. EPA's rationale. i. Coliform treatment technique. The advisory committee indicated that the conditions leading to a monthly/non-acute MCL under the current TCR should trigger an assessment under the RTCR for several reasons. First, the advisory committee recognized that presence of total coliform indicates the potential presence of a pathway for contamination from external sources such as source water or through a loss of distribution system integrity. The change to a coliform treatment technique construct that uses total coliforms as an indicator of distribution system integrity places the emphasis on systems to take more preventive actions to address problems. These actions would better protect public health than the additional monitoring with no corrective action that is required under the current TCR. To address the high and constant number of PWSs with violations over the years under the current TCR, the proposed changes would be more protective by requiring systems to correct deficiencies associated with the non-acute MCL (see Exhibit VI.1 in section VI.C.1 of this preamble).

Second, the advisory committee indicated that the public notice associated with non-acute violations is confusing because the presence of total coliforms is not necessarily an indication of a potential public health threat; however, it is an indicator of a potential pathway for fecal contamination to enter into the distribution system. Under the treatment technique requirement, the presence of total coliforms (at the level equivalent to a non-acute violation) indicates a need to assess whether a problem exists. When a system fails to conduct the assessment, the system will then incur a violation and be required to issue public notification. If the system does conduct

the assessment and satisfies the requirements of the treatment technique (including corrective action when a sanitary defect is identified), no public notification is required. Third, the occurrence of total coliforms in the context of the coliform treatment technique requirement continues to inform and further the original objectives of the TCR: to evaluate the effectiveness of treatment, determine the integrity of the distribution system, and signal the possible presence of fecal contamination. Finally, total coliform presence indicates the potential presence of a pathway for contaminants from external sources such as source water or through a loss of distribution system integrity.

ii. Assessment. The proposed rule requires assessments to ensure that specific action is taken to identify whether potential pathways of contamination into the distribution system exist. The advisory committee indicated that assessments are significant actions that protect public health. Under the current rule, when a system has a non-acute MCL violation and if any subsequent sampling did not detect total coliform, the problem may persist due to the intermittent nature of total coliform and remain unaddressed. However, the absence of total coliform-positive samples subsequent to an initial positive finding is not a reliable indicator that a contamination pathway no longer exists. In contrast, the proposed revisions would ensure that systems examine and assess the cause of the total coliform occurrence (that is equivalent to the current non-acute MCL level) and take any corrective action if necessary.

Under the proposed rule, the system will also be required to conduct an assessment if it fails to conduct repeat monitoring following an initial total coliform-positive sample result. As discussed in section III.A.4 of this preamble, repeat monitoring is critical in identifying the extent, source, and characteristics of fecal contamination in a timely manner. Since the revised rule proposes to eliminate additional routine monitoring for systems that monitor at least monthly and decrease the number of additional routine monitoring and repeat monitoring samples for the smallest systems, the need to conduct repeat monitoring is more crucial than ever in providing immediate and useful information needed to protect public health. The cost for collecting and analyzing a repeat sample would be considerably less than the cost for conducting a Level 1 assessment. EPA expects that systems will want to ensure that assessments are conducted only when potential problems may exist rather than for failure to take repeat samples.

The proposed rule specifies two different levels of assessments—Level 1 and Level 2—to recognize that a higher level of effort to diagnose a problem applies to situations of greater potential of public health concern such as repeated Level 1 triggers or an E. coli MCL violation. Level 2 assessments are conducted by a party approved by the State, which may be the PWS where it has staff or management with the certification or qualifications as determined by the State. The Level 2 assessments may also be conducted by the State or a third party approved by the State.

To make more transparent what the Level 1 and Level 2 assessments entail and to facilitate consistent implementation among States, the proposed rule specifies minimum elements for these assessments. The advisory committee recommended that the minimum elements identified previously in this preamble be included in the Level 1 and Level 2 assessments to identify potential flaws in monitoring or specific pathways of contamination. Although the proposed RTCR specifies the same minimum elements for both the Level 1 and Level 2 assessments, the Level 2 assessment involves a more in-depth examination of these elements compared to a Level 1 assessment. Specific examples of how the Level 2 assessments are more in-depth than Level 1 assessments may be found in Appendix X of the AIP (USEPA 2008c).

EPA recognizes that not every assessment will identify a sanitary defect or find a reason or cause for the presence of total coliforms. If no sanitary defect is identified, the system must document that fact in the completed assessment form and provide supporting evidence for this conclusion. EPA expects that only systems that adhere to proper procedures and standards set by the State are eligible to arrive at this determination, and only after providing sufficient supporting evidence.

The advisory committee recommended that the Level 1 and Level 2 assessments be conducted as soon as practical after the PWS receives notice that the system has exceeded the treatment technique trigger. The advisory committee also recommended that systems submit the completed assessment forms to the State within 30 days after determination that the PWS has exceeded the trigger. The rationale for the 30-day interval is to allow sufficient time for problem identification and potential remediation of the problem in conjunction with the follow-up assessment, in most cases.

To help States and PWSs conduct assessments, EPA intends to develop a draft assessment and corrective action guidance manual and to make it available for public comment prior to promulgation of the final rule and to finalize the guidance manual after the rule is finalized.

iii. Corrective action. The advisory committee recognized that not every assessment will identify a sanitary defect. However, the advisory committee recommended that the RTCR require all sanitary defects be corrected by the system in a timely manner. The system, in consultation with the State as needed, identifies and determines the specific corrective action.

Under the proposed rule, the State may allow the PWS additional time to conduct the corrective action if needed. EPA recognizes that some systems may not be able to fix sanitary defects before submitting the completed assessment form within the 30-day interval due to the extent and cost of the corrective action.

In such situations, EPA encourages the State and PWS to work together to determine the appropriate schedule for corrective actions (which may include additional or more detailed assessment or engineering studies) to be completed as soon as possible. The system must comply with the agreed upon schedule and notify the State when each scheduled corrective action is completed.

Either the PWS or the State may request consultation with the other party to determine the appropriate actions to be taken. EPA is not requiring this to be a mandatory consultation to provide ease of implementation for States. In many cases, consultation may not be necessary because the type of corrective action for the sanitary defect will be clear and can be fixed right away (for example, replacement of a missing screen).

c. Request for comment. EPA requests comment on the: (1) Proposed change from the non-acute MCL for total coliforms to a coliform treatment technique requirement that uses total coliforms as an indicator of a pathway of contamination; (2) proposed requirement for systems to conduct an assessment following a trigger condition; (3) proposed levels of assessment required; and (4) proposed requirement for systems to correct all sanitary defects found during an assessment. In addition, EPA requests comment on how to ensure that a Level 2 assessment is more comprehensive than a Level 1 assessment (e.g., should a Level 2 assessment include additional elements such as asset management and capacity development?). Should EPA provide more detail, either in guidance or rule language, on the elements and differences between a Level 1 and Level 2 assessments? If in rule language, how should the rule language distinguish the two levels of assessments? Please provide examples. Additionally, should EPA provide additional guidance on how systems might address the situation where a Level 1 or Level 2 assessment fails to identify any sanitary defects (i.e., the trigger event remains unexplained). If so, what should such guidance say?

6. Violations

a. Provisions. EPA is proposing to modify the definition of the existing MCL violation, establish a treatment technique violation, and revise the monitoring and reporting violations. EPA is proposing that public notice be required for each type of violation (see section III.A.7 of this preamble for detail information on public notification).

i. E. coli MCL violation. A violation of the E. coli MCL occurs when:

- ✓ A routine sample is total coliform-positive and one of its associated repeat samples is E. coli- positive; or
- ✓ A routine sample is E. coli-positive and one of its associated repeat samples is total coliform-positive; or
- ✓ A system fails to take all required repeat samples following a routine sample that is positive for E. coli; or
- ✓ A system fails to test for E. coli when any repeat sample tests positive for total coliforms.

ii. Coliform treatment technique violation. A coliform treatment technique violation occurs when:

- ✓ A system fails to conduct a required assessment within 30 days of notification of the system exceeding the trigger (see section III.A.5 of this preamble for conditions under which monitoring results trigger a required assessment); or
- ✓ A system fails to correct any sanitary defect found through either a Level 1 or 2 assessment within 30 days (see also section III.A.6 of this preamble) or in accordance with State-derived schedule.

There would be no treatment technique violation associated solely with a system exceeding one or more action triggers (Level 1 or Level 2 triggers).

iii. Monitoring violation. Under the current TCR, a monitoring violation occurs when a system fails to comply with the total coliform monitoring requirements, including the sanitary survey requirement. Under the proposed RTCR, a monitoring violation occurs when a system fails to take every required routine or additional routine sample in a compliance period, or when it fails to test for E. coli following a routine sample that is total coliform-positive.

In addition, if a system on quarterly monitoring has a monitoring violation in two or more quarters, or if a system on annual monitoring misses its annual monitoring, it must begin monthly monitoring until it meets criteria for less frequent monitoring. See section III.A.3 of this preamble for a detailed discussion on monitoring frequency.

iv. Reporting violation. A reporting violation occurs when a system that properly conducts monitoring or an assessment fails to submit a monitoring report or a correctly completed assessment form by the required deadline. The PWS is responsible for reporting this information to the State regardless of any arrangement with a laboratory. A reporting violation also occurs when a system fails to notify the State following an E. coli-positive sample.

b. EPA's rationale. To define violations, the advisory committee built upon the principles underlying the current TCR violations and current TCR public notification and suggested changes to improve public health protection where they saw a specific need. This proposal specifies responses to different degrees of potential public health concern. As described in the next section on providing information and notification to the public, Tier 1, Tier 2, and Tier 3 public notices are required following violations corresponding to the severity of each violation type.

i. E. coli MCL violation. An E. coli MCL violation (which includes failure to take all required repeat samples following an E. coli-positive sample) creates concern of an immediate potential public health threat. For this reason, an E. coli MCL violation is considered an acute violation requiring immediate response by the system. Including an E. coli MCL violation condition for systems failing to collect all repeat samples following an initial E. coli- positive sample enhances public health protection by preventing a system from incurring only a monitoring violation when there is an indication of fecal contamination. As discussed previously in section II.D of this preamble, the presence of E. coli indicates a pathway of fecal contamination and should be taken seriously. Systems need to follow up with repeat samples to characterize the extent and source of such contamination. Failure to take the required repeat samples following an initial E. coli-positive sample is not protective of public health and is a serious violation.

ii. Coliform treatment technique violation. A coliform treatment technique violation occurs when a potential pathway of contamination in the distribution system is unexplored and/or uncorrected. Performing the Level 1 and 2 assessments and taking corrective action are essential aspects of compliance with the treatment technique. A system which neglects to perform the prescribed assessment or corrective action is in violation of the proposed RTCR's treatment technique requirements.

iii. Monitoring violation and reporting violation. Monitoring and reporting violations occur when a system fails to comply with the routine monitoring requirements or when a system fails to submit monitoring reports or completed assessment forms. EPA believes that monitoring violations and reporting violations need to be addressed so that a system is held accountable to take actions to reduce public health risk, including regular monitoring of water quality.
c. Request for comment. EPA requests comment on the proposed violation determinations.

7. Providing Notification and Information to the Public
a. Provisions. To correspond to the changes in the proposed revised rule, EPA is proposing some modifications to the public notice (PN) requirements contained in 40 CFR part 141 subpart Q. Tier 1 PN is required for an E. coli MCL violation. Tier 2 PN is required for a treatment technique violation for failure to conduct assessments or corrective actions. Tier 3 PN is required for a monitoring violation or a reporting violation.

In the current TCR, if a system has an acute MCL violation which is based on the presence of fecal coliforms or E. coli, or which is based on the system's failure to test for fecal coliforms or E. coli following a total coliform-positive repeat sample, the system is required to publish Tier 1 PN. Under the proposed RTCR, a system is required to publish Tier 1 PN when it has an E. coli MCL violation (see section III.A.6 of this preamble for what constitutes an E. coli MCL violation). In addition, the system will continue to be required to notify the State after learning of an E. coli-positive sample, as currently is required under the TCR. As mentioned earlier in section III.A.2 of this preamble, EPA is proposing to eliminate the MCL for fecal coliforms. Under the proposed rule, the standard health effects language, which is required to be included in all public notification actions, is modified to delete the reference to the fecal coliform MCL and fecal coliforms. The language for a non-acute violation under the current TCR is modified to apply to a violation of the assessments and corrective action requirements of the coliform treatment technique.

In the current TCR, a system is required to publish a Tier 2 PN when the system has a non-acute MCL violation, which is based on total coliform presence. Under the proposed rule, a system is required to publish a Tier 2 PN if the system violates the coliform treatment technique requirements. Also, EPA is proposing to modify the standard health effects language for coliform to emphasize the assessment and corrective action requirements of the proposed rule.

In the current TCR, a system is required to publish a Tier 3 PN when the system has a monitoring or reporting violation. In the proposed rule, the Tier 3 PN requirements are changed to

incorporate the recommendation in the AIP that monitoring violations be considered distinct from reporting violations under the proposed RTCR. Both types of violations require Tier 3 PN.

Consumer confidence report (CCR) requirements are also modified. Health effects language for the CCR, which is identical to the health effects language required for PN, is updated in the same way as described for PN. In addition, the proposed RTCR removes the CCR requirements that require the inclusion of total numbers of positive samples, or highest monthly percentage of positive samples for total coliforms as well as total number of positive samples for fecal coliforms. These provisions are replaced by requirements to include the number of Level 1 and Level 2 assessments required and completed, the corrective actions required and completed, and the total number of positive samples for E. coli. Unchanged and consistent with existing provisions under the current TCR, a CWS may provide Tier 3 PN using the annual CCR.

b. EPA's rationale. The proposed public notification requirements are consistent with the AIP language as well as with the tier system described in 40 CFR part 141 subpart Q. These changes are appropriate because some of the types of violations in the proposed RTCR are different from the current TCR. The standard health effects language for the public notification is also revised as appropriate given the changes to what constitutes a violation under the proposed RTCR.

The proposed Tier 1 PN requirement for an E. coli MCL violation is consistent with the current TCR. Tier 1 PN is required for NPDWR violations and situations with significant potential to have serious adverse effects on human health as a result of short term exposure. The existing Tier 1 PN requires public notice as soon as possible but no later than 24 hours after the system learns of the violation. Exposure to E. coli in drinking water can possibly result in serious, acute health effects, such as diarrhea, cramps, nausea, headaches, or other symptoms and possible greater health risks for infants, young children, some of the elderly, and people with severely compromised immune systems.

Tier 2 PN is required for all NPDWR violations and situations with potential to have serious adverse effects on human health not requiring Tier 1 PN.

The system must provide public notice as soon as practical, but no later than 30 days after the system learns of the violation. A treatment technique violation under the proposed RTCR meets these criteria because it is an indication that the public water system failed to conduct an assessment or complete corrective action following identification of sanitary defects. Identification of a sanitary defect indicates that a problem may exist in the distribution system that has potential to cause public health concern.

Tier 3 PN is required for all other NPDWR violations and situations not included in Tier 1 or Tier 2. The existing Tier 3 PN requires a system to provide public notice no later than one year after the system learns of the violation or situation or begins operating under a variance or exemption. Monitoring violations and reporting violations meet these criteria because, while they do represent a violation of the proposed RTCR, the risk to public health is not as clearly linked as those that are Tier 1 or 2. Therefore, EPA believes that a public notice given at least annually fulfills the public's right-to-know about these violations.

Consumer confidence report requirements are updated to reflect the advisory committee's recommendations that total coliforms be used as an indicator to start an evaluation process that, where necessary, will require the PWS to correct sanitary defects. EPA believes it is most appropriate to inform the public about actions taken, in the form of assessments and corrective actions, since failure to conduct these activities lead to treatment technique violations under the proposed RTCR. Because the proposed RTCR no longer includes the total coliform MCL but now includes a trigger, EPA believes that systems no longer need to report the number of total coliform-positive samples via the CCR, since that could cause confusion or inappropriate changes in behavior among consumers. In addition, the CCR requirements will also reflect the removal of fecal coliform provisions under the proposed RTCR.

c. Request for comment. EPA requests comment on whether the PN and CCR language revisions are consistent with the provisions of the proposed RTCR that reflect the use of total coliforms as an indicator within a coliform treatment technique. Since EPA is not aware of health effects resulting solely from exposure to total coliforms, the proposed RTCR eliminates the public notification requirement for detection of total coliforms, but provides for public notification upon detection of E. coli, and for violation of the coliform treatment technique.
The Agency does request comment, however, on the loss of information to consumers resulting from elimination of public notification requirements following positive sample results for total coliforms.

EPA also requests comment on whether the proposed RTCR should require special notice to the public of sanitary defects, in addition to the PN requirements, similar to the GWR special notice requirements. This would be consistent with current requirements for other regulations that limit pathogens in ground water systems. Under 40 CFR 141.403(a)(7)(i), a CWS must inform the public of the significant deficiency and/or fecal indicator-positive sample. The CWS must continue to inform the public annually until the significant deficiency is corrected or the fecal contamination in the ground water source has been determined by the State to be corrected. Under 40 CFR 141.403(a)(7)(ii), an NCWS that receives notice from the State of a significant deficiency must inform the public of any significant deficiency that has not been corrected within 12 months of being notified by the State, or earlier if directed by the State. The NCWS must continue to inform the public annually until the significant deficiency is corrected.

8. Reporting and Recordkeeping Requirements for Systems
a. Provisions. i. Reporting. In addition to the existing general reporting requirements provided in 40 CFR 141.31, the proposed RTCR requires a PWS to:

- ✓ Notify the State no later than the end of the next business day after it learns of an E. coli-positive sample.

- ✓ Report to the State an E. coli MCL violation no later than the end of the next business day after learning of the violation. The PWS is also required to notify the public according to the provisions laid out in 40 CFR part 141 subpart Q.

- ✓ Report to the State a treatment technique violation no later than the end of the next business day after it learns of the violation. The PWS must also notify the public in accordance with 40 CFR part 141 subpart Q.

- ✓ Report to the State monitoring violations within ten days after the system discovers the violation, and notify the public in accordance with 40 CFR part 141 subpart Q.

- ✓ Notify the State when each scheduled corrective action is completed for corrections not completed by the time of the submission of the assessment form.

In addition, systems triggered into conducting an assessment are required to submit the completed assessment form within 30 days after determination that the coliform treatment technique trigger has been exceeded (see section III.A.3 of this preamble for additional discussion).

ii. Recordkeeping. EPA is proposing to maintain the current TCR requirements regarding retention of sample results and records of decisions related to monitoring schedules found in 40 CFR 141.33, including provisions that address the new requirements of the proposed RTCR pertaining to reduced and increased monitoring, treatment technique, etc. In addition, systems are required to maintain on file for State review the assessment form or other available summary documentation of the sanitary defects and corrective actions taken. Systems are required to

maintain these documents for a period not less than five years after completion of the assessment or corrective action.

b. EPA's rationale. In the case of an E. coli-positive sample, the proposed RTCR maintains the current TCR requirement that systems must notify the State by the end of the day when they are notified of the E. coli-positive result or by the end of the next business day if the State office is already closed. The advisory committee believed that this requirement is important to maintain because of the potential for immediate public health risk associated with E. coli presence and the desire for States to consider quickly whether additional actions might be appropriate. The same rationale applies to E. coli MCL violations.

Since there are new requirements for conducting assessments and corrective actions, and new conditions for obtaining increased or reduced monitoring provisions, the proposed rule includes reporting and recordkeeping requirements to facilitate tracking of Level 1 and Level 2 triggers and compliance with treatment technique requirements. Systems are required to maintain these files no less than five years. Since systems have to maintain these files no shorter than the maximum period allowed between sanitary surveys (i.e., five years; see 40 CFR 142.16(b)(3) and 40 CFR 142.16(o)(2)), States have the opportunity to look at and review these files during sanitary surveys and/or annual visits. The five year period is also consistent with the recordkeeping requirements for microbiological analyses under 40 CFR 141.33(a).

The timeframe by which reporting and recordkeeping are required under the proposed rule is consistent with EPA's practice regarding reporting and recordkeeping requirements in other regulations under SDWA.

c. Request for comment. EPA requests comment on whether the timeframe required for reporting and recordkeeping requirements are appropriate.

9. Analytical Methods

a. AIP-related method issues. i. Evaluation of currently-approved methods. The AIP contains several recommendations by the advisory committee regarding the analytical methods approved under the proposed RTCR. The advisory committee noted that the methods currently approved under the current TCR have varying sensitivities and specificities, and recommended that " * * * the Agency evaluate all currently approved coliform analytical methods to determine whether these methods continue to be appropriate for use in drinking water compliance monitoring" (USEPA 2008c, AIP p. 7).

In the twenty years since the current TCR was promulgated, many methods have been developed and approved for use. Most of the approved methods that are used to support the current TCR were evaluated under EPA's Alternate Test Procedure (ATP) process. Under this process, a proposed method is evaluated in comparison to a reference method. A favorable comparison serves as the basis for subsequent approval of the method for use in regulatory compliance monitoring.

The ATP evaluations are designed based on the ATP Microbiology Protocol (USEPA 2004), an EPA guidance document that outlines how the evaluation study should be conducted. In the years the ATP program has been in place, the ATP guidance document has been revised several times. As a result of different protocols being used over time, the current set of approved methods have not all been evaluated under identical conditions.

In addition to the concerns expressed by the advisory committee that the approved methods may not be equivalent to each other, EPA notes that there have been additional concerns with some of the methods currently approved. This includes allegations that some of the approved methods may have been modified since approval without EPA's knowledge. EPA is also aware of reports of varying performance of some enzyme-based methods (Oldstadt et al. 2007; Fricker et al. 2003). Lastly, EPA is aware of at least one circumstance where the manufacturer of an approved

method placed a “product hold” and recall on the medium after the product was reported to be experiencing reduced recovery of E. coli.

For these reasons, EPA believes that additional information may be needed regarding the performance of the currently approved methods in order to justify their continued approval. Among the options, EPA is considering a complete, side-by-side method evaluation study, whereby all the methods are compared to each other under identical conditions, according to the same protocol.

EPA is considering an approach under which vendors of all currently approved methods would have the option of voluntarily participating in an independent, third-party laboratory evaluation through EPA's Environmental Technology Verification (ETV) Program. The goal of the ETV Program is to provide independent, objective, and credible performance data for commercial-ready environmental technologies. More information on this program is available on EPA's Web site at www.epa.gov/etv/index.

Under the ETV approach, EPA anticipates that participating vendors would generally fund the majority of the cost of their method evaluation. Based on the results of the ETV study, as documented in the verification report, EPA would judge the appropriateness of each analytical method and would determine which should continue to be approved for future monitoring. EPA would then make any changes to the analytical methods approved under the RTCR through later rulemaking.

If a vendor chooses not to participate in the ETV study, EPA would allow the vendor to propose, for EPA's consideration, an equivalent alternative approach for method evaluation. EPA will determine whether the proposed approach will provide an independent, effective, and credible evaluation. EPA emphasizes that any alternative approach would need to be equivalent in scope and rigor to the ETV program. As with the ETV study, EPA would use the results from an alternative study to judge the appropriateness of each analytical method and would determine which methods warrant approval for future monitoring under this regulation.

iii. Approval of “24-hour” methods. The AIP also recommends that EPA “consider approving methods that allow the timely (e.g. on the order of 24 hours) analytical results for E. coli and TC and that provide relatively concurrent analyses, without significantly sacrificing accuracy, precision and specificity” (USEPA 2008c, AIP p. 7). EPA notes that many of the approved methods that may be used in compliance with the proposed rule can be completed in approximately 24 hours. However, the methods that detect lactose fermentation include a confirmation step that involves transfer of a presumptively positive culture into a more inhibitory confirmation medium which serves to ensure the initial positive was correct. As a result of this confirmatory step, lactose fermentation methods can take up to 96 hours to obtain a result. The enzyme based methods do not require this confirmation step, and their results can be obtained in a 24 to 48 hour time period.

EPA is aware of some concerns that methods with a 24 hour incubation time may not be able to detect as many coliform bacteria as methods with a 48 hour incubation period. Since many of the coliform bacteria found in a distribution system are injured or stressed due to disinfection practices, and since injured/stressed organisms may take longer to detect than 24 hours, this concern is of interest to EPA.

As part of, or in addition to, the method evaluation previously described, EPA may therefore further investigate the impact of incubation time on the recovery of stressed/injured organisms in drinking water using approved media. At this time, EPA believes that it is premature to conclude that either enzyme-based or lactose-based methods are inherently preferable.

As discussed during the advisory committee meetings, the analysis time of the analytical methods is just one aspect in the overall amount of time it takes before a PWS obtains sample results from

the laboratory and subsequently collects repeat samples. Factors that can impact how quickly the PWS receives notification of a positive result include whether the PWS uses an in-house laboratory or must ship the sample to a distant contract laboratory, and whether the sample results are reported via an electronic means or via traditional mail. In addition, the turnaround time for repeat sampling can be affected by such factors as the laboratory daily hours of operation. The current TCR specifies that repeat samples be collected within 24 hours, but States currently have the flexibility to extend this timeline. The current TCR does not contain provisions for how quickly the laboratory must notify the PWS when a positive result is obtained. This proposal does not change these provisions.

iv. Elimination of fecal coliforms under the proposed RTCR. The AIP also contains a recommendation that EPA remove all provisions related to fecal coliforms under the proposed RTCR. Consistent with this recommendation, and for the following reasons, EPA is proposing to eliminate all fecal coliform provisions in the RTCR.

First, the fecal coliform group can contain bacteria not associated with fecal contamination. E. coli is the most prominent member of the fecal coliform group. However, other coliform bacteria, such as thermotolerant strains of Klebsiella spp., have been shown to occur in the fecal coliform group (Warren et al. 1978). These non-E. coli bacteria are often found in environmental sources (for example, soil, vegetation, water) and, therefore, are not exclusively associated with feces. Due to the presence of these non-fecal bacteria, the fecal coliform group may not always provide the public water system with meaningful data regarding the vulnerability of their distribution system to fecal contamination.

Secondly, when the current TCR was developed, there were few E. coli methods available. Many public water systems were familiar with and preferred to use the fecal coliform methods. However, since the current TCR was promulgated, many E. coli methods have been developed and approved for use. EPA believes that most systems nationwide currently test for E. coli, while few test for fecal coliform bacteria. Since the methods used to test for E. coli have approximately the same cost as those used to test for fecal coliform bacteria, this proposed change is not expected to create an additional burden on PWSs.
EPA is proposing to eliminate all analytical method provisions for fecal coliforms that are included in the current TCR. EPA proposes instead to allow testing only for E. coli following a total coliform-positive sample. This change will provide the water system with more meaningful information regarding potential fecal contamination of the distribution system.

The current TCR specifies a number of analytical methods that can be used for compliance sample analysis (in 40 CFR 141.21(f)). Since fecal coliform bacteria are not regulated contaminants under this proposed rule, the analytical methods for fecal coliforms are no longer applicable and are removed from the list of analytical methods. All other methods used for compliance with the current TCR are maintained for compliance sample analysis under the proposed RTCR.

v. Request for comment on AIP-related method issues. EPA is requesting comment on the following RTCR analytical method issues related to recommendations from the TCRDSAC in its AIP:

- The use of an ETV approach for a reevaluation of analytical methods.
- Whether the RTCR should include provisions to ensure a more expedited results notification process. The RTCR could, for example, include language requiring that PWSs arrange to be notified of a positive result by their laboratory within 24 hours.
- Whether the RTCR should require repeat samples be taken within 24 hours of a total coliform-positive with no (or limited) exceptions.

b. Other method issues. In addition to addressing the recommendations of the advisory committee, EPA is proposing some minor technical changes related to analytical methods.

Many of these changes document practices that are already followed by PWSs and laboratories, and are consistent with the Manual for the Certification of Laboratories Analyzing Drinking Water (referred to as the "Laboratory Certification Manual") (USEPA 2005), an EPA document that outlines method requirements and good laboratory practices for certified laboratories conducting drinking water compliance sample analyses.

Some of these changes were brought to the attention of EPA by EPA Regions and States involved in the implementation of the drinking water certification program. Other minor changes have been proposed to make the analytical methods section of this regulation easier to understand and implement. Each proposed change is described as follows with a discussion of the rationale for the change.

i. Holding time. The proposed RTCR continues to provide a 30-hour holding time limit for the samples collected in compliance with this regulation (40 CFR 141.21(f)(3)). However, EPA is proposing to change the definition for holding time from "the time from sample collection to initiation of analysis may not exceed 30 hours" to "the time from sample collection to initiation of test medium incubation may not exceed 30 hours."

ii. Dechlorinating agent for sample preservation of chlorinated water supplies. The proposed RTCR establishes the following provision: "If chlorinated water is to be analyzed, sufficient sodium thiosulfate (Na 2 S 2 O 3) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample." Dechlorination procedures are addressed in section 9060A.2 of Standard Methods for the Examination of Water and Wastewater (20th and 21st editions) (Clesceri et al. 1998; Eaton et al. 2005).

iii. Filtration funnels. EPA is proposing to add the following footnote to the analytical methods table (§ 141.852) under the revised rule:

All filtration series must begin with membrane filtration equipment that has been sterilized by autoclaving. Exposure of membrane filtration equipment to UV light is not adequate to ensure sterilization. Subsequent to the initial autoclaving, exposure of the filtration equipment to UV light may be used to sanitize the funnels between filtrations within a filtration series.
iv. Analytical methods table changes. EPA is proposing the following changes to the analytical methods table:

- ✓ The table is organized by methodology (e.g., lactose-fermentation methods vs. enzyme-substrate methods).
- ✓ E. coli methods are included in the table.
- ✓ 18th and 19th editions of Standard Methods for the Examination of Water and Wastewater are no longer approved and have been removed.
- ✓ The references to Standard Methods 9221A and 9222A are removed.
- ✓ The reference to Standard Methods 9221B is changed to 9221B.1, B.2.
- ✓ The reference to Standard Methods 9221D is changed to 9221D.1, D.2.
- ✓ The table proposes to allow Standard Methods 9221D in the multiple tube format as described in Standard Methods 9221B.
- ✓ The citation for MI agar is changed to EPA Method 1604 for clarity and consistency.

- ✓ The table clarifies that Standard Methods 9221 F.1 and 9222 G.1 a (1), (2) may be used for E. coli analysis.

- ✓ The table clarifies the correct formulation for EC-MUG broth, when used in conjunction with Standard Methods 9222G.1 a (2), through the addition of the following footnote:

The following changes must be made to the EC broth with MUG (EC-MUG) formulation: Potassium dihydrogen phosphate, KH_2PO_4 must be 1.5g and 4-methylumbelliferyl-Beta-D-glucuronide must be 0.05 g.

- ➢ The table reflects the approval of a modified Colitag method for the simultaneous detection of E. coli and other total coliforms.

v. EPA's rationale for proposed changes related to other method issues. (a). Holding time. The current rule states "The time from sample collection to initiation of analysis may not exceed 30 hours" (40 CFR 141.21 (f)(3)). Since promulgation of the current TCR, some States and EPA Regions have commented that "initiation of analysis" may be interpreted several different ways, which can lead to the sample being held longer than the 30 hours intended by the rule. The proposed language more clearly defines the amount of time that the sample may be held and is consistent with section 6.4.1 of the Manual for the Certification of Laboratories Analyzing Drinking Water which states: "For the analysis of total coliform in drinking water, the time between sample collection and the placement of sample in the incubator must not exceed 30 hours."

EPA believes that changing the definition of holding time from "the time from sample collection to initiation of analysis" to "the time from sample collection to initiation of test medium incubation" may slightly decrease the amount of time a PWS has to get the sample to a laboratory. EPA does not believe that this change will significantly reduce the amount of time a water system has to get a sample to the laboratory, as most of the methods approved under this rule require 30 minutes or less to process and prepare the sample for the incubation step. Thus, the initial analytical steps should not constitute a large portion of the holding time as a whole. EPA recommends that PWSs that have difficulty meeting the holding time notify the laboratory that the samples are in transit and need to be given priority. The laboratory could begin analysis immediately upon sample arrival so that the samples could be placed in the incubator in time to meet the 30 hour holding time. EPA notes that a laboratory may have to make specific accommodations in their processes in order to properly analyze a sample received close to the end of the holding time. EPA believes that this is feasible with proper planning.

(b). Dechlorinating agent for sample preservation of chlorinated water supplies. Under this proposal, EPA would require that chlorinated water samples be collected in bottles that contain the dechlorinating agent sodium thiosulfate. This is consistent with section 3.15.4 of the Laboratory Certification Manual, which states "If chlorinated water is to be analyzed, sufficient sodium thiosulfate (Na 2 S 2 O 3) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample." Neutralization ceases the bactericidal action of the chlorine during sample transit, thus allowing a more accurate assessment of what the true microbial content of the water sample was at the time of sample collection. Implementation of this new requirement should be straightforward since PWSs need only ask the laboratory for pre-treated sample containers. EPA does not believe this provision will cause an increase in cost to PWSs, as the cost of the bottles with the sodium thiosulfate is essentially the same as the cost of the bottles without the sodium thiosulfate.

(c). Filtration funnels. Under this proposal, EPA is requiring that membrane filtration equipment be autoclaved before beginning a filtration series. This requirement is consistent with section 4.1.3 of the Laboratory Certification Manual, which states: "Membrane filter equipment must be autoclaved before the beginning of a filtration series."

Under the current TCR, not all of the approved membrane filtration methods require that a filtration series begin with membrane filtration units that have been sterilized by autoclave. Some of the approved methods allow the laboratory to use ultraviolet (UV) radiation exposure in lieu of autoclaving to sterilize filtration units between filtration series. EPA does not believe that ultraviolet radiation is sufficient to properly sterilize the membrane filtration equipment. Additionally, EPA believes that when ultraviolet radiation is used, not all areas of the membrane filtration equipment are exposed, and therefore microorganisms may persist and contaminate other water samples and the laboratory. For these reasons, EPA is proposing to include a footnote to the analytical methods table in order to ensure proper sterilization.

EPA does, however, believe that ultraviolet light can be used to sanitize the filtration equipment between filtrations within a filtration series, as stated in section 4.1.4 of the Laboratory Certification Manual: "Ultraviolet light (254 nm) may be used to sanitize equipment (after initial autoclaving for sterilization), if all supplies are pre-sterilized. Ultraviolet light may be used to reduce bacterial carry-over between samples during a filtration series."

(d). Analytical methods table. In this proposal, EPA is identifying a number of changes to the analytical methods table for clarity and accuracy.

In the current TCR, the methods are listed by date approved and the E. coli methods are listed in a text format. In this proposal, the analytical methods table is organized by methodology (e.g., lactose-fermentation methods vs. enzyme-substrate methods), and the E. coli methods are included in the table.
Standard Methods for the Examination of Water and Wastewater is a reference document designed to represent "the best current practice of American water analysts." Periodically, new editions are published in order to incorporate improvements in the methods contained within this manual. Thus, new editions of this publication contain more current and improved versions of the methods. Under the current TCR, four editions of this publication are approved, resulting in different, oftentimes outdated, versions of the same method being approved.

Having multiple editions of this manual approved under this regulation also creates a burden for the laboratory certification officers who must understand the differences between the versions of the method for which the laboratory may be seeking certification. For these reasons, EPA is proposing to remove the 18th and 19th editions of Standard Methods for the Examination of Water and Wastewater for use in compliance sample analysis under the RTCR. EPA expects that the burden associated with this change will be minimal as most laboratories have already procured the newer editions or have arranged for access to the online publication.

In this proposed regulation, the reference to Standard Methods 9221A and 9222A are removed. These sections of the methods contain only introductory information, not any actual methodology. They do not represent methods approved for use under this regulation.

The references to Standard Methods 9221B and 9221D are modified in this proposed regulation. In the current TCR, the methods are referenced as 9221B and 9221D with footnote 5 denoting that the "completed phase" called for in the methods is not required. By more specifically citing Standard Methods 9221 B.1, B.2, and 9221 D.1, D.2 (which contain the applicable, required steps of these methods) EPA is able to eliminate the original footnote and improve clarity.

EPA is proposing to allow Standard Methods 9221D (Presence-Absence broth) to be used in a multiple tube format. This method has traditionally been used in a single bottle, allowing only the qualitative detection of total coliforms. However, there are published reports showing this method can be used in a multiple tube format for the quantitative detection of total coliforms (Rice et al. 1987; Rice et al. 1993). This medium would be used in the same manner that Lauryl Tryptose Broth (LTB) is described as being used in Standard Methods 9221B. Allowing the use of this method in a multiple tube format would allow PWSs that use this method to quantitate any total coliforms that may occur in the water sample.

EPA is proposing to change the citation for MI Agar. Under the current TCR, this method is cited as Standard Methods 9222, with a footnote citing the Applied and Environmental Microbiology article where the method was initially described. In this proposal, the method is now cited as EPA Method 1604, consistent with section 5.4.2.1.3 of the Laboratory Certification Manual. EPA Method 1604 is identical to the citation in the TCR and does not require the use of the original footnote. This change is also consistent with the citation of this method as listed in the Ground Water Rule (see 40 CFR 141.402).

The current TCR describes the use of "EC medium supplemented with 50 µg/mL of 4-methylumbelliferyl-Beta-D-glucuronide (MUG)" (see 40 CFR 141.21(f)(6)(i)). This proposal clarifies that this medium, included in both Standard Methods 9221F and Standard Methods 9222G.1 a (2), is approved for use under this regulation. This is consistent with the Laboratory Certification Manual, particularly section 5.1.8, which describes both of these methods as approved for use in the detection of E. coli under this regulation.

Lastly, EPA is clarifying the formulation for EC broth with MUG (EC-MUG) given in Standard Methods 9222G.1 a (2) to correct an error in the publication. The Standard Methods 9222G.1 a (2) formulation calls for 0.1 g of 4-methylumbelliferyl-Beta-D-glucuronide, and 1.4g KH 2 PO 4. This formulation differs from that given in Standard Methods 9221F.1, which calls for 0.05 g and 1.5 g, respectively. EPA believes that the correct formulation is given in Standard Methods 9221F and has confirmed this with Standard Methods committee members (Rice 2009). Accordingly, EPA has added a footnote to the 9222G.1 a (2) stating the proper formulation.

EPA anticipates that these changes to the analytical methods table will not cause any additional burden to the PWSs.

vi. Request for comment regarding holding temperature. The current TCR states the following regarding sample shipment: "Systems are encouraged but not required to hold samples below 10 deg. C during transit." Other national primary drinking water regulations requiring microbial sampling require that the samples be shipped in cold conditions, and require the sample be maintained at a temperature of 10 degrees Celsius (C) or less. Maintaining the sample temperature below 10 degrees C serves to preserve the bacterial population by minimizing both bacterial cell death and cell multiplication, thus allowing for a more accurate representation of the microbial population in the sample at the time of sample collection. Also, Standard Methods for Examination of Water and Wastewater, 21st edition (Eaton et al. 2005) recommends that samples be shipped at less than 8 degrees C but not frozen.

In the years since the promulgation of the current TCR, EPA has heard concern that at times, samples collected under the TCR may reach high temperatures during transit to the laboratory due to the lack of a requirement to ship samples on ice. High temperatures that may be reached during transit could have a deleterious or prolific effect on the bacterial cells present in the samples such that the samples may no longer represent the microbial content of the water at the time of sample collection.

EPA recognizes that requiring the samples under the proposed RTCR to be held at 10 degrees C or less, but above freezing, would result in an increased cost to the water systems (for shipping, supplies, etc.), but believes the extra burden may be warranted. EPA is seeking public comment on whether this passage should remain as is in the current TCR or whether the RTCR should require that the samples collected for compliance with this regulation be shipped in cold conditions, i.e., requiring a temperature of 10 degrees C or less, but above freezing to be maintained for better sample preservation. EPA also welcomes comments and supporting data on what the acceptable temperature range should be when samples are in transit.

. Proposed Compliance Date
Consistent with SDWA section 1412(b)(10), EPA proposes that the compliance date of the final RTCR be three years from the date on which the regulation is promulgated (i.e., the publication date of the final rule in the Federal Register). PWSs must comply with the requirements of the rule by the compliance date.

EPA believes that capital improvements generally are not necessary to ensure compliance with the proposed RTCR. However, a State may allow individual systems up to two additional years to comply with the RTCR if the State determines that additional time is necessary for capital improvements, in accordance with SDWA section 1412(b)(10).

EPA requests comment on the proposed compliance date of the proposed RTCR.

C. Links to Other Drinking Water Rule Requirements
The proposed RTCR recognizes that existing NPDWRs contain linkages among monitoring requirements in different rules. The current residual disinfectant monitoring must be conducted at the same time and location at which TCR samples are taken, as provided for in the Surface Water Treatment Rule (SWTR) (USEPA 1989b, 54 FR 27486, June 29, 1989) and the Stage 1 Disinfectants and Disinfection Byproducts Rule (Stage 1 DBPR) (USEPA 1998a, 63 FR 69389, December 16, 1998). Under the GWR, TCR distribution system monitoring results determine whether a system is required to conduct source water monitoring. Under the SWTR, high measurements of turbidity in an unfiltered subpart H system of this part trigger additional total coliform samples. Sanitary survey provisions exist in surface water and ground water drinking water regulations. The proposed RTCR does not change the existing sanitary survey requirements except to add the special monitoring evaluation that States must conduct at systems serving 4,100 or fewer people. These evaluations do not increase the burden to conduct sanitary surveys because of the relatively simple nature of these systems and their monitoring requirements.

1. SWTR, Stage 1 and Stage 2 DBPRs, ADWR
After considering the possible linkages among the proposed RTCR and the SWTR, Stage 1 DBPR, Stage 2 DBPR (USEPA 2006e, 71 FR 388, January 4, 2006), and Airline Drinking Water Rule (ADWR) (USEPA 2009), EPA has concluded that the only necessary revision is to update the reference to the current TCR at 40 CFR 141.21, which is superseded by 40 CFR part 141 subpart Y beginning three years following publication of the final rule. EPA is also proposing several revisions to other NPDWRs, discussed below, that are not necessary but would facilitate implementation of all applicable NPDWRs.

2. GWR
As with the other drinking water rules mentioned above, EPA is proposing to update the references in the GWR to the current TCR at 40 CFR 141.21, which will be superseded by 40 CFR part 141 subpart Y.

3. Sanitary Surveys
Sanitary survey requirements are not included in the proposed RTCR. Under the current TCR, community water systems and non-community water systems that serve 4,100 or fewer people are required to conduct periodic sanitary surveys. Since the promulgation of the TCR in 1989, new sanitary survey requirements for surface water systems and ground water systems have been established for all system sizes and types under the Interim Enhanced Surface Water Treatment Rule (IESWTR) (USEPA 1998b, 63 FR 69477, December 16, 1998) (40 CFR 142.16(b)(3)), and the Ground Water Rule (GWR) (40 CFR 142.16(o)(2)(i)). Public water systems began implementing the IESWTR sanitary survey requirements in 2001. Therefore, for surface water systems, the current TCR sanitary survey requirements have phased out since that time. Implementation of the GWR sanitary survey requirements began in December 2009 for ground water systems. Therefore, for ground water systems, the GWR sanitary survey requirements will be in effect by the time the RTCR is finalized.

D. Best Available Technology (BAT)

1. Provisions

The proposed RTCR would maintain the provisions set forth in 40 CFR 141.63(d) (proposed to be in § 141.63(e)), regarding the best technology, treatment techniques, or other means available for achieving compliance with the MCL of either total coliforms or E. coli. EPA is proposing the following modifications:

- ✓ 40 CFR 141.63(d)(1) (proposed § 141.63(e)(1)) would be modified by replacing "coliforms" with "fecal contaminants."

- ✓ 40 CFR 141.63(d)(3) (proposed § 141.63(e)(3)) would be modified by including "cross connection control" in the list of proper maintenance practices for the distribution system.

- ✓ 40 CFR 141.63(d)(4) (proposed § 141.63(e)(4)) would be modified by including the subparts P, T, and W that describe filtration and/or disinfection of surface water, and subpart S for disinfection of ground water.

2. EPA's Rationale

a. Change "coliform" to "fecal contaminants." This change reflects the approach of the proposed RTCR that the presence of total coliforms does not necessarily have a direct public health implication. Instead, total coliform is used as an indicator of a potential pathway of contamination within a treatment technique requirement. For additional discussion on this topic, see section III.A.2 of this preamble.

b. Inclusion of cross connection control. EPA believes that adding cross connection control to the list of proper maintenance practices for distribution systems is appropriate because of the significant contribution of cross connections and backflow to waterborne disease outbreaks. From 1981 to 1998, the CDC documented 9, 734 detected and reported illnesses from 57 waterborne outbreaks related to cross connections (NRC 2006). From 1970 to 2001, approximately 12,000 illnesses resulted from 459 incidents of waterborne outbreaks from backflow events (NRC 2006).

c. Addition of other relevant subparts of 141. This change adds references to subparts that contain provisions for the other drinking water rules promulgated since 1989 when the TCR was promulgated (in particular, subpart P for the IESWTR, subpart S for the GWR, subpart T for the Long Term Enhanced Surface Water Treatment Rule (USEPA 2002, 67 FR 1812, January 14, 2002, and subpart W for the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA 2006d, 71 FR 654, January 5, 2006)). These drinking water rules contain updated filtration and disinfection standards that were not part of the current TCR when it was promulgated in 1989.

3. Request for Comment

EPA requests comment on the modifications to the existing BATs and whether there is a need to add or otherwise update the list of BATs.

E. Variances and Exemptions

1. Provisions

EPA is proposing to not allow variances or exemptions to the E. coli MCL. EPA is also proposing to eliminate the variance provisions in 40 CFR 141.4(b) that allow systems to demonstrate to the State that the violation of the monthly/non-acute total coliform MCL is due to biofilm and not fecal or pathogenic contamination. This change will also result in a parallel change in 40 CFR 142.63(b).

2. EPA's Rationale

Under the proposed RTCR, E. coli is used as an indicator of fecal contamination that may contain waterborne pathogens. To the extent a variance or exemption would permit the continued presence of E. coli, the potential for pathogens to be present also would remain. EPA believes that water which exceeds the MCL for E. coli poses an unreasonable risk to public health.

Therefore, EPA is not allowing any variances or exemptions to the E. coli MCL. This provision is consistent with the existing requirement, since the provision that allows variances applies only to the monthly/non-acute total coliform MCL violation and not to the acute violation associated with the presence of E. coli.

Under the current TCR, EPA allows variances to the MCL for total coliforms when a system has demonstrated to the State that the violation of the total coliform MCL is due to a persistent growth of total coliforms in the distribution system (i.e., biofilm) rather than fecal or pathogenic contamination, a treatment lapse or deficiency, or a problem in the operation or maintenance of the distribution system.

EPA is proposing to eliminate the variance in 40 CFR 141.4(b) because under the proposed RTCR, there would no longer be an MCL for total coliforms (see section III.A.2 of this preamble). The current TCR MCL for total coliforms was based on the presence or absence of total coliforms in a sample (see 40 CFR 141.63 for details). In the proposed RTCR, the presence of total coliforms at a certain level requires the system to comply with the coliform treatment technique requirements (see section III.A.5 of this preamble). The assessment and corrective action requirements under this proposed rule include the possibility of recognizing that the total coliform presence is associated with biofilm. EPA plans to include this information in a new assessment and corrective action guidance manual related to the RTCR.

3. Request for Comment

EPA requests comment on its proposal to allow no variance or exemption to the E. coli MCL and to eliminate the variance provisions associated with the monthly/non-acute total coliform MCL.

F. Request for Comment on Other Issues Related to the Proposed RTCR

1. Consistency Between the Proposed RTCR and the GWR

EPA requests comment on the need for general consistency between the proposed RTCR and the GWR. Please provide specific examples. For example, under the current TCR, States are required to keep records of their decision to either waive or extend the 24-hour limit for collecting samples (that is, for repeat samples following a total coliform-positive sample, or for follow-up samples after high levels of turbidity) (see 40 CFR 142.14(a)(5)(i)(A) and 142.14(a)(5)(ii)(D)). The proposed RTCR also requires States to keep records of decisions to either waive or extend the 24-hour limit for repeat samples following a total coliform-positive sample, for samples following invalidation, or for follow-up samples after high levels of turbidity (see§§ 142.14(a)(10)(i)(A) and 142.14(a)(10)(ii)(D) of the proposed RTCR). Under the GWR, there are no recordkeeping requirements for the decision to waive or extend the 24-hour limit. Instead, the GWR includes special primacy requirements to describe criteria the State will use to extend the 24-hour limit (see 40 CFR 142.16(o)(3)(i)). EPA requests comment on whether it is appropriate to have States describe their criteria for waiving or extending the 24-hour limit as a primacy condition, or instead have States keep records of decisions to waive and/or extend the 24-hour limit.

2. Storage Tank Inspection and Cleaning

EPA requests comment on the value and cost of periodic storage tank inspection and cleaning. There are instances of storage tanks being the source of waterborne disease outbreaks at PWSs. In December 1993, a Salmonella typhimurium outbreak in Gideon, Missouri resulted in over 600 people affected by diarrhea, 31 cases of laboratory-confirmed salmonellosis and seven deaths of nursing home residents who had exhibited diarrheal illness (four deaths were confirmed by culture). The larger of the two storage tanks had a breach in the roof hatch that allowed pigeon droppings to be carried into the tank and likely accumulated in the several inches of sediment. This contaminated sediment, more than likely, was pulled into the distribution system by a flushing program that drained the tank (Clark et al. 1996). Salmonella typhimurium was isolated from the sediment of one of the towers, and tap water tested positive for fecal coliforms (CDC 1996).

In March 2008, Alamosa, Colorado (with a population of about 9,000 people) experienced a waterborne disease outbreak associated with Salmonella. The report released by the Colorado Department of Public Health and Environment (Falco and Williams 2009) indicated that the outbreak resulted in 442 reported cases of illnesses, 122 of which were laboratory confirmed, and one fatality. The State epidemiologist estimated that a total of 1,300 people may have been ill. Two storage tanks in Alamosa had several inches of sediment and breaches; one tank had breaches large enough for birds and animals to enter. Some of the key factors that contributed to these two outbreaks include significant levels of sediment (several inches to feet) and the presence of breaches of the integrity of the storage tank.

Sediment accumulation occurs within storage facilities due to quiescent conditions which promote particle setting. Over time sediment continues to accumulate in a tank, even if the finished water is consistently treated to below 0.1 nephelometric turbidity unit (NTU). For surface water systems, it is not uncommon to have1/4to1/2inch or more of sediment accumulate after two to three years (Kirmeyer et al. 1999). While there are no turbidity regulations for ground water systems (except for ground water under the direct influence of surface water (GWUDI)), the levels of turbidity can be significant in the water pumped from an aquifer. Sand particles, if allowed to accumulate, provide pore spaces that house diverse populations of biota (which may include pathogenic microorganisms) (Kirmeyer et al. 1999; van der Kooij 2003). Periodic high flows in the storage tank may scour, stir up, and suspend the sediment (along with entrapped bacteria and pathogens) and carry it into the distribution system, with greater accumulation of sediment being a more significant concern. Other water quality problems associated with sediment accumulation include increased disinfectant demand and disinfection byproduct formation.

The storage tank's vulnerability to contamination increases when breaches of the storage tank allow insects, animals, and birds and their associated diseases to enter. Contamination from bird and other animal excrement can potentially transmit disease-causing organisms to the finished water. Waterfowl, for example, are known carriers of many different waterborne pathogens including Vibrio cholerae (Ogg et al. 1989).

Based on the potential public health implications associated with poorly maintained storage tanks (e.g., as indicated by significant sediment accumulation and breaches), EPA is interested in receiving comments and supporting information regarding the state and condition of tanks that have been cleaned and inspected, costs of storage tank inspection and cleaning, and how public health can be better protected. EPA requests information on whether there are States that recommend or require periodic inspection and cleaning of storage tanks. If so, what are the requirements, the frequency of inspection and cleaning, and how successful are they? Are inspections and cleaning done by individual PWSs or by contractors?

3. States Under EPA Direct Implementation

EPA does not have the authorities provided to other primacy agencies under 40 CFR part 142 to use in implementing rules in direct implementation entities (e.g., Tribal systems and Wyoming). To provide EPA the flexibility of other primacy agencies to modify monitoring requirements as necessary to protect public health (e.g., to require more stringent monitoring or to develop criteria such as those that primacy States develop under the special primacy conditions requirement in 40 CFR 142.16) and facilitate implementation of this rule. EPA is requesting comment on whether the Agency should have the same authorities specified in subpart Y, as States have in 40 CFR 142.16, for PWSs for which the Agency has direct implementation responsibilities. EPA is requesting comment on whether this authority should be added to subpart Y specifically.

G. Limitations to the Public Comment on the Proposed RTCR

The proposed revisions to other drinking water regulations (SWTR, Stage 1 DBPR, Stage 2 DBPR, and ADWR) are made solely to update the reference to the current TCR at 40 CFR 141.21, which will be superseded by 40 CFR part 141 subpart Y beginning three years following publication of the final rule. This proposed rule would not change any substantive requirements of those rules and EPA is not soliciting public comments on those rules other than their proposed

revised references to the current TCR or any other references to the current TCR that EPA may need to revise.
IV.

The proposed RTCR provides States with flexibility to implement the requirements of the rule in a manner that maximizes the efficiency of the rule for the States and water systems while increasing the effectiveness of the rule to protect public health. While the proposed rule provides some reduction in monitoring relative to the current TCR, overall, the proposed rule is more stringent and better protects public health. As a result, States must adopt these revisions, when final, or adopt or maintain more stringent requirements, in order to maintain primacy. This section describes the regulations and other procedures and policies States must adopt in order to obtain primacy to implement the RTCR, if finalized as proposed today.

SDWA section 1413 establishes requirements that States or eligible Indian Tribes must meet to assume and maintain primary enforcement responsibility (primacy) for its PWSs. These requirements include:

- ✓ Adopting drinking water regulations that are no less stringent than Federal drinking water regulations;
- ✓ Adopting and implementing adequate procedures for enforcement;
- ✓ Keeping records and making reports available on activities that EPA requires by regulation;
- ✓ Issuing variances and exemptions (if allowed by the State), under conditions no less stringent than allowed under SDWA; and
- ✓ Adopting and being capable of implementing an adequate plan for the provisions of safe drinking water under emergency situations.

States may adopt more stringent requirements (e.g., requiring all systems to conduct routine monthly monitoring). Many States have used this authority in the past to improve public health protection and/or simplify implementation.

Section 1413(a)(1) of SDWA provides two years (plus up to two more years if the Administrator approves) after promulgation of the final RTCR for the State to adopt corresponding drinking water regulations in order to obtain primacy for the final RTCR. To implement the final RTCR, States would be required to adopt or maintain requirements that are at least as stringent as the following revisions to 41 CFR part 141:

- ➢ Section 141.4—Variances and exemptions (if allowed by the State).
- ➢ Section 141.21—Coliform sampling.
- ➢ Section 141.52—Maximum contaminant level goals for microbiological contaminants.
- ➢ Section 141.63—Maximum contaminant levels (MCLs) for microbiological contaminants.
- ➢ Section 141.74—Analytical and monitoring requirements.
- ➢ Section 141.132—Monitoring requirements.
- ➢ Subpart 141.153—Content of the reports.
- ➢ Subpart 141.202—Tier 1 Public Notice—Form, manner, and frequency of notice.

- Subpart 141.203—Tier 2 Public Notice—Form, manner, and frequency of notice.
- Subpart 141.204—Tier 3 Public Notice—Form, manner, and frequency of notice.
- Subpart O—Consumer Confidence Reports, Appendix A, Regulated Contaminants.
- Subpart Q—Public Notification of Drinking Water Violations, Appendix A, NPDWR Violations and Other Situations.
- Subpart Q—Public Notification of Drinking Water Violations, Appendix B, NPDWR Violations and Other Situations.
- Subpart Y—Revised Total Coliform Rule.

EPA's regulation at 40 CFR part 142 sets out the specific program implementation requirements for States to obtain primacy for the public water supply supervision program as authorized under SDWA section 1413. In addition to adopting basic primacy requirements specified in 40 CFR part 142, States may be required to adopt special primacy provisions pertaining to specific regulations where implementation of the rule involves activities beyond general primacy provisions. States must include these regulation-specific provisions in their application for approval of their program revision. States must continue to meet all other conditions of primacy for all other rules in 40 CFR part 142. Primacy requirements for the proposed RTCR are described below.

The advisory committee recognized that this rule will require more tracking to ensure effective implementation. Therefore, EPA plans to release an upgrade to SDWIS/State and SDWIS/FED (the State and Federal versions of the Safe Drinking Water Information System, respectively) within 18 months of final rule promulgation to accommodate monitoring data, tracking, compliance determinations and reporting of all rule related requirements, as appropriate.

A. State Special Primacy Requirements

To ensure that a State program includes all the elements necessary for an effective and enforceable program under the proposed RTCR, a State primacy application must include a description of how the State will perform the following:

- ✓ Sample Siting Plans—States must describe the frequency and process used to review and revise sample siting plans in accordance with 40 CFR 141, subpart Y to determine adequacy.
- ✓ Reduced Monitoring Criteria—The primacy application must indicate whether the State will adopt the reduced monitoring provisions of subpart Y. If the State adopts the reduced monitoring provisions, it must describe the specific types or categories of water systems that will be covered by reduced monitoring and whether the State will use all or a reduced set of the optional criteria. For each of the reduced monitoring criteria, both mandatory and optional, the State must describe how the criteria will be evaluated to determine when systems qualify.
- ✓ Assessments and Corrective Actions—States must describe their process to implement the new assessment and corrective action phase of the rule. The description must include examples of sanitary defects, examples of assessment forms or formats, and methods that systems may use to consult with the State on appropriate corrective actions.
- ✓ Invalidation of routine and repeat samples collected under subpart Y—States must describe their criteria and process to invalidate total coliform-positive and E. coli- positive samples under subpart Y. This includes criteria to determine if a sample was improperly processed by the laboratory, reflects a domestic or other non-distribution system plumbing problem or reflects circumstances or condition that does not reflect water quality in the distribution system.

- ✓ Approval of individuals allowed to conduct subpart Y Level 2 assessments—States must describe their criteria and process for approval of individuals allowed to conduct subpart Y Level 2 assessments.
- ✓ Special monitoring evaluation—States must describe how they will perform special monitoring evaluations during sanitary surveys for ground water systems serving 1,000 or fewer people to determine whether systems are on an appropriate monitoring schedule.
- ✓ Seasonal systems—States must describe how they will identify seasonal systems, how they will determine when systems on less than monthly monitoring must monitor, and what will be the seasonal system start-up provisions.
- ✓ Additional criteria for reduced monitoring—States must describe how they will require systems on reduced monitoring to demonstrate:
  —Continuous disinfection entering the distribution system and a residual in the distribution system;
  —Cross connection control;
  —Other enhancements to water system barriers; and
  —Procedures for seasonal systems to start up operations at the beginning of each season.

B. State Recordkeeping Requirements

The current regulations in 40 CFR 142.14 require States with primacy to keep records, including: Analytical results to determine compliance with MCLs, MRDLs, and treatment technique requirements; PWS inventories; State approvals; enforcement actions; and the issuance of variances and exemptions. The proposed RTCR requires States to keep additional records, including all supporting information and an explanation of the technical basis for each decision as follows. Records of the following decisions or activities must be retained for five years, consistent with recordkeeping requirements for existing regulations:

- ✓ Any decision to waive the 24-hour time limit for collecting repeat samples after a total coliform-positive routine sample, or to extend the 24-hour limit for collection of samples following invalidation, or for an unfiltered subpart H system of this part to collect a total coliform sample following a turbidity measurement exceeding 1 NTU.
- ✓ Any decision to allow a system to waive the requirement for three routine samples the month following a total coliform-positive sample. The record of the waiver decision must contain all the items listed in §§ 141.854(j) and 141.855(f) of the proposed RTCR.
- ✓ Any decision to invalidate a total coliform-positive sample. If the State decides to invalidate a total coliform-positive sample as provided in § 141.853(c)(1) of the proposed RTCR, the record of the decision must contain all the items listed in that paragraph.
- ✓ Completed and approved 40 CFR part 141 subpart Y assessments, including reports from the system that corrective action has been completed.

States must retain records of each of the following decisions in such a manner so that each system's current status may be determined at any time:

- ➢ Any decision to reduce the total coliform monitoring frequency for a community water system serving 1,000 or fewer people to less than once per month, as provided in § 141.855(d) of the proposed RTCR; and what the reduced monitoring frequency is. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to reduce the total coliform monitoring frequency for a non-community water system using only ground water and serving 1,000 or fewer people to less than once per quarter, as provided in § 141.854(e) of the proposed RTCR, and what the reduced monitoring frequency is. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to reduce the total coliform monitoring frequency for a non-community water system using only ground water and serving more than 1,000 persons during any month the system serves 1,000 or fewer people, as provided in § 141.857(d) of the proposed RTCR. A copy of the reduced monitoring frequency must be provided to the system.
- ➢ Any decision to waive the 24-hour limit for taking a total coliform sample for a public water system that uses surface water, or ground water under the direct influence of

surface water, and that does not practice filtration in accordance with part 141, subparts H, P, T, and W, and that measures a source water turbidity level exceeding 1 NTU near the first service connection.

- Any decision to allow a public water system to forgo E. coli testing on a total coliform-positive sample if that system assumes that the total coliform-positive sample is E. coli-positive.

C. State Reporting Requirements

EPA currently requires at 40 CFR 142.15 that States report to EPA information such as violations, variance and exemption status, and enforcement actions. The proposed RTCR requires States to develop and maintain a list of public water systems that the State is allowing to monitor less frequently than once per month for community water systems or less frequently than once per quarter for non-community water systems, including the compliance date (the date that reduced monitoring was approved) of the reduced monitoring requirement for each system.

D. Interim Primacy

On April 28, 1998, EPA amended its State primacy regulations at 40 CFR 142.12 to incorporate the new process identified in the 1996 SDWA Amendments for granting primary enforcement authority to States while their applications to modify their primacy programs are under review (USEPA 1998c, 63 FR 23361, April 28, 1998). The new process grants interim primary enforcement authority for a new or revised regulation during the period in which EPA is making a determination with regard to primacy for that new or revised regulation. This interim enforcement authority begins on the date of the primacy application submission or the effective date of the new or revised State regulation, whichever is later, and ends when EPA makes a final determination. However, this interim primacy authority is only available to a State that has primacy (including interim primacy) for every existing NPDWR in effect when the new regulation is promulgated.

As a result, States that have primacy (including interim primacy) for every existing NPDWR already in effect may obtain interim primacy for the RTCR, beginning on the date that the State submits the application for this rule to EPA, or the effective date of its revised regulations, whichever is later. A State that wishes to obtain interim primacy for future NPDWRs must obtain primacy for this rule.

E. Request for Comment

EPA requests comment on the adequacy of the proposed RTCR requirements for State implementation, including but not limited to State special primacy requirements and State reporting and recordkeeping requirements. Specifically, EPA requests comment on whether there are any requirements that should be added to assure proper State oversight, or any that can be removed without detriment to implementation of the rule.

V. Distribution System Research and Information Collection Activities

A. Research and Information Collection Partnership

The advisory committee recommended that a Research and Information Collection Partnership (RICP) be formed to inform and support the drinking water community in developing future national risk management decisions pertaining to drinking water distribution systems. The advisory committee recommended seven priority areas for research and information collection. These seven priority areas are: (1) Cross-connection and backflow of contaminated water; (2) contamination due to storage facility design, operation, or maintenance; (3) contamination due to main installation, repair, or rehabilitation practices; (4) contaminant intrusion due to pressure conditions and physical gaps in distribution system infrastructure; (5) significance and control of biofilm and microbial growth; (6) nitrification issues that lead to public health effects; and (7) accumulation and release of contaminants from distribution system scales and sediments (USEPA 2008c, AIP p. 30).

In January 2009, EPA and the Water Research Foundation (WRF or the Foundation) signed a memorandum of understanding (MOU) to form the RICP in response to recommendations from the advisory committee contained in the AIP (USEPA and WRF 2009). The MOU conveys the partners' agreement to collaborate and identify, define, prioritize, coordinate, and communicate critical decision-relevant distribution system research and information collection needs of the drinking water community. The RICP is directed by a steering committee comprised of nine members: Three members from EPA, three members from water utilities, and three additional members representing the public health, environmental advocate, and State regulator perspectives.

B. Distribution System Optimization Activities

As part of the AIP, the advisory committee encouraged "the development of national and regional distribution system optimization partnerships that focus on protecting the integrity of drinking water quality once it is delivered to the distribution system. The purpose of the partnerships should be to inform and inspire proactive systems to implement best management practices that emphasize protection of public health. These partnerships, comprised, for example, of representatives from utilities, communities, academia, and regulatory organizations could develop continuous improvement programs that encompass water distribution optimization principles and practices for system design, operations, and maintenance. These partnerships should foster continuous review of distribution system issues and should define excellence in distribution system operation in terms of processes, systems, procedures, as well as measures. The optimization partnerships should encourage voluntary program participation of all drinking water utilities regardless of system size" (USEPA 2008c, AIP p. 25).

EPA is aware of two distribution system optimization programs that are currently being developed. EPA and the Foundation are concurrently developing distribution system optimization programs that focus on protecting public health in the distribution system. Developmental activities to support these efforts are occurring through the EPA's National Area Wide Optimization Program (AWOP) and the Foundation's project #4109. While these programs are being developed independently with differing measures of performance, both are founded on the optimization principles of improving water systems, and go beyond the regulatory requirements, while using existing staff and facilities. These principles and practices are currently being used through the in-plant treatment optimization programs operated through AWOP and the American Water Works Association's (AWWA) Partnership for Safe Water (the Partnership).

The goal of EPA's optimization program is to protect public health by addressing both the technical and management issues that limit the water system's ability to meet water quality performance goals. EPA has started developing a distribution system optimization program, which is currently focused on improving water treatment plant finished water quality while maintaining disinfectant residual and minimizing disinfection byproduct formation in the distribution system. Future work may focus on other water quality parameters or issues of concern. An outcome of this effort will be the identification of the key technical and management skills, practices, and tools that a water system should implement to achieve long-term distribution system optimization. Ultimately, participating AWOP States will be introduced to distribution system optimization methods developed by EPA. At this time, additional development activities are needed before a distribution system optimization program will be available for State implementation.

In 2007, the Foundation initiated project #4109 to identify a limited number of straightforward criteria that can be used by water utilities to measure distribution system optimization performance and to develop a self-assessment approach using standards of excellence. The results from this project will also be used to expand the Partnership for Safe Water Program treatment plant optimization program into distribution system optimization. The Foundation anticipates project #4109 to be completed by early 2010. With the results of project #4109, the Partnership anticipates finalizing a preliminary set of optimization goals and a model assessment process in calendar year 2010. Prior to finalizing the goals and assessment process, the

Partnership will conduct trials at several volunteer utilities. The optimization goals and assessment process will be evaluated and refined based on those trials prior to consideration by the Partnership for adoption and implementation.

C. Request for Comment
EPA requests comment about these distribution system optimization projects and information about or suggestions for other possible approaches to distribution system optimization.

VI. Economic Analysis (Health Risk Reduction and Cost Analysis)
This section summarizes the Health Risk Reduction and Cost Analysis (HRRCA) in support of the proposed RTCR as required by section 1412(b)(3)(C) of the SDWA. EPA has prepared the RTCR Economic Analysis (EA) (USEPA 2010a) to comply with this requirement. The EA document for the proposed RTCR is available in the docket and is also published on the government's Web site at http://www.regulations.gov.

The HRRCA consists of seven elements:
(1) quantifiable and nonquantifiable health risk reduction benefits;
(2) quantifiable and nonquantifiable health risk reduction benefits from reductions in co-occurring contaminants;
(3) quantifiable and nonquantifiable costs that are likely to occur solely as a result of compliance;
(4) incremental costs and benefits of rule options;
(5) effects of the contaminant on the general population and sensitive subpopulations including infants, children, pregnant women, elderly, and individuals with a history of serious illness;
(6) any increased health risks that may occur as a result of compliance, including risks associated with co-occurring contaminants; and
(7) other relevant factors such as uncertainties in the analysis and factors with respect to the degree and nature of risk. See SDWA section 1412(b)(3)(C). A summary of these elements is provided in this section of the preamble, and a complete discussion can be found in the Proposed RTCR EA (USEPA 2010a).

The benefits described in this section are discussed qualitatively, and reductions in detection of total coliforms and E. coli and in Level 2 assessments are used to describe the benefits, as described later in this section. The costs discussed in this section are presented as annualized present values in 2007 dollars. Both benefit and cost measures are adjusted using social discounting. In social discounting, future values of a rule's or policy's effects are multiplied by discount factors. The discount factors reflect both the amount of time between the present and the point at which these events occur and the degree to which current consumption is more highly valued than future consumption (USEPA 2000c). This process allows comparison of cost and benefit streams that are variable over a given time period. EPA uses social discount rates of both three percent and seven percent to calculate present values from the stream of benefits and costs and also to annualize the present value estimates. Historically, the use of three percent is based on rates of return on relatively risk-free financial instruments, while seven percent is generally an estimate of before-tax rate of return to incremental private investment. For further information, see USEPA 2000c and OMB 1996.

In the Proposed RTCR EA (USEPA 2010a), EPA also presents the undiscounted stream of benefits and costs over the 25-year time frame (i.e., the year-to-year realization of benefits and costs presented in constant terms).

The time frame used for both benefit and cost comparisons in this rule is 25 years. This time interval accounts for rule implementation activities occurring soon after promulgation (e.g., States adopting the criteria of the regulation) and the time for different types of compliance actions (e.g., assessments and corrective actions) to be realized up through the 25th year following rule promulgation.

EPA was unable to quantify health benefits for the proposed RTCR because there are insufficient data reporting the co-occurrence in a single sample of fecal indicator E. coli and pathogenic organisms. In addition, the available fecal indicator E. coli data from the Six-Year Review 2 dataset (USEPA 2010e) described in this preamble were limited to presence-absence data because the current TCR requires only the reporting of presence or absence of fecal indicator E. coli using EPA-approved standard methods.

However, as discussed in chapter 6 of the Proposed RTCR EA (USEPA 2010a), even though health benefits could not be directly quantified, the potential benefits from the proposed RTCR include avoidance of a full range of health effects from the consumption of fecally contaminated drinking water, including the following: acute and chronic illness, endemic and epidemic disease, waterborne disease outbreaks, and death. Also, since fecal contamination may contain waterborne pathogens including bacteria, viruses, and parasitic protozoa, in general, a reduction in fecal contamination should reduce the risk from these other contaminants.

The net costs of the rule stem mostly from the new assessment and corrective action requirements as well as the revised monitoring provisions described earlier in this preamble.

This section of the preamble includes elements as follows: (A) Regulatory Options Considered, (B) Major Sources of Data and Information used in Supporting Analyses, (C) Occurrence and Predictive Modeling, (D) Baseline Profiles, (E) Anticipated Benefits of the Proposed RTCR, (F) Anticipated Costs of the Proposed RTCR, (G) Potential Impact of the Proposed RTCR on Households, (H) Incremental Costs and Benefits, (I) Benefits from Simultaneous Reduction of Co-occurring Contaminants, (J) Change in Risk from Other Contaminants, (K) Effects of Fecal Contamination and/or Waterborne Pathogens on the General Population and Sensitive Subpopulations, (L) Uncertainties in the Benefit and Cost Estimates for the Proposed RTCR, (M) Benefit Cost Determination for the Proposed RTCR, and (N) Request for Comment.

A. Regulatory Options Considered

EPA evaluated the following three regulatory options as part of this revised rule proposal: (1) The current TCR option, (2) the AIP option, and (3) an Alternative option. EPA discusses the three regulatory options briefly in this preamble and in greater detail in the Proposed RTCR EA (USEPA 2010a).

First, the current TCR option reflects EPA's understanding of how the current TCR (USEPA 1989a, 54 FR 27544, June 29, 1989) is currently being implemented. That is, the current TCR option is assumed to include “status quo” PWS and State implementation practices. Next, the AIP option is a revised TCR based on the recommendations of the advisory committee. The provisions of this proposed rule are based on the AIP option and are described in detail in section III of this preamble. Third, the Alternative option parallels the AIP in most ways but includes variations of some of the provisions that were discussed by the advisory committee before consensus was reached on the AIP.

The Alternative option differs from the AIP option in two ways. First, under the Alternative option, at the compliance date all PWSs are required to sample monthly for an initial period until they meet the eligibility criteria for reduced monitoring. EPA assumes that eligibility for reduced monitoring is determined during the next sanitary survey following the RTCR compliance date.
This more stringent approach differs from the AIP option that allows PWSs to continue to monitor at their current frequencies (with an additional annual site visit or voluntary Level 2 assessment requirement for PWSs wishing to remain on annual monitoring) until they are triggered into an increased sampling frequency. Second, under the Alternative option, no PWSs are allowed to reduce monitoring to an annual basis. EPA defined the Alternative option this way and included it in the Proposed RTCR EA (USEPA 2010a) to assess the relative impacts of a more stringent rule and to better understand the balance between costs and public health protection.

To understand the relative impacts of the options, EPA gathered available data and information to develop and provide input into an occurrence and predictive model. EPA estimated both baseline conditions and changes to these conditions anticipated to occur over time as a result of these revised rule options. The analysis is described in more detail in the Proposed RTCR EA (USEPA 2010a).

B. Major Sources of Data and Information Used in Supporting Analyses

This section of the preamble briefly discusses the data sources that EPA used in its supporting analyses for the proposed RTCR. For a more detailed discussion, see chapter 4 of the Proposed RTCR EA (USEPA 2010a).

1. Safe Drinking Water Information System Federal Version Data

Safe Drinking Water Information System Federal Version (SDWIS/FED) is EPA's national regulatory compliance database for the drinking water program and is the main source of PWS inventory and violation data for the proposed RTCR baseline. SDWIS/FED contains information on each of the approximately 155,000 active PWSs as reported by primacy agencies, EPA Regions, and EPA headquarters personnel. SDWIS/FED includes records of MCL violations and monitoring and reporting (MR) violations (both routine and repeat and minor and major). It does not include sample results. It also contains information to characterize the US inventory of PWSs including system name and location, retail population served, source water type (ground water (GW), surface water (SW), or ground water under the direct influence of surface water (GWUDI)), disinfection status, and PWS type (community water system (CWS), transient non-community water system (TNCWS), and non-transient non-community water system (NTNCWS)).

To create the PWS and population baseline, EPA used the fourth quarter of SDWIS/FED 2007 (USEPA 2007b), which was the most current PWS inventory data available when EPA began developing the Proposed RTCR EA. These data represent all current, active PWSs and the population served by these systems.

EPA also used the MCL violation data from SDWIS/FED to validate model predictions for systems serving 4,100 or fewer people and to predict E. coli (acute)_-MCL violations (current TCR, AIP, and Alternative option), total coliform (non-acute or monthly) MCL violations (current TCR), and Level 1 and Level 2 assessment triggers (AIP and Alternative option) for systems serving more than 4,100 people.

2. Six-Year Review 2 Data

Through an Information Collection Request (USEPA 2006b), States voluntarily submitted electronically available TCR monitoring data (sample results) that were collected between January 1998 and December 2005. EPA requested the TCR monitoring results with the intent of conducting analyses and developing models to assess the potential impacts of changes to the current TCR. EPA received data from 46 States, Tribes, and territories. A Data Quality Report (USEPA 2010c) describes how TCR monitoring data were obtained, evaluated, and modified where necessary to make the database internally consistent and usable for analysis. Exhibit 2.1 in the Data Quality Report provides a complete list of States or territories that submitted data and a description of the use of these data.

In this EA, EPA included data from 37 primacy agencies (35 States and 2 Tribes). Records included data for:

- PWS information (system type, population served, source water type)
- Sample type (routine, repeat, special purpose)
- Analytical result
- Sampling location—entry point, distribution system and, for repeat samples, original location, downstream, upstream, and other
- Analytical method
- Disinfectant residual data collected at TCR monitoring sites

As discussed in greater detail in section 4.2.2.1 of the Proposed RTCR EA (USEPA 2010a), EPA used 2005 data exclusively in the analyses supporting the proposed RTCR because the 2005 data set was the most complete year of data among the Six-Year Review 2 data (USEPA 2010e). The 2005 data was also the most recent data available suggesting that it may be the most representative of present conditions.

The Six-Year Review 2 data (USEPA 2010e) also informed EPA's assumptions regarding the proportions of GWSs serving 1,000 or fewer people that sample monthly, quarterly, or annually.

Disinfection Byproduct Regulations

Drinking water chlorination has contributed to a dramatic decline in waterborne disease rates and increased life expectancy in the United States. Largely because of this success, many Americans take it for granted that their tap water will be free of disease-causing organisms. In recent years, regulators and the general public have focused greater attention on potential health risks from chemical contaminants in drinking water. One such concern relates to disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with certain organic matter in water.

In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. Concerned that these chemicals may be carcinogenic to humans, EPA set the first regulatory limits for THMs in 1979. Since that time, a wealth of research has improved our understanding of how DBPs are formed, their potential health risks, and how they can be controlled. It is now recognized that all chemical disinfectants form some potentially harmful byproducts. The byproducts of chlorine disinfection are by far the most thoroughly studied.

While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formation are available and should be adopted where possible. However, the International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environment Programme, the International Labor Organization, and the World Health Organization (IPCS 2000, p. 13) strongly cautions:

The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts.

Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic matter prior to disinfection, while ensuring that microbial protection remains the top priority. DBPs and Human Cancer Risk Toxicology studies have reported that high doses of some DBPs, including THMs and haloacetic acids (HAAs), can cause cancer in laboratory animals. Based largely on these animal data, EPA considers individual THMs and HAAs to be either possible or probable human carcinogens, although any risk from the low levels found in drinking water would be slight. After reviewing the full body of toxicology studies, the IPCS concluded, “None of the chlorination byproducts studied to date is a potent carcinogen at concentrations normally found in drinking water” (IPCS 2000, p. 376).

Some epidemiology studies have reported an association between human exposure to DBPs and elevated cancer risks, while other studies have found no association. EPA evaluated the existing cancer epidemiology studies and found that only for bladder cancer were associations with chlorinated water somewhat consistent. Even in these studies, cancer risks were not strongly correlated to measured THM levels, indicating that other factors cannot be ruled out (Craun et al., 2001). EPA has concluded, “The present epidemiologic data do not support a causal relationship between exposure to chlorinated drinking water and development of cancer at this time” (EPA 1998). The IPCS reached a similar conclusion in 2000, noting that a causal relationship between DBPs and increased cancer remains an open question (IPCS 2000).

Balancing DBP and Microbial Risks

Continuing evidence of waterborne disease occurrence suggests that microbial risks should receive a much higher level of attention than disinfection byproducts. For this reason, The American Academy of Microbiology (Ford and Colwell, 1996) has recommended, the health risks posed by microbial pathogens should be placed as the highest priority in water treatment to

protect public health. A report published by the International Society of Regulatory Toxicology and Pharmacology (Coulston and Kolbye, 1994) stated "The reduction in mortality due to waterborne infectious diseases, attributed largely to chlorination of potable water supplies, appears to outweigh any theoretical cancer risks (which may be as low as zero) posed by the minute quantities of chlorinated organic chemicals reported in drinking waters disinfected with chlorine."

The IPCS (IPCS 2000, p. 375) reached similar conclusions:
Disinfection is unquestionably the most important step in the treatment of water for drinking water supplies. The microbial quality of drinking water should not be compromised because of concern over the potential long-term effects of disinfectants and DBPs. The risk of illness and death resulting from exposure to pathogens in drinking water is very much greater than the risks from disinfectants and DBPs.

Controlling Disinfection Byproducts
Treatment techniques are available that provide water suppliers the opportunity to maximize potable water safety and quality while minimizing the risk of DBP risks. Generally, the best approach to reduce DBP formation is to remove natural organic matter precursors prior to disinfection. EPA has published a guidance document for water system operators entitled, Controlling Disinfection byproducts and Microbial Contaminants in Drinking Water (EPA, 2001).

The EPA guidance discusses three processes to effectively remove natural organic matter prior to disinfection:

1. Coagulation and Clarification
Most treatment plants optimize their coagulation process for turbidity (particle) removal. However, coagulation processes can also be optimized for natural organic matter removal with higher doses of inorganic coagulants (such as alum or iron salts), and optimization of pH.

2. Absorption
Activated carbon can be used to absorb soluble organics that react with disinfectants to form byproducts.

3. Membrane Technology
Membranes, used historically to desalinate brackish waters, have also demonstrated excellent removal of natural organic matter. Membrane processes use hydraulic pressure to force water through a semi-permeable membrane that rejects most contaminants. Variations of this technology include reverse osmosis (RO), nanofilitration (low pressure RO), and microfiltration (comparable to conventional sand filtration).

Other conventional methods of reducing DBP formation include changing the point of chlorination and using chloramines for residual disinfection. EPA predicts that most water systems will be able to achieve compliance with new DBP regulations through the use of one or more of these relatively low cost methods (EPA, 1998).

Water system managers may also consider switching from chlorine to alternative disinfectants to reduce formation of THMs and HAAs. However, all chemical disinfectants form some DBPs. Much less is known about the byproducts of these alternatives than is known about chlorination byproducts. Furthermore, each disinfection method has other distinct advantages and disadvantages.

Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration IMS/FA

1.0 Scope and Application

1.1 This method is for determination of the identity and concentration of *Cryptosporidium* (CAS Registry number 137259-50-8) and *Giardia* (CAS Registry number 137259-49-5) in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. *Cryptosporidium* and *Giardia* may be confirmed using 4',6-diamidino-2-phenylindole (DAPI) staining and differential interference contrast (DIC) microscopy. The method has been validated in surface water, but may be used in other waters, provided the laboratory demonstrates that the method's performance acceptance criteria are met.

1.2 This method is designed to meet the survey and monitoring requirements of the U.S. Environmental Protection Agency (EPA). It is based on laboratory testing of recommendations by a panel of experts convened by EPA. The panel was charged with recommending an improved protocol for recovery and detection of protozoa that could be tested and implemented with minimal additional research.

1.3 This method will not identify the species of *Cryptosporidium* or *Giardia* or the host species of origin, nor can it determine the viability or infectivity of detected oocysts and cysts.

1.4 This method is for use only by persons experienced in the determination of *Cryptosporidium* and *Giardia* by filtration, IMS, and FA. Experienced persons are defined in Section 22.2 as analysts. Laboratories unfamiliar with analyses of environmental samples by the techniques in this method should gain experience using water filtration techniques, IMS, fluorescent antibody staining with monoclonal antibodies, and microscopic examination of biological particulates using bright-field and DIC microscopy.

1.5 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 *CFR* Part 141.27.

2.0 Summary of Method

2.1 A water sample is filtered and the oocysts, cysts, and extraneous materials are retained on the filter. Although EPA has only validated the method using laboratory filtration of bulk water samples shipped from the field, field-filtration also can be used.

2.2 Elution and separation

2.2.1 Materials on the filter are eluted and the eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated.

2.2.2 The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to anti-*Cryptosporidium* and anti-*Giardia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts.

2.3 Enumeration

2.3.1 The oocysts and cysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy.

2.3.2 Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of Cryptosporidium oocysts or Giardia cysts. Potential oocysts or cysts are confirmed through DAPI staining characteristics and DIC microscopy. Oocysts and cysts are identified when the size, shape, color, and morphology agree with specified criteria and examples in a photographic library.

2.3.3 Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

2.4 Quality is assured through reproducible calibration and testing of the filtration, immunomagnetic separation (IMS), staining, and microscopy systems. Detailed information on these tests is provided in Section 9.0.

3.0 Definitions

3.1 *Cryptosporidium* is defined as a protozoan parasite potentially found in water and other media. The six species of *Cryptosporidium* and their potential hosts are *C. parvum* (mammals, including humans); *C. baileyi* and *C. meleagridis* (birds); *C. muris* (rodents); *C. serpentis* (reptiles); and *C. nasorum* (fish).

3.2 *Giardia* is defined as a protozoan parasite potentially found in water and other media. The two species of *Giardia* and their potential hosts are *G. intestinalis* (humans) and *G. muris* (mice).

3.3 Definitions for other terms used in this method are given in the glossary (Section 22.0).

4.0 Contamination, Interferences, and Organism Degradation

4.1 Turbidity caused by inorganic and organic debris can interfere with the concentration, separation, and examination of the sample for *Cryptosporidium* oocysts and *Giardia* cysts. In addition to naturally-occurring debris, such as clays and algae, chemicals, such as iron and alum coagulants and polymers, may be added to finished waters during the treatment process, which may result in additional interference.

4.2 Organisms and debris that autofluoresce or demonstrate non-specific fluorescence, such as algal and yeast cells, when examined by epifluorescent microscopy, may interfere with the detection of oocysts and cysts and contribute to false positives by immunofluorescence assay (FA).

4.3 Solvents, reagents, labware, and other sample-processing hardware may yield artifacts that may cause misinterpretation of microscopic examinations for oocysts and cysts. All materials used shall be demonstrated to be free from interferences under the conditions of analysis by running a method blank (negative control sample) initially and a minimum of every week or after changes in source of reagent water. Specific selection of reagents and purification of solvents and other materials may be required.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the water being sampled. Experience suggests that high levels of algae, bacteria, and other protozoa can interfere in the identification of oocysts and cysts (Reference 20.1).

4.5 Freezing samples, filters, eluates, concentrates, or slides may interfere with the detection and/or identification of oocysts and cysts.

4.6 All equipment should be cleaned according to manufacturers' instructions. Disposable supplies should be used wherever possible.

5.0 Safety

5.1 The biohazard associated with, and the risk of infection from, oocysts and cysts is high in this method because live organisms are handled. This method does not purport to address all of the safety problems associated with its use. It is the responsibility of the laboratory to establish appropriate safety and health practices prior to use of this method. In particular, laboratory staff must know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms while preparing, using, and disposing of sample concentrates, reagents and materials, and while operating sterilization equipment.

5.2 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration regulations regarding the safe handling of the chemicals specified in this method. A reference file of Safety Data Sheet (formerly MSDS)s should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 20.2 through 20.5.

5.3 Samples may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves and opened in a biological safety cabinet to prevent exposure. Reference materials and standards containing oocysts and cysts must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Do not mouth-pipette.

5.4 Laboratory personnel must change gloves after handling filters and other contaminant-prone equipment and reagents. Gloves must be removed or changed before touching any other laboratory surfaces or equipment.
5.5 Centers for Disease Control (CDC) regulations (42 CFR 72) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. Unless the sample is known or suspected to contain *Cryptosporidium*, *Giardia*, or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by CDC or state regulations, the sample should be shipped in accordance with these regulations.

6.0 Equipment and Supplies

NOTE*: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection equipment for shipment of bulk water samples for laboratory filtration. Collapsible LDPE cubitainer for collection of 10-L bulk sample(s)—Cole Parmer cat. no. U-06100-30 or equivalent. Fill completely to ensure collection of a full 10-L sample. Discard after one use.
6.2 Equipment for sample filtration. Three options have been demonstrated to be acceptable for use with Method 1623. Other options may be used if their acceptability is demonstrated according to the procedures outlined in Section 9.1.2.
6.2.1 Cubitainer spigot to facilitate laboratory filtration of sample (for use with any filtration option)—Cole Parmer cat. no. U-06061-01, or equivalent.
6.2.2 Envirochek™ sampling capsule equipment requirements for use with the procedure described in Section 12.0. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

6.2.2.1 Sampling capsule—Envirochek™, Pall Gelman Laboratory, Ann Arbor, MI, product 12110
6.2.2.2 Laboratory shaker with arms for agitation of sampling capsules
6.2.2.2.1 Laboratory shaker—Lab-Line model 3589, VWR Scientific cat. no. 57039-055, Fisher cat. no. 14260-11, or equivalent
6.2.2.2.2 Side arms for laboratory shaker—Lab-Line Model 3587-4, VWR Scientific cat. no. 57039-045, Fisher cat. no. 14260-13, or equivalent
6.2.3 CrypTest™ capsule filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).
6.2.3.1 Capsule filter—CrypTest™, Whatman Inc, Clifton, NJ, product no. 610064
6.2.3.2 Cartridge housing—Ametek 5-in. clear polycarbonate, Whatman cat. no. 71503, or equivalent
6.2.3.3 Ultrasonic bath—VWR Model 75T#21811-808, or equivalent
6.2.3.4 Laboratory tubing—Tygon formula R-3603, or equivalent

6.2.4 Filta-Max™ foam filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 50-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.4.1 Foam filter—Filta-Max™, IDEXX, Westbrook, ME. Filter module and membrane: product code FMC 10601; filter membranes (100 pack), product code FMC 10800

NOTE: Check at least one filter per batch to ensure that the filters have not been affected by improper storage or other factors that could result in brittleness or other problems. At a minimum confirm that the test filter expands properly in water before using the batch or shipping filters to the field.

6.2.4.2 Filter processing equipment—Filta-Max starter kit, IDEXX, Westbrook, ME, cat. no. FMC 11002. Includes all equipment required to run and process Filta-Max filter modules (manual wash station (FMC 10102) including plunger head (FMC 12001), elution tubing set (FMC 10301), vacuum set (FMC 10401), filter housing (FMC 10501), and magnetic stirrer (FMC 10901).

6.3 Ancillary sampling equipment

6.3.1 Tubing—Glass, polytetrafluoroethylene (PTFE), high-density polyethylene (HDPE), or other tubing to which oocysts and cysts will not easily adhere—Tygon formula R-3603, or equivalent. If rigid tubing (glass, PTFE, HDPE) is used and the sampling system uses a peristaltic pump, a minimum length of compressible tubing may be used in the pump. Before use, the tubing must be autoclaved, thoroughly rinsed with detergent solution, followed by repeated rinsing with reagent water to minimize sample contamination. Alternately, decontaminate using hypochlorite solution, sodium thiosulfate, and multiple reagent water rinses; dispose of tubing when wear is evident. Dispose of tubing after one use whenever possible.

6.3.2 Flow control valve—0.5 gpm (0.03 L/s), Bertram Controls, Plast-O-Matic cat. no. FC050B½-PV, or equivalent; or 0.4- to 4-Lpm flow meter with valve—Alamo Water Treatment, San Antonio, TX, cat. no. R5310, or equivalent.

6.3.3 Centrifugal pump—Grainger, Springfield, VA, cat. no. 2P613, or equivalent

6.3.4 Flow meter—Sameco cold water totalizer, E. Clark and Associates, Northboro, MA, product no. WFU 10.110, or equivalent.

6.4 Equipment for spiking samples in the laboratory

6.4.1 10-L carboy with bottom delivery port (½")—Cole-Palmer cat. no. 06080-42, or equivalent; calibrate to 10.0 L and mark level with waterproof marker.

6.4.2 Stir bar—Fisher cat. no. 14-511-93, or equivalent.

6.4.3 Stir plate—Fisher cat. no. 14-493-120S, or equivalent.

6.4.4 Hemacytometer—Neubauer type, Hauser Scientific, Horsham, PA, cat. no. 3200 or 1475, or equivalent.

6.4.5 Hemacytometer coverslip—Hauser Scientific, cat. no. 5000 (for hemacytometer cat. no. 3200) or 1461 (for hemacytometer cat. no 1475), or equivalent.

6.4.6 Lens paper without silicone—Fisher cat. no. 11-995, or equivalent.

6.4.7 Polystyrene or polypropylene conical tubes with screw caps—15- and 50-mL.

6.4.8 Equipment required for enumeration of spiking suspensions using membrane filters.

6.4.8.1 Glass microanalysis filter holder—25-mm-diameter, with fritted glass support, Fisher cat. no. 09-753E, or equivalent. Replace stopper with size 8, one-hole rubber stopper, Fisher Cat. No. 14-135M, or equivalent.

6.4.8.2 Three-port vacuum filtration manifold and vacuum source—Fisher Cat. No. 09-753-39A, or equivalent.

6.4.8.3 Cellulose acetate support membrane—1.2-µm-pore-size, 25-mm-diameter, Fisher cat. no. A12SP02500, or equivalent.

6.4.8.4 Polycarbonate track-etch hydrophilic membrane filter—1-µm-pore-size, 25-mm-diameter, Fisher cat. no. K10CP02500, or equivalent.

6.4.8.5 100 × 15 mm polystyrene Petri dishes (bottoms only).

6.4.8.6 60 × 15 mm polystyrene Petri dishes.

6.4.8.7 Glass microscope slides—1 in. × 3 in or 2 in. × 3 in.

6.4.8.8 Coverslips—25 mm^2

6.5 Immunomagnetic separation (IMS) apparatus
6.5.1 Sample mixer—Dynal Inc., Lake Success, NY, cat. no. 947.01, or equivalent.
6.5.2 Magnetic particle concentrator for 10-mL test tubes—Dynal MPC-1® , cat. no. 120.01, or equivalent.
6.5.3 Magnetic particle concentrator for microcentrifuge tubes—Dynal MPC-M®, cat. no. 120.09, or equivalent.
6.5.4 Flat-sided sample tubes—16 × 125 mm Leighton-type tubes with 60 × 10 mm flat-sided magnetic capture area, Dynal L10, cat. no. 740.03, or equivalent.
6.6 Powder-free latex gloves—Fisher cat no. 113945B, or equivalent.
6.7 Graduated cylinders, autoclavable—10-, 100-, and 1000-mL.

6.8 Centrifuges

6.8.1 Centrifuge capable of accepting 15- to 250-mL conical centrifuge tubes and achieving 1500 × G—International Equipment Company, Needham Heights, MA, Centrifuge Size 2, Model K with swinging bucket, or equivalent.
6.8.2 Centrifuge tubes—Conical, graduated, 1.5-, 50-, and 250-mL.

6.9 Microscope

6.9.1 Epifluorescence/differential interference contrast (DIC) with stage and ocular micrometers and 20X (N.A.=0.4) to 100X (N.A.=1.3) objectives—Zeiss™ Axioskop, Olympus™ BH, or equivalent.
6.9.2 Excitation/band-pass filters for immunofluorescence assay (FA)—Zeiss™ 487909 or equivalent, including, 450- to 490-nm exciter filter, 510-nm dicroic beam-splitting mirror, and 515- to 520-nm barrier or suppression filter.
6.9.3 Excitation/band-pass filters for DAPI—Filters cited below (Chroma Technology, Brattleboro, VT), or equivalent.

Microscope model	Fluoro-chrome	Excitation filter (nm)	Dichroic beam-splitting mirror (nm)	Barrier or suppression filter (nm)	Chroma catalog number
Zeiss™ - Axioskop	DAPI (UV)	340-380	400	420	CZ902
Zeiss™ -IM35	DAPI (UV)	340-380	400	420	CZ702
Olympus™ BH	DAPI (UV)	340-380	400	420	11000
			Filter holder		91002
Olympus™ BX	DAPI (UV)	340-380	400	420	11000
			Filter holder		91008
Olympus™ IMT2	DAPI (UV)	340-380	400	420	11000
			Filter holder		91003

6.10 Ancillary equipment for microscopy

6.10.1 Well slides— Spot-On well slides, Dynal cat. no. 740.04; treated, 12-mm diameter well slides, Meridian Diagnostics Inc., Cincinnati, OH, cat. no. R2206; or equivalent.
6.10.2 Glass coverslips—22 × 50 mm.
6.10.3 Nonfluorescing immersion oil.
6.10.4 Micropipette, adjustable: 0- to 10-µL with 0- to 10-µL tips 10- to 100-µL, with 10- to 200-µL tips 100- to 1000-µL with 100- to 1000-µL tips
6.10.5 Forceps—Splinter, fine tip.
6.10.6 Forceps—Blunt-end.
6.10.7 Desiccant—Drierite™ Absorbent, Fisher cat. no. 07-577-1A, or equivalent
6.10.8 Humid chamber—A tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

6.11 Pipettes—Glass or plastic

6.11.1 5-, 10-, and 25-mL.
6.11.2 Pasteur, disposable.

6.12 Balances

6.12.1 Analytical—Capable of weighing 0.1 mg.
6.12.2 Top loading—Capable of weighing 10 mg.

6.13 pH meter

6.14 Incubator—Fisher Scientific Isotemp™, or equivalent.

6.15 Vortex mixer—Fisons Whirlmixer, or equivalent.

6.16 Vacuum source—Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.17 Miscellaneous labware and supplies

6.17.1 Test tubes and rack.
6.17.2 Flasks—Suction, Erlenmeyer, and volumetric, various sizes.
6.17.3 Beakers—Glass or plastic, 5-, 10-, 50-, 100-, 500-, 1000-, and 2000-mL.
6.17.4 Lint-free tissues.
6.18 10- to 15-L graduated container—Fisher cat. no. 02-961-50B, or equivalent; calibrate to 9.0, 9.5, 10.0, 10.5, and 11.0 L and mark levels with waterproof marker.
6.19 Filters for filter-sterilizing reagents—Sterile Acrodisc, 0.45 µm , Gelman Sciences cat no. 4184, or equivalent.

7.0 Reagents and Standards

7.1 Reagents for adjusting pH
7.1.1 Sodium hydroxide (NaOH)—ACS reagent grade, 6.0 N and 1.0 N in reagent water 7.1.2 Hydrochloric acid (HCl)—ACS reagent grade, 6.0 N, 1.0 N, and 0.1 N in reagent water.

NOTE: Due to the low volumes of pH-adjusting reagents used in this method, and the impact that changes in pH have on the immunofluorescence assay, the laboratory should purchase standards at the required normality directly from a vendor. Normality should not be adjusted by the laboratory.

7.2 Solvents—Acetone, glycerol, ethanol, and methanol, ACS reagent grade

7.3 Reagent water—Water in which oocysts and cysts and interfering materials and substances, including magnetic minerals, are not detected by this method.

7.4 Reagents for eluting filters

7.4.1 Reagents for eluting Envirochek™ sampling capsules (Section 6.2.2)

7.4.1.1 Laureth-12—PPG Industries, Gurnee, IL, cat. no. 06194, or equivalent. Store Laureth-12 as a 10% solution in reagent water. Weigh 10 g of Laureth-12 and dissolve using a microwave or hot plate in 90 mL of reagent water. Dispense 10-mL aliquots into sterile vials and store at room temperature for up to 2 months, or in the freezer for up to a year.

7.4.1.2 1 M Tris, pH 7.4—Dissolve 121.1 g Tris (Fisher cat. no. BP152) in 700 mL of reagent water and adjust pH to 7.4 with 1 N HCl or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize through a 0.2-µm membrane into a sterile plastic container and store at room temperature.

7.4.1.3 0.5 M EDTA, 2 Na, pH 8.0—Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate (Fisher cat. no. S311) in 800 mL and adjust pH to 8.0 with 6.0 N HCl or NaOH. Dilute to a final volume of 1000 mL with reagent water and adjust to pH 8.0 with 1.0 N HCl or NaOH.

7.4.1.4 Antifoam A—Sigma Chemical Co. cat. no. A5758, or equivalent

7.4.1.5 Preparation of elution buffer solution—Add the contents of a pre-prepared Laureth-12 vial (Section 7.4.1.1) to a 1000-mL graduated cylinder. Rinse the vial several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution (Section 7.4.1.2), 2 mL of EDTA solution (Section 7.4.1.3), and 150 µL Antifoam A (Section 7.4.1.4). Dilute to 1000 mL with reagent water.

7.4.2 Reagents for eluting CrypTest™ capsule filters (Section 6.2.3). To 900 mL of reagent water add 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 ($12H_2O$) 0.2 g KCl, 0.2 g sodium lauryl sulfate (SDS), 0.2 mL Tween 80, and 0.02 mL Antifoam A (Sigma Chemical Co. cat. no. A5758, or equivalent). Adjust volume to 1 L with reagent water and adjust pH to 7.4 with 1 N NaOH or HCl.

7.4.3 Reagents for eluting Filta-Max™ foam filters (Section 6.2.4)

7.4.3.1 Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4.

7.4.3.2 Tween 20—Sigma Chemical Co. cat. no. P-7949, or equivalent.

7.4.3.3 High-vacuum grease—BDH/Merck. cat. no. 636082B, or equivalent.

7.4.3.4 Preparation of PBST elution buffer. Add the contents of one sachet of PBS to 1.0 L of reagent water. Dissolve by stirring for 30 minutes. Add 100 µL of Tween 20. Mix by stirring for 5 minutes.

7.5 Reagents for immunomagnetic separation (IMS)—Dynabeads® GC-Combo, Dynal cat. nos. 730.02, 730.12, or equivalent.

7.6 Direct antibody labeling reagents for detection of oocysts and cysts. Store reagents at 0 ^{o}C to 8 ^{o}C and return promptly to this temperature after each use. Do not allow any of the reagents to freeze. The reagents should be protected from exposure to light. Diluted, unused working reagents should be discarded after 48 hours. Discard reagents after the expiration date is reached. The labeling reagents in Sections 7.6.1-7.6.3 have been approved for use with this method.

7.6.1 Merifluor Cryptosporidium/Giardia, Meridian Diagnostics cat. no. 250050, Cincinnati, OH, or equivalent.

7.6.2 Aqua-Glo™ G/C Direct FL, Waterborne cat. no. A100FLR, New Orleans, LA, or equivalent.

7.6.3 Crypt-a-Glo™ and Giardi-a-Glo™, Waterborne cat. nos. A400FLR and A300FLR, respectively, New Orleans, LA, or equivalent.

NOTE: If a laboratory will use multiple types of labeling reagents, the laboratory must demonstrate acceptable performance through an initial precision and recovery test (Section 9.4) for each type, and must perform positive and negative staining controls for each batch of slides stained using each product. However, the laboratory is not required

to analyze additional ongoing precision and recovery samples or method blank samples for each type.

7.6.4 Diluent for labeling reagents—Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4. Filter-sterilize (Section 6.19) or autoclave. Discard if growth is detected or after 6 months, whichever comes first.

7.7 4',6-diamidino-2-phenylindole (DAPI) stain—Sigma Chemical Co. cat. no. A5758, or equivalent.

7.7.1 Stock solution—Dissolve 2 mg/mL DAPI in absolute methanol. Prepare volume consistent with minimum use. Store at 0 °C to 8 °C in the dark. Do not allow to freeze. Discard unused solution when positive staining control fails.

7.7.2 Staining solution (1/5000 dilution in PBS [Section 7.6.4])—Add 10 µL of 2 mg/mL DAPI stock solution to 50 mL of PBS. Prepare daily. Store at 0 °C to 8 °C in the dark except when staining. Do not allow to freeze. The solution concentration may be increased up to 1µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.

7.8 Mounting medium

7.8.1 DABCO/glycerol mounting medium (2%)—Dissolve 2 g of DABCO (Sigma Chemical Co. cat no. D-2522, or equivalent) in 95 mL of warm glycerol/PBS (60% glycerol, 40% PBS [Section 7.6.4]). After the DABCO has dissolved completely, adjust the solution volume to 100 mL by adding an appropriate volume of glycerol/PBS solution. Alternately, dissolve the DABCO in 40 mL of PBS, then add azide (1 mL of 100X, or 10% solution), then 60 mL of glycerol.

7.8.2 Mounting medium supplied with Merifluor direct labeling kit (Section 7.6.1)

7.9 Clear fingernail polish or clear fixative, PGC Scientifics, Gaithersburg, MD, cat. no. 60-4890, or equivalent.

7.10 Oocyst and cyst suspensions for spiking

7.10.1 Enumerated spiking suspensions prepared by flow cytometer—not heat-fixed or formalin fixed: Wisconsin State Laboratory of Hygiene Flow Cytometry Unit or equivalent

7.10.2 Materials for manual enumeration of spiking suspensions

7.10.2.1 Purified Cryptosporidium oocyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Sterling Parasitology Laboratory, University of Arizona, Tucson, or equivalent

7.10.2.2 Purified Giardia cyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Waterborne, Inc., New Orleans, LA; Hyperion Research, Medicine Hat, Alberta, Canada; or equivalent

7.10.2.3 Tween-20, 0.01%—Dissolve 1.0 mL of a 10% solution of Tween-20 in 1 L of reagent water

7.10.2.4 Storage procedure—Store oocyst and cyst suspensions at 0 °C to 8 °C, until ready to use; do not allow to freeze

7.11 Additional reagents for enumeration of spiking suspensions using membrane filtration (Section 11.3.6)—Sigmacote® Sigma Company Product No. SL-2, or equivalent

8.0 Sample Collection and Storage

8.1 Samples are collected as bulk samples and shipped to the laboratory for processing through the entire method, or are filtered in the field and shipped to the laboratory for processing from elution (Section 12.2.6) onward. Samples must be shipped via overnight service on the day they are collected. Chill samples as much as possible between collection and shipment by storing in a refrigerator or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment. Samples should be shipped at 0 °C to 8 °C, unless the time required to chill the sample to 8 °C would prevent the sample from being shipped

overnight for receipt at the laboratory the day after collection. Samples must not be allowed to freeze. Upon receipt, the laboratory should record the temperature of the samples and store them refrigerated at 0 ^{o}C to 8 ^{o}C until processed. Results from samples shipped overnight to the laboratory and received at >8 ^{o}C should be qualified by the laboratory.

NOTE: See transportation precautions in Section 5.5.

8.2 Sample holding times. Sample processing should be completed as soon as possible by the laboratory. The laboratory should complete sample filtration, elution, concentration, purification, and staining the day the sample is received wherever possible. However, the laboratory is permitted to split up the sample processing steps if processing a sample completely in one day is not possible. If this is necessary, sample processing can be halted after filtration, application of the purified sample onto the slide, or staining. Table 1, in Section 21.0 provides a breakdown of the holding times for each set of steps. Sections 8.2.1 through 8.2.4 provide descriptions of these holding times.
8.2.1 Sample collection and filtration. Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).
8.2.2 Sample elution, concentration, and purification. The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.
8.2.3 Staining. The sample must be stained within 72 hours of application of the purified sample to the slide.
8.2.4 Examination. Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In
addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.
8.5 Spiking suspension enumeration holding times. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometer sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance (QA) program (Reference 20.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability through performance of the initial precision and recovery (IPR) test (Section 9.4), analysis of spiked samples to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
9.1.1 A test of the microscope used for detection of oocysts and cysts is performed prior to examination of slides. This test is described in Section 10.0.
9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to modify certain method procedures to improve recovery or lower the costs of measurements, provided that all required quality control (QC) tests are performed and

all QC acceptance criteria are met. Method procedures that can be modified include front-end techniques, such as filtration or immunomagnetic separation (IMS). The laboratory is not permitted to use an alternate determinative technique to replace immunofluorescence assay in this method (the use of different determinative techniques are considered to be different methods, rather than modified version of this method). However, the laboratory is permitted to modify the immunofluorescence assay procedure, provided that all required QC tests are performed (Section 9.1.2.1) and all QC acceptance criteria are met (see guidance on the use of multiple labeling reagents in Section 7.6).

9.1.2.1 Method modification validation/equivalency demonstration requirements.
9.1.2.1.1 Method modifications at a single laboratory. Each time a modification is made to this method for use in a single laboratory, the laboratory is required to validate the modification according to Tier 1 of EPA's performance-based measurement system (PBMS) (Table 2 and Reference 20.7) to demonstrate that the modification produces results equivalent or superior to results produced by this method as written. Briefly, each time a modification is made to this method, the laboratory is required to demonstrate acceptable modified method performance through the IPR test (Section 9.4). IPR results must meet the QC acceptance criteria in Tables 3 and 4 in Section 21.0, and should be comparable to previous results using the unmodified procedure. Although not required, the laboratory also should perform a matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate the performance of the modified method in at least one real-world matrix before analyzing field samples using the modified method. The laboratory is required to perform MS samples using the modified method at the frequency noted in Section 9.1.8.
9.1.2.1.2 Method modifications for nationwide approval. If the laboratory or a manufacturer seeks EPA approval of a method modification for nationwide use, the laboratory or manufacturer must validate the modification according to Tier 2 of EPA's PBMS (Table 2 and Reference 20.7). Briefly, at least three laboratories must perform IPR tests (Section 9.4) and MS/MSD (Section 9.5) tests using the modified method, and all tests must meet the QC acceptance criteria specified in Tables 3 and 4 in Section 21.0. Upon nationwide approval, laboratories electing to use the modified method still must demonstrate acceptable performance in their own laboratory according to the requirements in Section 9.1.2.1.1.
9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
9.1.2.2.2 A listing of the analyte(s) measured (Cryptosporidium and Giardia).
9.1.2.2.3 9.1.2.2.4 A narrative stating reason(s) for the modification.
9.1.2.2.5 Results from all QC tests comparing the modified method to this method, including: (a) IPR (Section 9.4) (b) MS/MSD (Section 9.5) (c) Analysis of method blanks (Section 9.6) Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:
(a) Sample numbers and other identifiers
(b) Source of spiking suspensions, as well as lot number and date received (Section 7.10)
(c) Spike enumeration date and time
(d) All spiking suspension enumeration counts and calculations (Section 11.0)
(e) Sample spiking dates and times
(f) Volume filtered (Section 12.2.5.2)
(g) Filtration and elution dates and times
(h) Pellet volume, resuspended concentrate volume, resuspended concentrate volume transferred to IMS, and all calculations required to verify the percent of concentrate examined (Section 13.2)

(i) Purification completion dates and times (Section 3.3.3.11)
(j) Staining completion dates and times (Section 14.10)
(k) Staining control results (Section 15.2.1)
(l) All required examination information (Section 15.2.2)
(m) Examination completion dates and times (Section 15.2.4)
(n) Analysis sequence/run chronology
(o) Lot numbers of elution, IMS, and staining reagents
(p) Copies of bench sheets, logbooks, and other recordings of raw data
(q) Data system outputs, and other data to link the raw data to the results reported

9.1.3 The laboratory shall spike a separate sample aliquot from the same source to monitor method performance. This MS test is described in Section 9.5.1.

9.1.4 Analysis of method blanks is required to demonstrate freedom from contamination. The procedures and criteria for analysis of a method blank are described in Section 9.6.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample that the analysis system is in control. These procedures are described in Section 9.7.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Development of accuracy statements is described in Sections 9.5.1.4 and 9.7.3.

9.1.7 The laboratory shall analyze one method blank (Section 9.6) and one OPR sample (Section 9.7) each week during which samples are analyzed if 20 or fewer field samples are analyzed during this period. The laboratory shall analyze one laboratory blank and one OPR sample for every 20 samples if more than 20 samples are analyzed in a week.

9.1.8 The laboratory shall analyze one MS sample (Section 9.5.1) when samples are first received from a utility for which the laboratory has never before analyzed samples. The MS analysis is performed on an additional (second) sample sent from the utility. If the laboratory routinely analyzes samples from 1 or more utilities, 1 MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a given site, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 21st sample from this site, a separate aliquot of this 21st sample must be collected and spiked.

9.2 Micropipette calibration

9.2.1 Micropipettes must be sent to the manufacturer for calibration annually. Alternately, a qualified independent technician specializing in micropipette calibration can be used. Documentation on the precision of the recalibrated micropipette must be obtained from the manufacturer or technician.

9.2.2 Internal and external calibration records must be kept on file in the laboratory's QA logbook.

9.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. Ten replicates should be performed at each weight. Record the weight of the water (assume that 1.00 mL of reagent water weighs 1.00 g) and calculate the relative standard deviation (RSD) for each. If the weight of the reagent water is within 1% of the desired weight (mL) and the RSD of the replicates at each weight is within 1%, then the pipette remains acceptable for use.

9.2.4 If the weight of the reagent water is outside the acceptable limits, consult the manufacturer's instruction manual troubleshooting section and repeat steps described in Section 9.2.3. If problems with the pipette persist, the laboratory must send the pipette to the manufacturer for recalibration.

9.3 Microscope adjustment and certification: Adjust the microscope as specified in Section 10.0. All of the requirements in Section 10.0 must be met prior to analysis of IPRs, blanks, OPRs, field samples, and MS/SDS.

9.4 Initial precision and recovery (IPR)—To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.4.1 Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine four reagent water samples spiked with 100 to 500 oocysts and 100 to 500 cysts. If more than one process will be used for filtration and/or separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE: IPR tests must be accompanied by analysis of a method blank (Section 9.6).

9.4.2 Using results of the four analyses, calculate the average percent recovery and the relative standard deviation (RSD) of the recoveries for Cryptosporidium and for Giardia. The RSD is the standard deviation divided by the mean times 100.

9.4.3 Compare RSD and the mean with the corresponding limits for initial precision and recovery in Tables 3 and 4 in Section 21.0. If the RSD and the mean meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If the RSD or the mean falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test (Section 9.4.1).

9.5 Matrix spike (MS) and matrix spike duplicate (MSD):

9.5.1 Matrix spike—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's oocyst and cyst recovery. The MS shall be analyzed according to the frequency in Section 9.1.8.

9.5.1.1 Analyze an unspiked field sample according to the procedures in Sections 12.0 to 15.0. Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine a second field sample aliquot with the number of organisms used in the IPR or OPR tests (Sections 9.4 and 9.7).

9.5.1.2 For each organism, calculate the percent recovery (R) using the following equation.

$$R = 100 \times \frac{Nsp - Ns}{T}$$

where

R is the percent recovery

N_{sp} is the number of oocysts or cysts detected in the spiked sample

N_s is the number of oocysts or cysts detected in the unspiked sample T is the true value of the oocysts or cysts spiked

9.5.1.3 Compare the recovery for each organism with the corresponding limits in Tables 3 and 4 in Section 21.0.

NOTE: Some sample matrices may prevent the acceptance criteria in Tables 3 and 4 from being met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

9.5.1.4 As part of the QA program for the laboratory, method precision for samples should be assessed and records maintained. After the analysis of five samples for which the spike recovery for each organism passes the tests in Section 9.5.1.3, the laboratory should calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_r). Express the precision assessment as a percent recovery interval from P ˉ 2 s_r to P + 2 s_r for each matrix. For example, if P = 80% and s_r = 30%, the accuracy interval is expressed as 20% to 140%. The

precision assessment should be updated regularly across all MS samples and stratified by MS samples for each source.
9.5.2 Matrix spike duplicate—MSD analysis is required as part of nationwide approval of a modified version of this method to demonstrate that the modified version of this method produces results equal or superior to results produced by the method as written (Section 9.1.2.1.2). At the same time the laboratory spikes and analyzes the second field sample aliquot in Section 9.5.1.1, the laboratory shall spike and analyze a third, identical field sample aliquot.

NOTE: Matrix spike duplicate samples are only required for Tier 2 validation studies. They are recommended for Tier 1 validation, but not required.

9.5.2.1 For each organism, calculate the percent recovery (R) using the equation in Section 9.5.1.2.

9.5.2.2 Calculate the mean of the number of oocysts or cysts in the MS and MSD (X_{mean}) (= [MS+MSD]/2).

9.5.2.3 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \frac{| NMS - NMSD |}{Xmean}$$

where
RPD is the relative percent difference
N_{MS} is the number of oocysts or cysts detected in the MS
N_{MSD} is the number of oocysts or cysts detected in the MSD
X_{mean} is the mean number of oocysts or cysts detected in the MS and MSD

9.5.2.4 Compare the mean MS/MSD recovery and RPD with the corresponding limits in Tables 3 and 4 in Section 21.0 for each organism.

9.6 Method blank (negative control sample, laboratory blank): Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (Section 9.4) and OPR test (Section 9.7) and prior to analysis of samples for the week to demonstrate freedom from contamination.
9.6.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week (Section 9.1.7) according to the procedures in Sections 12.0 to 15.0. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.
9.6.2 If *Cryptosporidium* oocysts, *Giardia* cysts, or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more oocysts or cysts is assumed to be contaminated and should be recollected, if possible. Any method blank in which oocysts or cysts are not detected is assumed to be uncontaminated and may be reported.

9.7 Ongoing precision and recovery ([OPR]; positive control sample; laboratory control sample): Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water sample spiked with 100 to 500 oocysts and 100 to 500 cysts each week to verify all performance criteria. The laboratory must analyze one OPR sample for every 20 samples if more than 20 samples are analyzed in a week. If multiple method variations are used, separate OPR samples must be prepared for each method variation. Adjustment and/or

recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.
9.7.1 Examine the slide from the OPR prior to analysis of samples from the same batch.
9.7.1.1 Using 200X to 400X magnification, more than 50% of the oocysts or cysts must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the organisms. Determine the step or reagent that is causing damage to the organisms. Correct the problem and repeat the OPR test.

9.7.1.2 Identify and enumerate each organism using epifluorescence microscopy. The first three presumptive *Cryptosporidium* oocysts and three *Giardia* cysts identified in the OPR sample must be examined using FITC, DAPI, and DIC, as per Section 15.2, and the detailed characteristics (size, shape, DAPI category, and DIC category) reported on the *Cryptosporidium* and *Giardia* report form, as well as any additional comments on organism appearance, if notable.

9.7.2 For each organism, calculate the percent recovery (R) using the following equation:

$$R = 100 \times \frac{N}{T}$$

where:
R = the percent recovery
N = the number of oocysts or cysts detected
T = the number of oocysts or cysts spiked

9.7.3 Compare the recovery with the limits for ongoing precision and recovery in Tables 3 and 4 in Section 21.0. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, system performance is unacceptable. In this event, there may be a problem with the microscope or with the filtration or separation systems. Troubleshoot the problem using the procedures at Section 9.7.4 as a guide. After assessing the issue, reanalyze the OPR sample. All samples must be associated with an OPR that passes the criteria in Section 21.0. Samples that are not associated with an acceptable OPR must be flagged accordingly.

9.7.4 Troubleshooting. If an OPR sample has failed, and the cause of the failure is not known, the laboratory generally should identify the problem working backward in the analytical process from the microscopic examination to filtration.
9.7.4.1 Microscope system and antibody stain: To determine if the failure of the OPR test is due to changes in the microscope or problems with the antibody stain, re-examine the positive staining control (Section 15.2.1), check Köhler illumination, and check the fluorescence of the fluorescein-labeled monoclonal antibodies (Mabs) and 4',6-diamidino-2-phenylindole (DAPI). If results are unacceptable, re-examine the previously-prepared positive staining control to determine whether the problem is associated with the microscope or the antibody stain.
9.7.4.2 Separation (purification) system: To determine if the failure of the OPR test is attributable to the separation system, check system performance by spiking a 10-mL volume of reagent water with 100 - 500 oocysts and cysts and processing the sample through the IMS, staining, and examination procedures in Sections 13.3 through 15.0.
9.7.4.3 Filtration/elution/concentration system: If the failure of the OPR test is attributable to the filtration/elution/concentration system, check system performance by processing spiked reagent water according to the procedures in Section 12.2 through 13.2.2.1, and filter, stain, and examine the sample concentrate according to Section 11.3.6.

9.7.5 The laboratory should add results that pass the specifications in Section 9.7.3 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the

standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from R ¯2 s_r to R + 2 s_r. For example, if R = 95% and s_r = 25%, the accuracy is 45% to 145%.
9.8 The laboratory should periodically analyze an external QC sample, such as a performance evaluation or standard reference material, when available.

The laboratory also should periodically participate in interlaboratory comparison studies using the method.

9.9 The specifications contained in this method can be met if the analytical system is under control. The standards used for initial (Section 9.4) and ongoing (Section 9.7) precision and recovery should be identical, so that the most precise results will be obtained. The microscope in particular will provide the most reproducible results if dedicated to the settings and conditions required for the determination of Cryptosporidium and Giardia by this method.
9.10 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and duplicate spiked samples may be required to determine the precision of the analysis.

10.0 Microscope Calibration and Analyst Verification
10.1 In a room capable of being darkened to near-complete darkness, assemble the microscope, all filters, and attachments. The microscope should be placed on a solid surface free from vibration. Adequate workspace should be provided on either side of the microscope for taking notes and placement of slides and ancillary materials.
10.2 Using the manuals provided with the microscope, all analysts must familiarize themselves with operation of the microscope.

10.3 Microscope adjustment and calibration (adapted from Reference 20.6)

10.3.1 Preparations for adjustment

10.3.1.1 The microscopy portion of this procedure depends upon proper alignment and adjustment of very sophisticated optics. Without proper alignment and adjustment, the microscope will not function at maximal efficiency, and reliable identification and enumeration of oocysts and cysts will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.
10.3.1.2 While microscopes from various vendors are configured somewhat differently, they all operate on the same general physical principles. Therefore, slight deviations or adjustments may be required to make the procedures below work for a particular instrument.
10.3.1.3 The sections below assume that the mercury bulb has not exceeded time limits of operation, that the lamp socket is connected to the lamp house, and that the condenser is adjusted to produce Köhler illumination.
10.3.1.4 Persons with astigmatism should always wear contact lenses or glasses when using the microscope.

CAUTION: In the procedures below, do not touch the quartz portion of the mercury bulb with your bare fingers. Finger oils can cause rapid degradation of the quartz and premature failure of the bulb.
WARNING: Never look at the ultraviolet (UV) light from the mercury lamp, lamp house, or the UV image without a barrier filter in place. UV radiation can cause serious eye damage.

10.3.2 Epifluorescent mercury bulb adjustment: The purpose of this procedure is to ensure even field illumination. This procedure must be followed when the microscope is first used, when replacing bulbs, and if problems such as diminished fluorescence or uneven field illumination are experienced.

10.3.2.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.2.2 Using a prepared microscope slide, adjust the focus so the image in the oculars is sharply defined.
10.3.2.3 Replace the slide with a business card or a piece of lens paper.
10.3.2.4 Close the field diaphragm (iris diaphragm in the microscope base) so only a small point of light is visible on the card. This dot of light indicates the location of the center of the field of view.
10.3.2.5 Mount the mercury lamp house on the microscope without the UV diffuser lens in place and turn on the mercury bulb.
10.3.2.6 Remove the objective in the light path from the nosepiece. A primary (brighter) and secondary image (dimmer) of the mercury bulb arc should appear on the card after focusing the image with the appropriate adjustment.
10.3.2.7 Using the lamp house adjustments, adjust the primary and secondary mercury bulb images so they are side by side (parallel to each other) with the transmitted light dot in between them.
10.3.2.8 Reattach the objective to the nosepiece.
10.3.2.9 Insert the diffuser lens into the light path between the mercury lamp house and the microscope.
10.3.2.10 Turn off the transmitted light and replace the card with a slide of fluorescent material. Check the field for even fluorescent illumination. Adjustment of the diffuser lens probably will be required. Additional slight adjustments as in Section 10.3.2.7 above may be required.
10.3.2.11 Maintain a log of the number of hours the UV bulb has been used. Never use the bulb for longer than it has been rated. Fifty-watt bulbs should not be used longer than 100 hours; 100-watt bulbs should not be used longer than 200 hours.

10.3.3 Transmitted bulb adjustment: The purpose of this procedure is to center the filament and ensure even field illumination. This procedure must be followed when the bulb is changed.
10.3.3.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.3.2 Using a prepared microscope slide and a 40X (or similar) objective, adjust the focus so the image in the oculars is sharply defined.
10.3.3.3 Without the ocular or Bertrand optics in place, view the pupil and filament image at the bottom of the tube.
10.3.3.4 Focus the lamp filament image with the appropriate adjustment on the lamp house.
10.3.3.5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on the lamp house.
10.3.3.6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

10.3.4 Adjustment of the interpupillary distance and oculars for each eye: These adjustments are necessary so that eye strain is reduced to a minimum, and must be made for each individual using the microscope. Section 10.3.4.2 assumes use of a microscope with both oculars adjustable; Section 10.3.4.3 assumes use of a microscope with a single adjustable ocular. The procedure must be followed each time an analyst uses the microscope.
10.3.4.1 Interpupillary distance

10.3.4.1.1 Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.
10.3.4.1.2 Using both hands, move the oculars closer together or farther apart until a single circle of light is observed while looking through the oculars with both eyes. Note interpupillary distance.

10.3.4.2 Ocular adjustment for microscopes capable of viewing a photographic frame through the viewing binoculars: This procedure assumes both oculars are adjustable.

10.3.4.2.1 Place a card between the right ocular and eye keeping both eyes open. Adjust the correction (focusing) collar on the left ocular by focusing the left ocular until it reads the same as the interpupillary distance. Bring an image located in the center of the field of view into as sharp a focus as possible.
10.3.4.2.2 Transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the same image into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

10.3.4.3 Ocular adjustment for microscopes without binocular capability: This procedure assumes a single focusing ocular. The following procedure assumes that only the right ocular is capable of adjustment.

10.3.4.3.1 Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.
10.3.4.3.2 Transfer the card to between the left eye and ocular. Keeping both eyes open, bring the image for the right eye into sharp focus by adjusting the ocular collar at the top of the ocular without touching the coarse or fine adjustment.
10.3.5 Calibration of an ocular micrometer: This section assumes that a reticle has been installed in one of the oculars by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed, the ocular reticle should be left in place. The more an ocular is manipulated the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is a top lens on the microscope, the calibration procedure must be done for the respective objective at each top lens setting. The procedure must be followed when the microscope is first used and each time the objective is changed.
10.3.5.1 Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.
10.3.5.2 Adjust the stage and ocular with the micrometer so the 0 line on the ocular micrometer is exactly superimposed on the 0 line on the stage micrometer.
10.3.5.3 Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.
10.3.5.4 Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition. For example: Suppose 48 ocular micrometer spaces equal 0.6 mm.
10.3.5.5 Calculate the number of mm/ocular micrometer space. For example:
0.6 *mm* 0.0125 *mm* = 48 *ocular micrometer spaces ocular micrometer space*

10.3.5.6 Because most measurements of microorganisms are given in µm rather than mm, the value calculated above must be converted to µm by multiplying it by 1000 µm /mm. For example:

0.0125 mm 1,000 µm 12.5 µm x =
ocular micrometer space mm ocular micrometer space

10.3.5.7 Follow the procedure below for each objective. Record the information as shown in the example below and keep the information available at the microscope.

Item no.	Objective power	Description	No. of ocular micrometer spaces	No. of stage micrometer mm[1]	µm/ocular micrometer space[2]
1		10X		N.A.[3]=	
2		20X		N.A.=	
3		40X		N.A.=	
4		100X		N.A.=	

[1] 100 µm /mm [2] (Stage micrometer length in mm × (1000 µm /mm)) ÷ no. ocular micrometer spaces [3] N.A. refers to numerical aperture. The numerical aperture value is engraved on the barrel of the objective.

10.3.6 Köhler illumination: This section assumes that Köhler illumination will be established for only the 100X oil DIC objective that will be used to identify internal morphological characteristics in Cryptosporidium oocysts and Giardia cysts. If more than one objective is to be used for DIC, then each time the objective is changed, Köhler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Köhler illumination is not properly established, then DIC will not work to its maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore. The procedure must be followed each time an analyst uses the microscope and each time the objective is changed.

10.3.6.1 Place a prepared slide on the microscope stage, place oil on the slide, move the 100X oil objective into place, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.6.2 At this point both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.

10.3.6.3 Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the field to the center of the visual field.

10.3.6.4 Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up and down with the condenser focusing knob while you are looking through the binoculars. Once you have accomplished the precise focusing of the radiant field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

10.3.6.5 The aperture diaphragm of the condenser is now adjusted to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

10.3.6.6 After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish DIC

10.4 Protozoa libraries: Each laboratory is encouraged to develop libraries of photographs and drawings for identification of protozoa.

10.4.1 Take color photographs of Cryptosporidium oocysts and Giardia cysts by FA and 4',6-diamidino-2-phenylindole (DAPI) that the analysts (Section 22.2) determine are accurate (Section 15.2).

10.4.2 Similarly, take color photographs of interfering organisms and materials by FA and DAPI that the analysts believe are not Cryptosporidium oocysts or Giardia cysts. Quantify the size, shape, microscope settings, and other characteristics that can be used to differentiate oocysts and cysts from interfering debris and that will result in positive identification of DAPI positive or negative organisms.

10.5 Verification of performance: Until standard reference materials, such as National Institute of Standards and Technology standard reference materials, are available that contain a reliable number of DAPI positive or negative oocysts and cysts, this method shall rely upon the ability of the analyst for identification and enumeration of oocysts and cysts.

10.5.1 At least monthly when microscopic examinations are being performed, the laboratory shall prepare a slide containing 40 to 100 oocysts and 40 to 100 cysts. More than 50% of the oocysts and cysts must be DAPI positive.

10.5.2 Each analyst shall determine the total number of oocysts and cysts and the number that are DAPI positive or negative using the slide prepared in Section 10.5.1.

10.5.3 The total number and the number of DAPI positive or negative oocysts and cysts determined by each analyst (Section 10.5.2.) must be within ±10% of each other. If the number is not within this range, the analysts must identify the source of any variability

between analysts' examination criteria, prepare a new slide, and repeat the performance verification (Sections 10.5.1 to 10.5.2).

10.5.4 Document the date, name(s) of analyst(s), number of total, DAPI positive or negative oocysts and cysts determined by the analyst(s), whether the test was passed/failed and the results of attempts before the test was passed.

10.5.5 Only after an analyst has passed the criteria in Section 10.5.3, may oocysts and cysts in QC samples and field samples be identified and enumerated.

11.0 Oocyst and Cyst Suspension Enumeration and Spiking

11.1 This method requires routine analysis of spiked QC samples to demonstrate acceptable initial and ongoing laboratory and method performance (initial precision and recovery samples [Section 9.4], matrix spike and matrix spike duplicate samples [Section 9.5], and ongoing precision and recovery samples [Section 9.7]). The organisms used for these samples must be enumerated to calculate recoveries and precision. EPA recommends that flow cytometry be used for this enumeration, rather than manual techniques. Flow cytometer–sorted spikes generally are characterized by a relative standard deviation of ≤ 2.5%, versus greater variability for manual enumeration techniques (Reference 20.8). Guidance on preparing spiking suspensions using a flow cytometer is provided in Section 11.2. Manual enumeration procedures are provided in Section 11.3. The procedure for spiking bulk samples in the laboratory is provided in Section 11.4.

11.2 Flow cytometry enumeration guidelines. Although it is unlikely that many laboratories performing Method 1623 will have direct access to a flow cytometer for preparing spiking suspensions, flow-sorted suspensions are available from commercial vendors and other sources (Section 7.10.1). The information provided in Sections 11.2.1 through 11.2.4 is simply meant as a guideline for preparing spiking suspensions using a flow cytometer. Laboratories performing flow cytometry must develop and implement detailed standardized protocols for calibration and operation of the flow cytometer.

11.2.1 Spiking suspensions should be prepared using unstained organisms that have not been heat-fixed or formalin-fixed.

11.2.2 Spiking suspensions should be prepared using Cryptosporidium parvum oocysts <3 months old, and Giardia intestinalis cysts <2 weeks old.

11.2.3 Initial calibration. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The oocyst and cyst levels used for the initial calibration should be the same as the levels used for the spiking suspensions. Each initial calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the laboratory should perform the initial calibration again, until the RSD of the 10 counts is ≤ 2.5%. In addition to counting the organisms, the laboratory also should evaluate the quality of the organisms using DAPI and DIC to confirm that the organisms are in good condition.

11.2.4 Ongoing calibration. When sorting the spiking suspensions for use in QC samples, the laboratory should perform ongoing calibration samples at a 10% frequency, at a minimum. The laboratory should sort the first run and every eleventh sample directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the laboratory should discard the batch.

11.2.5 Method blanks. Depending on the operation of the flow cytometer, method blanks should be prepared and examined at the same frequency as the ongoing calibration samples (Section 11.2.4).

11.2.6 Holding time criteria. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories

should use flow-cytometer-sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory.

11.3 Manual enumeration procedures. Two sets of manual enumerations are required per organism before purified Cryptosporidium oocyst and Giardia cyst stock suspensions (Sections 7.9.2.1 and 7.9.2.2) received from suppliers can be used to spike samples in the laboratory. First, the stock suspension must be diluted and enumerated (Section 11.3.3) to yield a suspension at the appropriate oocyst or cyst concentration for spiking (spiking suspension). Then, 10 aliquots of spiking suspension must be enumerated to calculate a mean spike dose. Spiking suspensions can be enumerated using hemacytometer chamber counting (Section 11.3.4), well slide counting (Section 11.3.5), or membrane filter counting (Section 11.3.6).

11.3.1 Precision criteria. The relative standard deviation (RSD) of the calculated mean spike dose for manually enumerated spiking suspensions must be ≤16% for *Cryptosporidium* and ≤19% for *Giardia* before proceeding (these criteria are based on the pooled RSDs of 105 manual *Cryptosporidium* enumerations and 104 manual *Giardia* enumerations submitted by 20 different laboratories under the EPA Protozoa Performance Evaluation Program).

11.3.2 Holding time criteria. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension or membrane filter to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

11.3.3 Enumerating and diluting stock suspensions

11.3.3.1 Purified, concentrated stock suspensions (Sections 7.10.2.1 and 7.10.2.2) must be diluted and enumerated before the diluted suspensions are used to spike samples in the laboratory. Stock suspensions should be diluted with reagent water/Tween-20, 0.01% (Section 7.10.2.3), to a concentration of 20 to 50 organisms per large hemacytometer square before proceeding to Section 11.3.3.2.

11.3.3.2 Apply a clean hemacytometer coverslip (Section 6.4.5) to the hemacytometer and load the hemacytometer chamber with 10 µL of vortexed suspension per chamber. If this operation has been properly executed, the liquid should amply fill the entire chamber without bubbles or overflowing into the surrounding moats. Repeat this step with a clean, dry hemacytometer and coverslip if loading has been incorrectly performed. See Section 11.3.3.13, below, for the hemacytometer cleaning procedure.

11.3.3.3 Place the hemacytometer on the microscope stage and allow the oocysts or cysts to settle for 2 minutes Do not attempt to adjust the coverslip, apply clips, or in any way disturb the chamber after it has been filled.

11.3.3.4 Use 200X magnification.

11.3.3.5 Move the chamber so the ruled area is centered underneath it.

11.3.3.6 Move the objective close to the coverslip while watching it from the side of the microscope, rather than through the microscope.

11.3.3.7 Focus up from the coverslip until the hemacytometer ruling appears.

11.3.3.8 At each of the four corners of the chamber is a 1-square-mm area divided into 16 squares in which organisms are to be counted (Figure 1). Beginning with the top row of four squares, count with a hand-tally counter in the directions indicated in Figure 2. Avoid counting organisms twice by counting only those touching the top and left boundary lines. Count each square millimeter in this fashion.

11.3.3.9 Use the following formula to determine the number of organisms per mL of suspension:

11.3.3.10 Record the result on a hemacytometer data sheet.

11.3.3.11 A total of six different hemacytometer chambers must be loaded, counted, and averaged for each suspension to achieve optimal counting accuracy.

11.3.3.12 Based on the hemacytometer counts, the stock suspension should be diluted to a final concentration of between 8000 and 12,000 organisms per mL (80 to 120 organisms per 10

$_{\mu L}$); however, ranges as great as 5000 to 15,000 organisms per mL (50 to 150 organisms per 10 µL) can be used.

NOTE: If the diluted stock suspensions (the spiking suspensions) will be enumerated using hemacytometer chamber counts (Section 11.3.4) or membrane filter counts (Section 11.3.6), then the stock suspensions should be diluted with 0.01% Tween-20. If the spiking suspensions will be enumerated using well slide counts (Section 11.3.3), then the stock suspensions should be diluted in reagent water.

To calculate the volume (in µL) of stock suspension required per mL of reagent water (or reagent water/Tween-20, 0.01%), use the following formula:

required number of organisms x 1000 µL volume of stock suspension (µL) required = number of organisms/mL of Stock suspension

If the volume is less than 10 µL , an additional dilution of the stock suspension is recommended before proceeding.

To calculate the dilution factor needed to achieve the required number of organisms per 10 µL , use the following formula:
Total volume (µL) number of organisms required x 10 µL predicted number of organisms per 10 µL (80 to 120)

To calculate the volume of reagent water (or reagent water/Tween-20, 0.01%) needed, use the following formula:
reagent water volume (µL) = total volume (µL) -stock suspension volume required (µL)
11.3.3.13 After each use, the hemacytometer and coverslip must be cleaned immediately to prevent the organisms and debris from drying on it. Since this apparatus is precisely machined, abrasives cannot be used to clean it, as they will disturb the flooding and volume relationships.
11.3.3.13.1 Rinse the hemacytometer and cover glass first with tap water, then 70% ethanol, and finally with acetone.
11.3.3.13.2 Dry and polish the hemacytometer chamber and cover glass with lens paper. Store it in a secure place.

11.3.3.14 Several factors are known to introduce errors into hemacytometer counts, including:

- Inadequate mixing of suspension before flooding the chamber.
- Irregular filling of the chamber, trapped air bubbles, dust, or oil on the chamber or coverslip.
- Total number of organisms counted is too low to provide statistical confidence in the result
- Error in recording tally.
- Calculation error; failure to consider dilution factor, or area counted.
- Inadequate cleaning and removal of organisms from the previous count.
- Allowing filled chamber to sit too long, so that the chamber suspension dries and concentrates.

11.3.4 Enumerating spiking suspensions using a hemacytometer chamber
NOTE: Spiking suspensions enumerated using a hemacytometer chamber must be used within 24 hours of enumeration.
11.3.4.1 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.
11.3.4.2 To an appropriate-size beaker containing a stir bar, add enough spiking suspension to perform all spike testing and the enumeration as described. The liquid volume and beaker relationship should be such that a spinning stir bar does not splash the sides of the beaker, the stir bar has unimpeded rotation, and there is enough room to draw sample from the beaker with a 10-µL micropipette without touching the stir bar. Cover the beaker with a watch glass or Petri dish to prevent evaporation between sample withdrawals.
11.3.4.3 Allow the beaker contents to stir for a minimum of 30 minutes before beginning enumeration.
11.3.4.4 While the stir bar is still spinning, remove a 10-µL aliquot and carefully load one side of the hemacytometer. Count all organisms on the platform, at 200X magnification using phase-

contrast or darkfield microscopy. The count must include the entire area under the hemacytometer, not just the four outer 1-mm^2 squares. Repeat this procedure nine times. This step allows confirmation of the number of organisms per 10 µL (Section 11.3.3.12). Based on the 10 counts, calculate the mean, standard deviation, and RSD of the counts. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts. Refer to Section 11.3.3.14 for factors that may introduce errors.

Enumerating spiking suspensions using well slides

NOTE: Spiking suspensions enumerated using well slides must be used within 24 hours of application of the spiking suspension to the slides.

11.3.5.1 Remove well slides from cold storage and lay the slides on a flat surface for 15 minutes to allow them to warm to room temperature.

11.3.5.2 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.5.3 Remove a 10-µL aliquot from the spiking suspension and apply it to the center of a well.

11.3.5.4 Before removing subsequent aliquots, cap the tube and gently invert it three times to ensure that the oocysts or cysts are in suspension.

11.3.5.5 Ten wells must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Air-dry the well slides. Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 °C to 42 °C also can be used.

11.3.5.6 Positive and negative controls must be prepared.

11.3.5.6.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts or cysts to the center of a well and distribute evenly over the well area.

11.3.5.6.2 For the negative control, pipette 50 µL of PBS onto the center of a well and spread it over the well area with a pipette tip.

11.3.5.6.3 Air-dry the control slides.

11.3.5.7 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

11.3.5.8 Follow the manufacturer's instructions (Section 7.6) in applying the stain to the slide.

11.3.5.9 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

11.3.5.10 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with a paper towel or other absorbent material. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

11.3.5.11 Add mounting medium (Section 7.8) to each well.

11.3.5.12 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.

11.3.5.13 Record the date and time that staining was completed. If slides will not be read immediately, store in a humid chamber in the dark at 0 °C to 8 °C until ready for examination.

11.3.5.14 After examination of the 10 wells, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or

dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6 Enumeration of spiking suspensions using membrane filters

NOTE: Spiking suspensions enumerated using membrane filters must be used within 24 hours of application of the filters to the slides.

11.3.6.1 Pre-coat the glass funnels with Sigmacote® by placing the funnel in a large Petri dish and applying 5-mL of Sigmacoat® to the funnel opening using a pipette and allowing it to run down the inside of the funnel. Repeat for all funnels to be used. The pooled Sigmacoat® may be returned to the bottle for re-use. Place the funnels at 35 ^{o}C or 41 ^{o}C for approximately 5 minutes to dry.

11.3.6.2 Place foil around the bottoms of the 100 × 15 mm Petri dishes.

11.3.6.3 Filter-sterilize (Section 6.19) approximately 10 mL of PBS pH 7.2 (Section 7. 9. 4). Dilute detection reagent (Section 7.7) as per manufacturer's instructions using sterile PBS. Multiply the anticipated number of filters to be stained by 100 mL to calculate total volume of stain required. Divide the total volume required by 5 to obtain the microliters of antibody necessary. Subtract the volume of antibody from the total stain volume to obtain the required microliters of sterile PBS to add to the antibody.

11.3.6.4 Label the tops of foil-covered, 60 × 15 mm Petri dishes for 10 spiking suspensions plus positive and negative staining controls and multiple filter blanks controls (one negative control, plus a blank after every five sample filters to control for carry-over). Create a humid chamber by laying damp paper towels on the bottom of a stain tray (the inverted foil-lined Petri dishes will protect filters from light and prevent evaporation during incubation).

11.3.6.5 Place a decontaminated and cleaned filter holder base (Section 6.4.8.1) into each of the three ports of the vacuum manifold (Section 6.4.8.2).

11.3.6.6 Pour approximately 10 mL of 0.01% Tween 20 into a 60 × 15 mm Petri dish.

11.3.6.7 Using forceps, moisten a 1.2-µm cellulose-acetate support membrane (Section 6.4.8.3) in the 0.01% Tween 20 and place it on the fritted glass support of one of the filter bases. Moisten a polycarbonate filter (Section 6.4.8.4) the same way and position it on top of the cellulose-acetate support membrane. Carefully clamp the glass funnel to the loaded filter support. Repeat for the other two filters.

11.3.6.8 Add 5 mL of 0.01% Tween 20 to each of the three filtration units and allow to stand.

11.3.6.9 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.6.10 Using a micropipettor, sequentially remove two, 10-µL aliquots from the spiking suspension and pipet into the 5 mL of 0.01% Tween 20 standing in the unit. Rinse the pipet tip twice after each addition. Apply 10 µL of 0.01% Tween 20 to the third unit to serve as the negative control. Apply vacuum at 2" Hg and allow liquid to drain to miniscus, then close off vacuum. Pipet 10 mL of reagent water into each funnel and drain to miniscus, closing off the vacuum. Repeat the rinse and drain all fluid, close off the vacuum.

11.3.6.11 Pipet 100 mL of diluted antibody to the center of the bottom of a 60 × 15 mm Petri dish for each sample.

11.3.6.12 Unclamp the top funnel and transfer each cellulose acetate support membrane/ polycarbonate filter combination onto the drop of stain using forceps (apply each membrane/filter combination to a different Petri dish containing stain). Roll the filter into the drop to exclude air. Place the small Petri dish containing the filter onto the damp towel and cover with the corresponding labeled foil-covered top. Incubate for approximately 45 minutes at room temperature.

11.3.6.13 Reclamp the top funnels, apply vacuum and rinse each three times, each time with 20 mL of reagent water.

11.3.6.14 Repeat Sections 11.3.6.4 through 11.3.6.10 for the next three samples (if that the diluted spiking suspension has sat less than 15 minutes, reduce the suspension vortex time to 60 seconds). Ten, 10-µL spiking suspension aliquots must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Include a filter blank sample at a

frequency of every five samples; rotate the position of filter blank to eventually include all three filter placements.
11.3.6.15 Repeat Sections 11.3.6.4 through 11.3.6.10 until the 10-µL spiking suspensions have been filtered. The last batch should include a 10-µL 0.01 Tween 20 blank control and 20 µL of positive control antigen as a positive staining control.
11.3.6.16 Label slides. After incubation is complete, for each sample, transfer the cellulose acetate filter support and polycarbonate filter from drop of stain and place on fritted glass support. Cycle vacuum on and off briefly to remove excess fluid. Peel the top polycarbonate filter off the supporting filter and place on labeled slide. Discard cellulose acetate filter support. Mount and apply coverslips to the filters immediately to avoid drying.
11.3.6.17 To each slide, add 20 µL of mounting medium (Section 7.8).
11.3.6.18 Apply a coverslip. Seal the edges of the coverslip onto the slide using clear nail polish. (Sealing may be delayed until cover slips are applied to all slides.)
11.3.6.19 Record the date and time that staining was completed. If slides will not be read immediately, store sealed slides in a closed container in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.
11.3.6.20 After examination of the 10 slides, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.
11.3.6.21 If oocysts or cysts are detected on the filter blanks, modify the rinse procedure to ensure that no carryover occurs and repeat enumeration.
11.4 Procedure for spiking samples in the laboratory with enumerated spiking suspensions.
11.4.1 Arrange a bottom-dispensing container to feed the filter.
11.4.2 For initial precision and recovery (Section 9.4) and ongoing precision and recovery (Section 9.7) samples, fill the container with a volume of reagent water equal to the volume of the field samples analyzed in the analytical batch. For matrix spike samples (Section 9.5), fill the container with the field sample to be spiked. Continuously mix the sample (using a stir bar and stir plate for smaller-volume samples and alternate means for larger-volume samples).
11.4.3 Vortex the spiking suspension(s) (Section 11.2 or Section 11.3) for a minimum of 2 minutes.

11.4.3.1 For flow cytometer–enumerated suspensions (where the entire volume of a spiking suspension tube will be used):

11.4.3.1.1 Add 500 µL of the diluted antifoam to the tube containing the spiking suspension and vortex for 2 minutes.
11.4.3.1.2 Pour the suspension into the sample container.
11.4.3.1.3 Add 20 mL of reagent water to the empty tube, cap, vortex 10 seconds to rinse, and add the rinsate to the carboy.
11.4.3.1.4 Repeat this rinse using another 20 mL of reagent water.
11.4.3.1.5 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet.
11.4.3.1.6 Proceed to Section 11.4.4.
11.4.3.2 For manually enumerated spiking suspensions:

11.4.3.2.1 Rinse a pipette tip with 0.01% Tween-20 once, then rinse with the well-mixed spiking suspension a minimum of five times before pulling an aliquot to be used to spike the container.

11.4.3.2.2 Add the spiking suspension(s) to the carboy, delivering the aliquot below the surface of the water.

11.4.3.2.3 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet. Proceed to Section 11.4.4

11.4.4 Allow the spiking suspensions to mix for approximately 1 minute in the container.

11.4.5 Turn on the pump and allow the flow rate to stabilize. Set flow at the rate designated for the filter being used. As the carboy is depleted, check the flow rate and adjust if necessary.
11.4.6 When the water level approaches the discharge port of the carboy, tilt the container so that it is completely emptied. At that time, turn off the pump and add sufficient reagent water to the container to rinse. Swirl the contents to rinse down the sides.
11.4.7 Turn on the pump. Allow all of the water to flow through the filter and turn off the pump.

12.0 Sample Filtration and Elution
12.1 A water sample is filtered according to the procedures in Section 12.2. Alternate procedures may be used if the laboratory first demonstrates that the alternate procedure provides equivalent or superior performance per Section 9.1.2.
NOTE: Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

12.2 Capsule filtration (adapted from Reference 20.9). This procedure was validated using 10-L sample volumes. Alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).
NOTE: The filtration procedures specified in Section 12.2.1 - 12.2.5.3 are specific to laboratory filtration of a bulk sample, and reflect the procedures used during the interlaboratory validation of this method (Reference 20.10). These procedures may require modification if samples will be filtered in the field.

12.2.1 Flow rate adjustment

12.2.1.1 Connect the sampling system, minus the capsule, to a carboy filled with reagent water (Figure 3).
12.2.1.2 Turn on the pump and adjust the flow rate to 2.0 L/min.
12.2.1.3 Allow 2 to 10 L of reagent water to flush the system. Adjust the pump speed as required during this period. Turn off the pump when the flow rate has been adjusted.

12.2.2 Install the capsule filter in the line, securing the inlet and outlet ends with the appropriate clamps/fittings.
12.2.3 Record the sample number, sample turbidity (if not provided with the field sample), sample type, and sample filtration start date and time on a bench sheet.

12.2.4 Filtration
12.2.4.1 Connect the sampling system to the field carboy of sample water, or transfer the sample water to the laboratory carboy used in Section
12.2.1.1. If the sample will be filtered from a field carboy, a spigot (Section 6.2.1) can be used with the carboy to facilitate sample filtration.
NOTE: If the bulk field sample is transferred to a laboratory carboy, the laboratory carboy must be cleaned and disinfected before it is used with another field sample.
12.2.4.2 Place the drain end of the sampling system tubing into an empty graduated container with a capacity of 10 to 15 L, calibrated at 9.0, 9.5, 10.0, 10.5, and 11.0 L (Section 6.18). This container will be used to determine the sample volume filtered. Alternately, connect a flow meter (Section 6.3.4) downstream of the filter, and record the initial meter reading.
12.2.4.3 Allow the carboy discharge tube and capsule to fill with sample water. Vent residual air using the bleed valve/vent port, gently shaking or tapping the capsule, if necessary. Turn on the pump to start water flowing through the filter. Verify that the flow rate is 2 L/min.
12.2.4.4 After all of the sample has passed through the filter, turn off the pump. Allow the pressure to decrease until flow stops. (If the sample was filtered in the field, and excess sample remains in the filter upon receipt in the laboratory, pull the remaining sample volume through the filter before eluting the filter [Section 12.2.6].)

12.2.5 Disassembly

12.2.5.1 Disconnect the inlet end of the capsule filter assembly while maintaining the level of the inlet fitting above the level of the outlet fitting to prevent backwashing and the loss of oocysts and cysts from the filter. Restart the pump and allow as much water to drain as possible. Turn off the pump.

12.2.5.2 Based on the water level in the graduated container or meter reading, record the volume filtered on the bench sheet to the nearest quarter liter. Discard the contents of the graduated container.

12.2.5.3 Loosen the outlet fitting, then cap the inlet and outlet fittings.

12.2.6 Elution

NOTE: The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.

12.2.6.1 Setup

12.2.6.1.1 Assemble the laboratory shaker with the clamps aligned vertically so that the filters will be aligned horizontally. Extend the clamp arms to their maximum distance from the horizontal shaker rods to maximize the shaking action.

12.2.6.1.2 Prepare sufficient elution buffer so that all samples to be eluted that day can be eluted with the same batch of buffer. Elution may require up to 275 mL of buffer per sample.

12.2.6.1.3 Designate at least one 250-mL conical centrifuge tube for each sample and label with the sample number.

12.2.6.2 Elution

12.2.6.2.1 Record the elution date and time on the bench sheet. Using a ring stand or other means, clamp each capsule in a vertical position with the inlet end up. Remove the inlet cap and allow the liquid level to stabilize.

12.2.6.2.2 Pour elution buffer through the inlet fitting. Sufficient elution buffer must be added to cover the pleated white membrane with buffer solution. Replace the inlet cap and clamp the cap in place.

12.2.6.2.3 Securely clamp the capsule in one of the clamps on the laboratory shaker with the bleed valve positioned at the top on a vertical axis (in the 12 o'clock position). Turn on the shaker and set the speed to maximum (approximately 900 rpm). Agitate the capsule for approximately 5 minutes. Time the agitation using a lab timer, rather than the timer on the shaker to ensure accurate time measurement.

12.2.6.2.4 Remove the filter from the shaker, remove the inlet cap, and pour the contents of the capsule into the 250-mL conical centrifuge tube.

12.2.6.2.5 Clamp the capsule vertically with the inlet end up and add sufficient volume of elution buffer through the inlet fitting to cover the pleated membrane. Replace the inlet cap.

12.2.6.2.6 Return the capsule to the shaker with the bleed valve positioned at the 4 o'clock position. Turn on the shaker and agitate the capsule for approximately 5 minutes.

12.2.6.2.7 Remove the filter from the shaker, but leave the elution buffer in the capsule. Re-clamp the capsule to the shaker at the 8 o'clock position. Turn on the shaker and agitate the capsule for a final 5 minutes.

12.2.6.2.8 Remove the filter from the shaker and pour the contents into the 250-mL centrifuge tube. Rinse down the inside of the capsule filter walls with reagent water or elution buffer using a squirt bottle inserted in the inlet end of the capsule. Invert the capsule filter over the centrifuge tube and ensure that as much of the eluate as possible has been transferred.

12.2.7 Proceed to Section 13.0 for concentration and separation (purification).

13.0 Sample Concentration and Separation (Purification)

13.1 During concentration and separation, the filter eluate is concentrated through centrifugation, and the oocysts and cysts in the sample are separated from other particulates through immunomagnetic separation (IMS). Alternate procedures and

products may be used if the laboratory first demonstrates equivalent or superior performance as per Section 9.1.2.

13.2 Adjustment of pellet volume

13.2.1 Centrifuge the 250-mL centrifuge tube containing the capsule filter eluate at 1500 × G for 15 minutes. Allow the centrifuge to coast to a stop—do not use the brake. Record the pellet volume (volume of solids) on the bench sheet.

NOTE: Recoveries may be improved if centrifugation force is increased to 2000 × G. However, do not use this higher force if the sample contains sand or other gritty material that may degrade the condition of any oocysts and/or cysts in the sample.

13.2.2 Using a Pasteur pipette, carefully aspirate the supernatant to 5 mL above the pellet. Extra care must be taken to avoid aspirating oocysts and cysts during this step, particularly if the sample is reagent water (e.g. initial or ongoing precision and recovery sample).

13.2.3 If the packed pellet volume is ≤ 0.5 mL, vortex the tube vigorously until pellet is completely resuspended. Swirl the centrifuge tube gently to reduce any foaming after vortexing. Record the resuspended pellet volume on the bench sheet. Proceed to Section 13.3.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts and/or cysts in the sample is not compromised.

13.2.4 If the packed pellet volume is > 0.5 mL, the concentrate needs to be separated into multiple subsamples (a subsample is equivalent to no greater than 0.5 mL of packed pellet material, the recommended maximum amount of particulate material to process through the subsequent purification and examination steps in the method). Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples:

$$\text{total volume (mL) required} = \frac{\text{pellet volume}}{0.5\ \text{mL}} \times 5\ \text{mL}$$

(For example, if the packed pellet volume is 1.2 mL, the total volume required is 12 mL.) Add reagent water to the centrifuge tube to bring the total volume to the level calculated above. Vortex the tube vigorously for 10 to 15 seconds to completely resuspend the pellet. Record the resuspended pellet volume on the bench sheet.

> **NOTE**: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts in the sample is not compromised.

13.2.4.1 Analysis of entire sample. If analysis of the entire sample is required, determine the number of subsamples to be processed independently through the remainder of the method:

13.2.4.1.1 Calculate number of subsamples: Divide the total volume in the centrifuge tube by 5 mL and round up to the nearest integer (for example, if the resuspended volume in Section 13.2.4 is 12 mL, then the number of subsamples would be 12 mL / 5 mL = 2.4, rounded = 3 subsamples).

13.2.4.1.2 Determine volume of resuspended concentrate per subsample. Divide the total volume in the centrifuge tube by the calculated number of subsamples (for 13.2.4.1.3 example, if the resuspended volume in Section 13.2.4 is 12 mL, then the volume to use for each subsample = 12 mL / 3 subsamples = 4 mL).

Process sub-samples through IMS. Proceed to Section 13.3, and transfer aliquots of the resuspended concentrate equivalent to the volume in the previous step to multiple, flat-sided sample tubes in Section 13.3.2.1. Process the sample as multiple, independent subsamples from Section 13.3 onward, including the preparation and examination of separate slides for each aliquot. Record the volume of resuspended concentrate transferred to IMS on the bench sheet

(this will be equal to the volume recorded in Section 13.2.4). Also record the number of subsamples processed independently through the method on the bench sheet.

13.2.4.2 Analysis of partial sample. If not all of the concentrate will be examined, proceed to Section 13.3, and transfer one or more 5-mL aliquots of the resuspended concentrate to one or more flat-sided sample tubes in Section 13.3.2.1. Record the volume of resuspended concentrate transferred to IMS on the bench sheet. To determine the volume analyzed, calculate the percent of the concentrate examined using the following formula:

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS}}{\text{total volume of resuspended concentrate in Section 13.2.4}} \times 100\%$$

Then multiply the volume filtered (Section 12.2.5.2) by this percentage to determine the volume analyzed.

13.3 IMS procedure (adapted from Reference 20.11)

NOTE: The IMS procedure should be performed on a bench top with all materials at room temperature, ranging from 15 ^{o}C to 25 ^{o}C.

13.3.1 Preparation and addition of reagents

13.3.1.1 Prepare a 1X dilution of SL-buffer-A from the 10X SL-buffer-A (clear, colorless solution) supplied. Use reagent water (demineralized; Section 7.3) as the diluent. For every 1 mL of 1X SL-buffer-A required, take 100 µL of 10X SL-buffer-A and make up to 1 mL with the diluent water. A volume of 1.5 mL of 1X SL-buffer-A will be required per sample or subsample on which the Dynal IMS procedure is performed.

13.3.1.2 For each sample or subsample (Section 13.2) to be processed through IMS, add 1 mL of the 10X SL-buffer-A (supplied—not the diluted 1X SL-buffer-A) to a flat-sided tube (Section 6.5.4).

13.3.1.3 For each subsample, add 1 mL of the 10X SL-buffer-B (supplied— magenta solution) to the flat-sided tube containing the 10X SL-buffer-A.

13.3.2 Oocyst and cyst capture

13.3.2.1 Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample concentrate from Section 13.2 to the flat-sided tube(s) containing the SL-buffer. If all of the concentrate is used, rinse the centrifuge tube twice with reagent water and add the rinsate to the flat-sided tube containing the concentrate (or to the tube containing the first subsample, if multiple subsamples will be processed). Each of the two rinses should be half the volume needed to bring the total volume in the flat-sided sample tube to 10 mL. (For example, if 5 mL was transferred after resuspension of the pellet, the centrifuge tube would be rinsed twice with 2.5 mL of reagent water to bring the total volume in the flat-sided tube to 10 mL.) Visually inspect the centrifuge tube after completing the transfer to ensure that no concentrate remains. If multiple subsamples will be processed, bring the volume in the remaining flat-sided tubes to 10 mL with reagent water. Label the flat-sided tube(s) with the sample number (and subsample letters).

13.3.2.2 Vortex the Dynabeads®Crypto-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the sample tube and making sure that there is no residual pellet at the bottom.

13.3.2.3 Add 100 µL of the resuspended Dynabeads®Crypto-Combo (Section 13.3.2.2) to the sample tube(s) containing the water sample concentrate and SL-buffer.

13.3.2.4 Vortex the Dynabeads®Giardia-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the tube and making sure that there is no residual pellet at the bottom.

13.3.2.5 Add 100 µL of the resuspended Dynabeads®Giardia-Combo (Section 13.3.2.4) to the sample tube(s) containing the water sample concentrate, Dynabeads®Crypto-Combo, and SL-buffer.

13.3.2.6 Affix the sample tube(s) to a rotating mixer and rotate at approximately 18 rpm for 1 hour at room temperature.

13.3.2.7 After rotating for 1 hour, remove each sample tube from the mixer and place the tube in the magnetic particle concentrator (MPC-1) with flat side of the tube toward the magnet.

13.3.2.8 Without removing the sample tube from the MPC-1, place the magnet side of the MPC-1 downwards, so the tube is horizontal and the flat side of the tube is facing down.

13.3.2.9 Gently rock the sample tube by hand end-to-end through approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 minutes with approximately one tilt per second.

13.3.2.10 Ensure that the tilting action is continued throughout this period to prevent binding of low-mass, magnetic or magnetizable material. If the sample in the MPC-1 is allowed to stand motionless for more than 10 seconds, repeat Section 13.3.2.9 before continuing to Section 13.3.2.11.

13.3.2.11 Return the MPC-1 to the upright position, sample tube vertical, with cap at top. Immediately remove the cap and, keeping the flat side of the tube on top, pour off all of the supernatant from the tube held in the MPC-1 into a suitable container. Do not shake the tube and do not remove the tube from MPC-1 during this step.

13.3.2.12 Remove the sample tube from the MPC-1 and resuspend the sample in 1-mL 1X SL-buffer-A (prepared from 10X SL-buffer-A stock—supplied). Mix very gently to resuspend all material in the tube. Do not vortex.

13.3.2.13 Quantitatively transfer (transfer followed by two rinses) all the liquid from the sample tube to a labeled, 1.5-mL microcentrifuge tube. Use 1 mL of 1X SL-buffer-A to perform the first rinse and 0.5 mL of reagent water for the second rinse. Liberally rinse down the sides of the Leighton tube before transferring. Allow the flat-sided sample tube to sit for a minimum of 1 minute after transfer of the second rinse volume, then use a pipette to collect any residual volume that drips down to the bottom of the tube to ensure that as much sample volume is recovered as possible. Ensure that all of the liquid and beads are transferred.

13.3.2.14 Place the microcentrifuge tube into the second magnetic particle concentrator (MPC-M), with its magnetic strip in place.

13.3.2.15 Without removing the microcentrifuge tube from MPC-M, gently rock/roll the tube through 180° by hand. Continue for approximately 1 minute with approximately one 180° roll/rock per second. At the end of this step, the beads should produce a distinct brown dot at the back of the tube.

13.3.2.16 Immediately aspirate the supernatant from the tube and cap held in the MPC-M. If more than one sample is being processed, conduct three 90° rock/roll actions before removing the supernatant from each tube. Take care not to disturb the material attached to the wall of the tube adjacent to the magnet. Do not shake the tube. Do not remove the tube from MPC-M while conducting these steps.

13.3.3 Dissociation of beads/oocyst/cyst complex

NOTE: Two acid dissociations are required.

13.3.3.1 Remove the magnetic strip from the MPC-M.

13.3.3.2 Add 50 µL of 0.1 N HCl, then vortex at the highest setting for approximately 50 seconds.

NOTE: The laboratory should use 0.1-N standards purchased directly from a vendor, rather than adjusting the normality in-house.

13.3.3.3 Place the tube in the MPC-M without the magnetic strip in place and allow to stand in a vertical position for at least 10 minutes at room temperature.

13.3.3.4 Vortex vigorously for approximately 30 seconds.

13.3.3.5 Ensure that all of the sample is at the base of the tube. Place the microcentrifuge tube in the MPC-M.

13.3.3.6 Replace magnetic strip in MPC-M and allow the tube to stand undisturbed for a minimum of 10 seconds.
13.3.3.7 Prepare a well slide for sample screening and label the slide.
13.3.3.8 Add 5 µL of 1.0 N NaOH to the sample wells of two well slides (add 10 µL to the sample well of one well slide if the volume from the two required dissociations will be added to the same slide).

NOTE: The laboratory should use 1.0-N standards purchased directly from a vendor rather than adjusting the normality in-house.
13.3.3.9 Without removing the microcentrifuge tube from the MPC-M, transfer all of the sample from the microcentrifuge tube in the MPC-M to the sample well with the NaOH. Do not disturb the beads at the back wall of the tube. Ensure that all of the fluid is transferred.
13.3.3.10 Do not discard the beads or microcentrifuge tube after transferring the volume from the first acid dissociation to the well slide. Perform the steps in Sections 13.3.3.1 through 13.3.3.9 a second time. The volume from the second dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.

NOTE: If one slide is used, exert extra care when using Dynal Spot-On slides to ensure that the sample stays within the smaller-diameter wells on these slides.

13.3.3.11 Record the date and time the purified sample was applied to the slide(s).
13.3.3.12 Air-dry the sample on the well slide(s). Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

14.0 Sample Staining

NOTE: The sample must be stained within 72 hours of application of the purified sample to the slide.

14.1 Prepare positive and negative controls.
14.1.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts and 200 to 400 cysts to the center of a well.
14.1.2 For the negative control, pipette 50 µL of 150 mM PBS (Section 7.6.4) into the center of a well and spread it over the well area with a pipette tip.
14.1.3 Air-dry the control slides (see Section 13.3.3.12 for guidance).
14.2 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.
14.3 Follow manufacturer's instructions in applying stain to slide.
14.4 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.
14.5 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.6 Apply 50 µL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.7.2) to each well. Allow to stand at room temperature for a minimum of 1 minute. (The solution concentration may be increased up to 1 µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.)

14.7 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.8 Add mounting medium (Section 7.8) to each well.
14.9 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.
14.10 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

15.0 Examination
NOTE: Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.

15.1 Scanning technique: Scan each well in a systematic fashion. An up-and-down or a side-to-side scanning pattern may be used (Figure 4).
15.2 Examination using immunofluorescence assay (FA), 4',6-diamidino-2-phenylindole (DAPI) staining characteristics, and differential interference contrast (DIC) microscopy. The minimum magnification requirements for each type of examination are noted below.
NOTE: All shape and measurements must be determined using 1000X magnification and reported to the nearest 0.5 µm.

Record examination results for Cryptosporidium oocysts on a Cryptosporidium report form; record examination results for Giardia cysts on a Giardia report form. All oocysts and cysts that meet the criteria specified in Sections 15.2.2 and 15.2.3, less atypical organisms specifically identified as non-target organisms by DIC or DAPI (e.g. possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.), must be reported.
15.2.1 Positive and negative staining control.
15.2.1.1 Each analyst must characterize a minimum of three Cryptosporidium oocysts and three Giardia cysts on the positive staining control slide before examining field sample slides. This characterization must be performed by each analyst during each microscope examination session.

FITC examination must be conducted at a minimum of 200X total magnification, DAPI examination must be conducted at a minimum of 400X, and DIC examination must be conducted at a minimum of 1000X. Size, shape, and DIC and DAPI characteristics of the three Cryptosporidium oocysts and Giardia cysts must be recorded by the analyst on a microscope log. The analyst also must indicate on each sample report form whether the positive staining control was acceptable.
15.2.1.2 Examine the negative staining control to confirm that it does not contain any oocysts or cysts (Section 14.1). Indicate on each sample report form whether the negative staining control was acceptable.
15.2.1.3 If the positive staining control contains oocysts and cysts within the expected range and at the appropriate fluorescence for both FA and DAPI, and the negative staining control does not contain any oocysts or cysts (Section 14.1), proceed to Sections 15.2.2 and 15.2.3.

15.2.2 Sample examination—Cryptosporidium
15.2.2.1 FITC examination (the analyst must use a minimum of 200X total magnification). Use epifluorescence to scan the entire well for apple-green fluorescence of oocyst and cyst shapes. When brilliant apple-green fluorescing ovoid or spherical objects 4 to 6 µm in diameter are observed with brightly highlighted edges, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.2.2), then to DIC (Section 15.2.2.3).
15.2.2.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one of the following characteristics: (a) Light blue internal staining (no distinct nuclei) with a green rim (b) Intense blue internal staining (c) Up to four distinct, sky-blue nuclei Record oocysts in category (a) as DAPI negative; record oocysts in categories (b) and (c) as DAPI positive.
15.2.2.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Cryptosporidium oocysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each apple-green fluorescing object as: (a) An empty Cryptosporidium oocyst (b) A Cryptosporidium oocyst with amorphous structure (c) A Cryptosporidium oocyst with internal structure (one to four sporozoites/oocyst)
Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of sporozoites (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics. Although not a defining characteristic, surface oocyst folds may be observed in some specimens.

NOTE: All measurements must be made at 1000X magnification.

15.2.3 Sample examination—Giardia
15.2.3.1 FITC examination (the analyst must use a minimum of 200X total magnification). When brilliant apple-green fluorescing round to oval objects (8 - 18 µm long by 5 - 15 µm wide) are observed, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.3.2) then to DIC (Section 15.2.3.3).
15.2.3.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one or more of the following characteristics: (a) Light blue internal staining (no distinct nuclei) and a green rim (b) Intense blue internal staining (c) Two to four sky-blue nuclei Record cysts in category (a) as DAPI negative; record cysts in categories (b) and (c) as DAPI positive.
15.2.3.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Giardia cysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each object meeting the criteria specified in Sections 15.2.3.1 - 15.2.3.3 as one of the following, based on DIC examination: (a) An empty Giardia cyst (b) A Giardia cyst with amorphous structure (c) A Giardia cyst with one type of internal structure (nuclei, median body, or axonemes), or (d) A Giardia cyst with more than one type of internal structure.
Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of nuclei and presence of median body or axonemes (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics.
NOTE: All measurements must be made at 1000X magnification.
15.2.4 Record the date and time that sample examination was completed on the report form.
15.2.5 Report Cryptosporidium and Giardia concentrations as oocysts/L and cysts/L.

16.0 Analysis of Complex Samples
16.1 Some samples may contain high levels (>1000/L) of oocysts and cysts and/or interfering organisms, substances, or materials. Some samples may clog the filter (Section 12.0); others will

not allow separation of the oocysts and cysts from the retentate or eluate; and others may contain materials that preclude or confuse microscopic examination.
16.2 If the sample holding time has not been exceeded and a full-volume sample cannot be filtered, dilute an aliquot of sample with reagent water and filter this smaller aliquot (Section 12.0). This dilution must be recorded and reported with the results.
16.3 If the holding times for the sample and for microscopic examination of the cleaned up retentate/eluate have been exceeded, the site should be re-sampled. If this is not possible, the results should be qualified accordingly.

17.0 Method Performance
17.1 Method acceptance criteria are shown in Tables 3 and 4 in Section 21.0. The initial and ongoing precision and recovery criteria are based on the results of spiked reagent water samples analyzed during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike and matrix spike duplicate criteria are based on spiked source water data generated during the interlaboratory validation study of Method 1623 involving 11 laboratories and 11 raw surface water matrices across the U.S. (Reference 20.10).

NOTE: Some sample matrices may prevent the MS acceptance criteria in Tables 3 and 4 to be met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

18.0 Pollution Prevention
18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.
18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management
19.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of these requirements can be found in the *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).
19.2 Samples, reference materials, and equipment known or suspected to have viable oocysts or cysts attached or contained must be sterilized prior to disposal.
19.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

20.0 References

20.1 Rodgers, Mark R., Flanigan, Debbie J., and Jakubowski, Walter, 1995. *Applied and Environmental Microbiology* 61 (10), 3759-3763.
20.2 Fleming, Diane O., et al.(eds.), *Laboratory Safety: Principles and Practices,* 2nd edition.1995. ASM Press, Washington, DC
20.3 "Working with Carcinogens," DHEW, PHS, CDC, NIOSH, Publication 77-206, (1977).
20.4 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 *CFR* 1910 (1976).
20.5 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety (1979).
20.6 *ICR Microbial Laboratory Manual*, EPA/600/R-95/178, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 Martin Luther King Drive, Cincinnati, OH 45268 (1996).
20.7 USEPA. *EPA Guide to Method Flexibility and Approval of EPA Water Methods,* EPA 821-D-96-004. Office of Water, Engineering and Analysis Division, Washington, DC 20460 (1996).
20.8 Connell, K., C.C. Rodgers, H.L. Shank-Givens, J Scheller, M.L Pope, and K. Miller, 2000. Building a Better Protozoa Data Set. Journal AWWA, 92:10:30.
20.9 "Envirochek™ Sampling Capsule," PN 32915, Gelman Sciences, 600 South Wagner Road, Ann Arbor, MI 48103-9019 (1996).
20.10 USEPA. Results of the Interlaboratory Method Validation Study for Determination of Cryptosporidium and Giardia Using USEPA Method 1623, EPA-821-R-01-028. Office of Water, Office of Science and Technology, Engineering and Analysis Division, Washington, DC (2001).
20.11 "Dynabeads® GC-Combo," Dynal Microbiology R&D, P.O. Box 8146 Dep., 0212 Oslo, Norway (September 1998, Revision no. 01).
20.12 USEPA. Implementation and Results of the Information Collection Rule Supplemental Surveys. EPA-815-R-01-003. Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division, Washington, DC (2001).
20.13 Connell, K., J. Scheller, K. Miller, and C.C. Rodgers, 2000. Performance of Methods 1622 and 1623 in the ICR Supplemental Surveys. Proceedings, American Water Works Association Water Quality Technology Conference, November 5 - 9, 2000, Salt Lake City, UT.

21.0 Tables and Figures
Table 1. Method Holding Times (See Section 8.2 for details)

Table 2. Tier 1 and Tier 2 Validation/Equivalency Demonstration Requirements

Test	Description	Tier 1 modification(1)	Tier 2 modification(2)
IPR (Section 9.4)	4 replicates of spiked reagent water	Required. Must be accompanied by a method blank.	Required per laboratory
Method blank (Section 9.6)	Unspiked reagent water	Required	Required per laboratory
MS (Section 9.5.1)	Spiked matrix water	Required on each water to which the modification will be applied and on every 20th sample of that water thereafter. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Not required
MS/MSD (Section 9.5)	2 replicates of spiked matrix water	Recommended, but not required. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Required per laboratory. Each laboratory must analyze a different water.

(1) If a modification will be used only in one laboratory, these tests must be performed and the results must meet all of the QC acceptance criteria in the method (these tests also are required the first time a laboratory uses the validated version of the method).
(2) If nationwide approval of a modification is sought for one type of water matrix (such as surface water), a minimum of 3 laboratories must perform the tests and the results from each lab individually must meet all QC acceptance criteria in the method. If more than 3 laboratories are used in a study, a minimum of 75% of the laboratories must meet all QC acceptance criteria.

NOTE: The initial precision and recovery and ongoing precision and recovery (OPR) acceptance criteria listed in Tables 3 and 4 are based on results from 293 Cryptosporidium OPR samples and 186 Giardia OPR samples analyzed by six laboratories during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike acceptance criteria are based on data generated through interlaboratory validation of Method 1623 (Reference 20.10).

Table 3. Quality Control Acceptance Criteria for *Cryptosporidium*

Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation) Ongoing precision and recovery (percent)	 9.7	24 - 100 55 11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery1, 2(as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	 13 - 111 61

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries from multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 4.
Quality Control Acceptance Criteria for *Giardia*

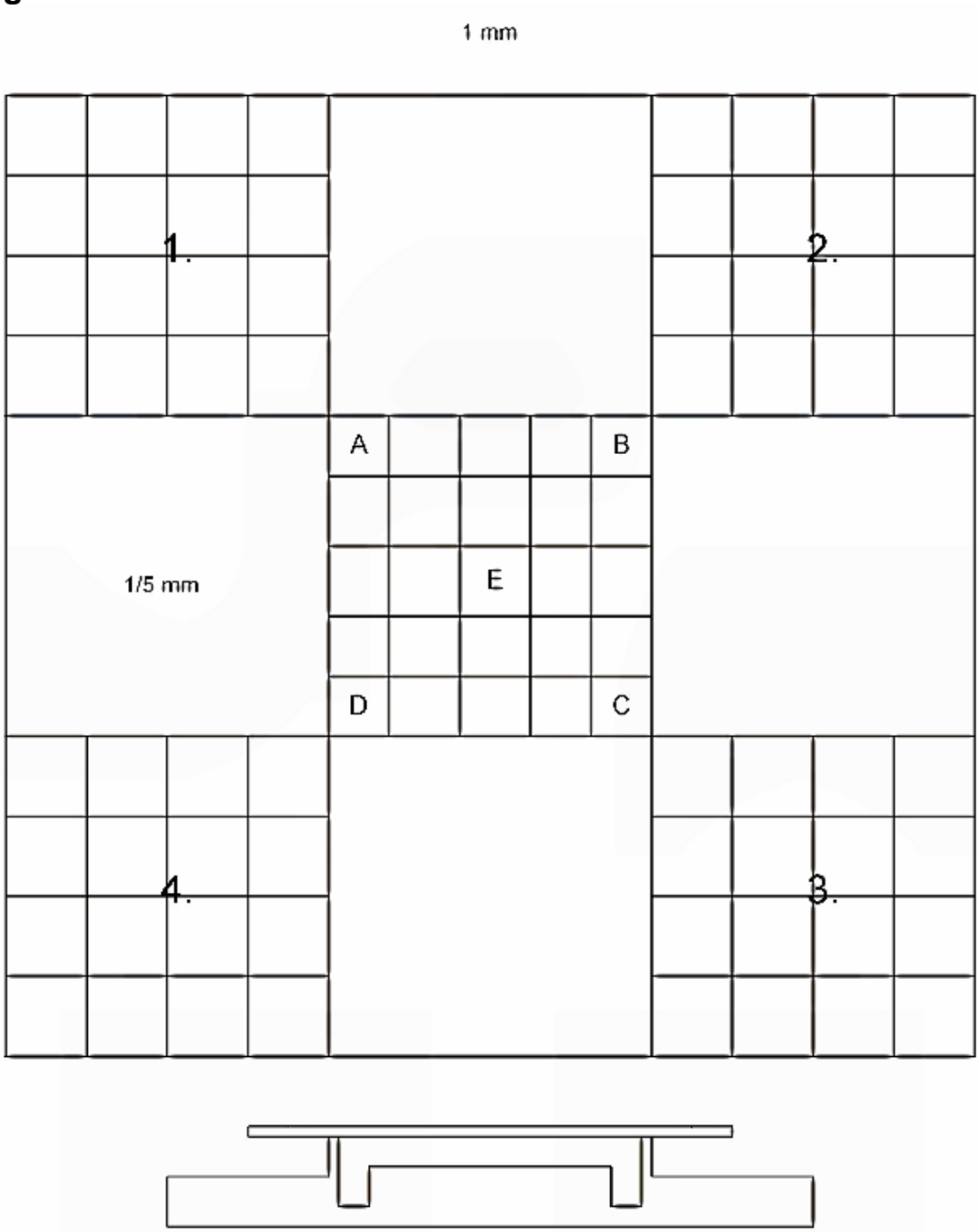
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation) Ongoing precision and recovery (percent)	 9.7	24 - 100 49 14 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery* (as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	 15 - 118 30

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries across multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 5. Distribution of Matrix Spike Recoveries from Multiple Samples Collected from 87 Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13)

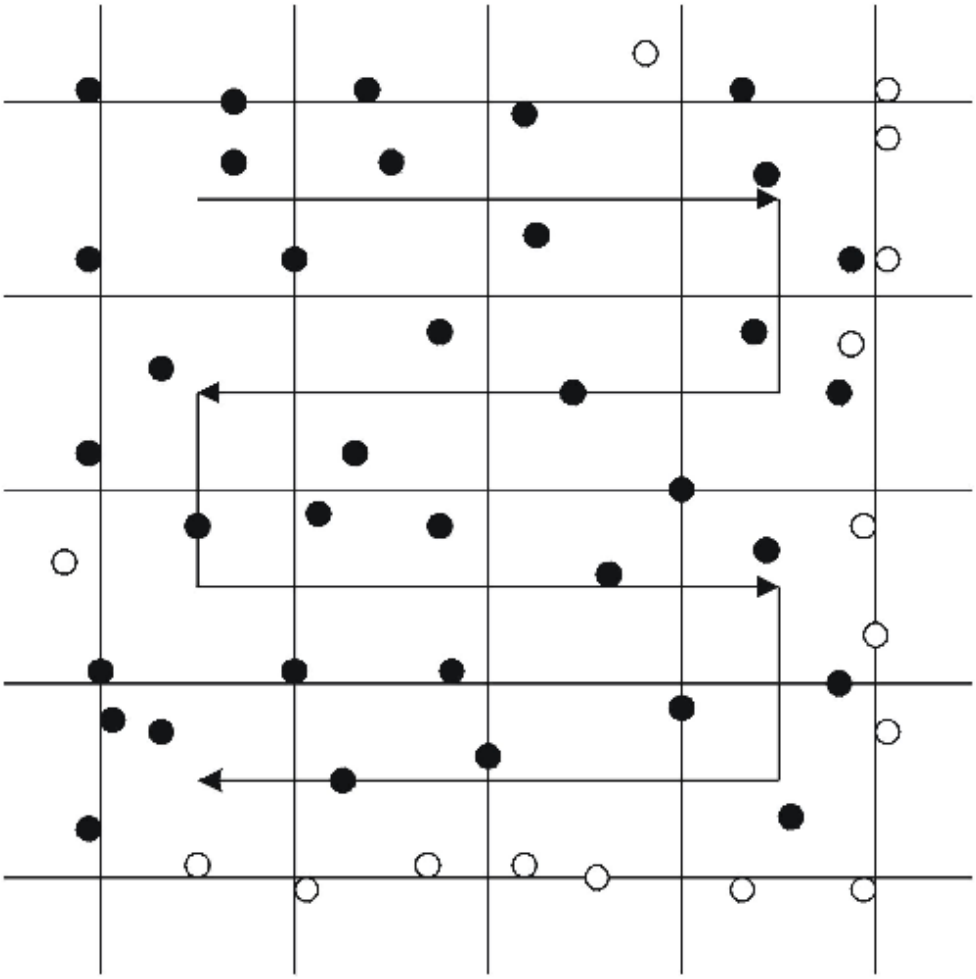
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium* MS Samples in Recovery Range	Percent of 270 *Giardia* MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 50%	18.4%	17.4%
>50% - 60%	17.4%	16.3%
>60% - 70%	11.2%	16.7%
>70% - 80%	8.4%	14.1%
>80% - 90%	2.3%	6.3%
>90%	0.2%	3.7%

Figure 1.



Hemacytometer Platform Ruling. Squares 1, 2, 3, and 4 are used to count stock suspensions of *Cryptosporidium*oocysts and *Giardia* cysts (after Miale, 1967)

Figure 2.



Manner of Counting Oocysts and Cysts in 1 Square mm. Dark organisms are counted and light organisms are omitted (after Miale, 1967).

Figure 3. Laboratory Filtration System

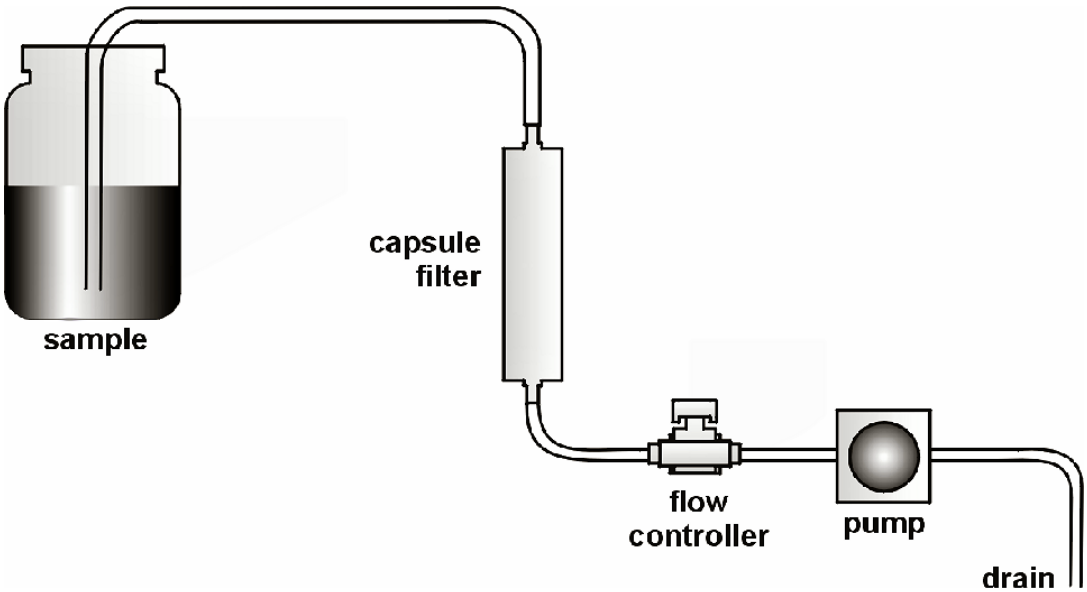
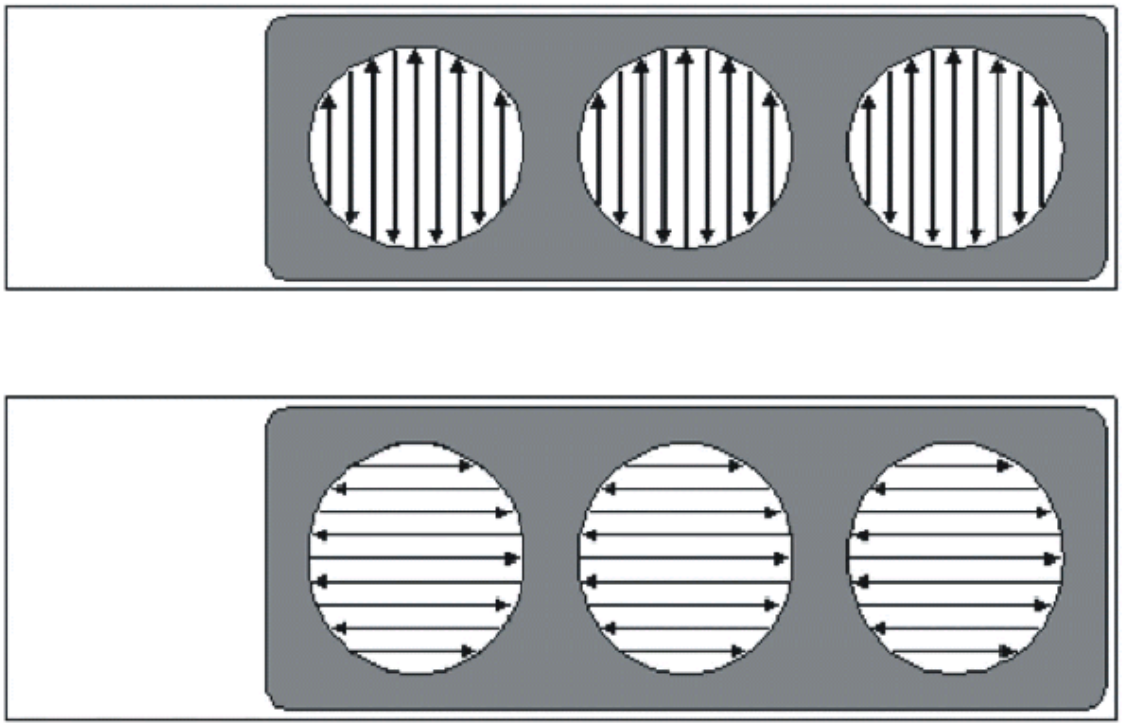


Figure 4. Methods for Scanning a Well Slide



Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using MI agar or MI broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl- β -D-galactopyranoside (MUGal) and a chromogen Indoxyl-β-D-glucuronide (IBDG), are included in the medium to detect the enzymes β -galactosidase and β -glucuronidase, respectively, produced by TC and *E. coli*, respectively.

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. coli* are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (References 16.8 - 16.10), will be used primarily by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human or veterinary), other environmental samples (*e.g.*, aerosols, soil, runoff, or sludge) and/or isolation and separation of transformants though the use of *E. coli lac Z* or *gus* A/*uid* reporter genes (Reference 16.11).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35°C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under long wave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β -galactosidase (Reference 16.8).

3.0 Definitions

3.1 Total coliforms (TC) - In this method, TC are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light (366 nm) after primary culturing on MI agar or broth (See Figure 1.). The fluorescent colonies can be completely blue-white (TC other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-green E. coli colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG (Reference 16.8).

3.2 Escherichia coli - In this method, the E. coli are those bacteria that produce blue colonies under ambient light after primary culturing on MI agar or broth (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under long wave ultraviolet light (366 nm) (Reference 16.8).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies which could interfere

with identification of target colonies. However, the blue E. coli colonies can often be counted on plates with heavy particulates or high concentrations of total bacteria (See Figures 2 and 3.) (Reference 16.8).
4.2 The presence of some lateral diffusion of blue color away from the target E. coli colonies can affect enumeration and colony picking on plates with high concentrations of E. coli. This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples (Reference 16.8).
4.3 Tiny, flat or peaked pinpoint blue colonies (# 0.5-mm in diameter on filters containing # 200 colonies) may be due to species other than E. coli. These colonies occur occasionally in low numbers and should be excluded from the count of the E. coli colonies, which are usually much larger in size (1-3-mm in diameter). The small colonies have never been observed in the absence of typical E. coli, but, if such should occur, the sample should not be considered E. coli-positive unless at least one colony has been verified by another method [e.g., EC medium with 4-Methylumbelliferyl- β -D-glucuronide (MUG) or API 20E strips] (Reference 16.8).
4.4 Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent TC colonies, may be species other than coliforms. These colonies, which generally occur in low numbers (# 5%) and can usually be distinguished from the TC, should be eliminated from the TC count. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium (Reference 16.8).

5.0 Safety
5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization equipment.
5.2 Mouth-pipetting is prohibited.
5.3 Avoid prolonged exposure to long wave or germicidal ultraviolet light.
5.4 Autoclave all contaminated plates and materials at the end of the analysis.

6.0 Equipment and Supplies
6.1 Incubator set at 35°C ± 0.5°C, with approximately 90% humidity if loose-lidded Petri dishes are used.
6.2 Stereoscopic microscope, with magnification of 10-15x, wide-field type.
6.3 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.
6.4 Hand tally.
6.5 Pipet container of stainless steel, aluminum, or Pyrex glass, for pipets.
6.6 Graduated cylinders (100-mL for drinking water), covered with aluminum foil or kraft paper and sterilized.
6.7 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel. These are wrapped with aluminum foil or kraft paper and sterilized.
6.8 Germicidal ultraviolet (254 nm) light box for sanitizing the filter funnels is desirable, but optional.
6.9 Line vacuum, electric vacuum pump, or aspirator is used as a vacuum source. In an emergency, a hand pump or a syringe can be used. Such vacuum-producing devices should be equipped with a check valve to prevent the return flow of air.
6.10 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.
6.11 Safety trap flask, placed between the filter flask and the vacuum source.
6.12 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.
6.13 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.
6.14 Bunsen or Fisher-type burner or electric incinerator unit.
6.15 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mLvolumes).

6.16 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ± 0.02-µm pore size, pretrial or sterilized for 10 minutes at 121°C (15-lb pressure).
6.17 Long wave ultraviolet lamp (366 nm), handheld 4-watt (preferred) or 6-watt, or microscope attachment.
6.18 Dilution water: Sterile phosphate-buffered dilution water, prepared in large volumes (e.g., 1 liter)for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks [Section 9050C in Standard Methods (Reference 16.2)].
6.19 Indelible ink marker for labeling plates.
6.20 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.
6.21 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids, or 15 x 60 mm, glass or plastic, with loose-fitting lids; 15 x 100 mm dishes may also be used.
6.22 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions (if needed). Dilution bottles marked at 90 mL, or tubes marked at 9 mL may be used for 1:10 dilutions.
6.23 Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, for agar preparation.
6.24 Waterbath maintained at 50°C for tempering agar.
6.25 Syringe filter, sterile, disposable, 25-mm diameter, 0.22-µm pore size, to filter cefsulodin for MI agar.
6.26 Syringe, sterile, plastic, disposable, 20-cc capacity. Autoclaved glass syringes are also acceptable.
6.27 Test tubes, sterile, screw-cap, 20 x 150-mm, borosilicate glass or plastic, with lids.
6.28 Sterilization filter units, presterile, disposable, 500- or 1000-mL capacity, 0.2-µm pore size, to filter stock buffer solutions.
6.29 Sterile 47-mm diameter absorbent pads (used with MI broth).
Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.0 Reagents and Standards
7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (Reference 16.1). The agar used in preparation of culture media must be of microbiological grade.
7.2 Whenever possible, use commercial culture media as a means of quality control.
7.3 Purity of Water: Reagent-grade distilled water conforming to Specification D1193, Type II water or better, ASTM Annual Book of Standards (Reference 16.3).
7.4 Buffered Dilution Water (Reference 16.2)
7.4.1 Stock Phosphate Buffer Solution (Reference 16.2):
Potassium Dihydrogen Phosphate (KH_2PO_4) 34.0 g Reagent-Grade Distilled Water 500 mL
7.4.2 Preparation of Stock Buffer Solution: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring volume to 1000 mL with reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.3 $MgCl_2$ Solution (Reference 16.2): Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2C6H_2O$) in one liter of reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.4 Storage of Stock Buffer and $MgCl_2$ Solutions: After sterilization of the stock solutions, store in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears in either stock, the solution should be discarded, and a fresh solution should be prepared.
7.4.5 Working Solution (Final pH 7.0 ± 0.2): Add 1.25 mL phosphate buffer stock (Section 7.4.2) and 5 mL $MgCl_2$ stock (Section 7.4.3) for each liter of reagent-grade distilled water prepared. Mix well, and dispense in appropriate amounts for dilutions in screw-cap dilution bottles or culture tubes, and/or into larger containers for use as rinse water. Autoclave at 121°C (15-lb pressure) for

15 minutes. Longer sterilization times may be needed depending on the container and load size and the amount of time needed for the liquid to reach 121°C.

7.5 MI Agar (Reference 16.8)
7.5.1 Composition:

Proteose Peptone #3	5.0 g
Yeast Extract	3.0 g
β -D-Lactose	1.0 g
4-Methylumbelliferyl- β -D-Galactopyranoside (MUGal) (Final concentration 100µg/mL)	0.1 g
Indoxyl- β -D-Glucuronide (IBDG) (Final concentration 320 µg/mL)	0.32 g
NaCl	7.5 g
K_2HPO_4	3.3 g
KH_2PO_4	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Agar	15.0 g
Reagent-Grade Distilled Water	1000 mL

7.5.2 Cefsulodin Solution (1 mg / 1 mL): Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-µm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time. Do not save the unused portion.
7.5.3 Preparation: Autoclave the medium for 15 minutes at 121°C (15-lb pressure), and add 5 mL of the freshly-prepared solution of Cefsulodin (5 µg/mL final concentration) per liter of tempered agar medium. Pipet the medium into 9 x 50-mm Petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks. The final pH should be 6.95 ± 0.2.
7.6 MI Broth: The composition of MI broth is the same as MI agar, but without the agar. The final pH of MI broth should be 7.05 ± 0.2. The broth is prepared and sterilized by the same methods described for MI agar in Sections 7.5.1, 7.5.2, and 7.5.3, except that absorbent pads are placed in 9 x 50 mm Petri dishes and saturated with 2-3 mL of MI broth containing 5 :g/mL final concentration of Cefsulodin. Alternately, the broth can be filter-sterilized. Excess broth is poured off before using the plates. Plates should be stored in the refrigerator and discarded after 96 hours (Reference 16.15).

7.7 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or equivalent) (TSA)

7.7.1 *Composition*:

Tryptone	15.0 g
Soytone	5.0 g
NaCl	5.0 g
Agar	15.0 g

7.7.2 *Preparation*: Add the dry ingredients listed above to 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121°C (15-lb pressure) for 15 minutes. Dispense the agar into 9 x 50-mm Petri dishes (5 mL/plate). Incubate the plates for 24 - 48 hours at 35°C to check for contamination. Discard any plates with growth. If > 5% of the plates show contamination, discard all plates, and make new medium. Store at 4°C until needed. The final pH should be 7.3 ± 0.2.

8.0 Sample Collection, Preservation, and Storage

8.1 Water samples are collected in sterile polypropylene sample containers with leakproof lids.

8.2 Sampling procedures are described in detail in Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Reference 16.2) or in the *USEPA Microbiology Methods Manual*, Section II, A (Reference 16.6). Residual chlorine in drinking water (or chlorinated effluent) samples should be neutralized with sodium thiosulfate (1 mL of a 10% solution per liter of water) at the time of collection. Adherence to sample preservation procedures and holding time limits are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.2.1 *Storage Temperature and Handling Conditions:* Ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water from melted ice during transit or storage.

8.2.2 *Holding Time Limitations:* Analyze samples as soon as possible after collection. Drinking water samples should be analyzed within 30 h of collection (Reference 16.13). Do not hold source water samples longer than 6 h between collection and initiation of analyses, and the analyses should be complete within 8 h of sample collection.

9.0 Calibration and Standardization

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits (Reference 16.14).

9.2 Check thermometers at least annually against an NIST-certified thermometer or one traceable to NIST. Check mercury columns for breaks.

10.0 Quality Control (QC)

10.1 Pretest each batch of MI agar or broth for performance (*i.e.*, correct enzyme reactions) with known cultures (*E. coli*, TC, and a non-coliform).

10.2 Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (Reference 16.7).

10.3 Perform specific filtration control tests each time samples are analyzed, and record the results.

10.3.1 *Filter Control*: Place one or more membrane filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the filter(s).

10.3.2 *Phosphate-Buffered Dilution Water Controls*: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the dilution water.

10.3.3 *Agar or Broth Controls*: Place one or more TSA plates and one or more MI agar plates or MI broth pad plates in the incubator for 24 hours at 35°C. Broth pad plates should be incubated *grid-side up*, not inverted like the agar plates. Absence of growth indicates sterility of the plates.

10.4 See recommendations on quality control for microbiological analyses in the "*Manual for the Certification of Laboratories Analyzing Drinking Water*: *Criteria and Procedures; Quality Assurance*" (Reference 16.15) and the *USEPA Microbiology Methods Manual*, part IV, C (Reference 16.6).

11.0 Procedure

11.1 Prepare MI agar or MI broth and TSA as described in Sections 7.5, 7.6, and 7.7. If plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. The crystals that form on MI agar after refrigeration will disappear as the plates warm up (Reference 16.8).

11.2 Label the bottom of the MI agar or MI broth plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar or MI broth sterility control plate(s).

11.3 Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.
11.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.
11.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.
11.6 Shake the sample container vigorously 25 times.
11.7 Measure an appropriate volume (100 mL for drinking water) or dilution of the sample with a sterile pipette or graduated cylinder, and pour it into the funnel. Turn on the vacuum, and leave it on while rinsing the funnel twice with about 30 mL sterile dilution water.
11.8 Remove the funnel from the base of the filter unit. A germicidal ultraviolet (254 nm) light box can be used to hold and sanitize the funnel between filtrations. At least 2 minutes of exposure time is required for funnel decontamination. Protect eyes from UV irradiation with glasses, goggles, or an enclosed UV chamber.
11.9 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the MI agar plate or MI broth pad plate. Slide the filter onto the agar or pad, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar or absorbent pad. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar or pad. Reseat the membrane if non-wetted areas occur due to air bubbles.
11.10 Invert the agar Petri dish, and incubate the plate at 35°C for 24 hours. Pad plates used with MI broth should be incubated grid-side up at 35°C for 24 hours. If loose-lidded plates are used for MI agar or broth, the plates should be placed in a humid chamber.
11.11 Count all blue colonies on each MI plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the E. coli count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete (Reference 16.14).
11.12 Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also E. coli)] (See Figure 1.). Record the data.

11.13 Add any blue, non-fluorescent colonies (if any) found on the same plate to the TC count (Reference 16.8).

12.0 Data Analysis and Calculations
12.1 Use the following general rules to calculate the E. coli or TC per 100 mL of sample:

12.1.1 Select and count filters with # 200 total colonies per plate.

12.1.2 Select and count filter with # 100 target colonies (ideally, 20-80).

12.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^{+} (TNTC)" and count the number of E. coli. If both target organisms are $ 200, record the results as "TC^{+} EC^{+} (TNTC)".

12.1.4 Calculate the final values using the formula:

$$\text{E. coli/100 mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{TC/100 mL} = \frac{\text{Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules (Reference 16.6).
12.3 Report results as E. coli or TC per 100 mL of drinking water.

13.0 Method Performance

13.1 The detection limits of this method are one E. coli and/or one total coliform per sample volume or dilution tested (Reference 16.8).

13.2 The false-positive and false-negative rates for E. coli are both reported to be 4.3% (Reference 16.8).

13.3 The single lab recovery of E. coli is reported (Reference 16.8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (Reference 16.2) and 115% of the R2A spread plate (Reference 16.2). For Klebsiella pneumoniae and Enterobacter aerogenes, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (Reference 16.8), respectively, and 89.3% and 85.8% of the R2A spread plate, respectively.

13.4 The specificities for *E. coli* and total coliforms are reported to be 95.7% and 93.1% (Reference 16.8), respectively.

13.5 The single lab coefficients of variation for *E. coli* and total coliforms are reported to be 25.1% and 17.6% (Reference 16.8), respectively, for a variety of water types.

13.6 In a collaborative study (References 16.4, 16.5, and 16.9), 19 laboratories concurrently analyzed six wastewater-spiked Cincinnati tap water samples, containing 3 different concentrations of *E. coli* (# 10, 11-30, and > 30 per 100 mL).

13.6.1 The single laboratory precision (coefficient of variation), a measure of the repeatability, ranged from 3.3% to 27.3% for *E. coli* and from 2.5% to 5.1% for TC for the six samples tested, while the overall precision (coefficient of variation), a measure of reproducibility, ranged from 8.6% to 40.5% and from 6.9% to 27.7%, respectively. These values are based on $\log_{10}$-transformed data (Reference 16.5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (Reference 16.9) results.

14.0 Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

16.0 References

16.1 American Chemical Society. 1981. Reagent Chemicals. In American Chemical Society Specifications, 6th edition. American Chemical Society, Washington, D.C. For suggestions on the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K. and the United States Pharmacopeia.

16.2 American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition. American Public Health Association, Washington, D.C.

16.3 American Society for Testing and Materials. 1993. Standard Specification for Reagent Water, Designation D1193-91, p. 45-47. In 1993 Annual Book of ASTM Standards: Water and Environmental Technology, Volume 11.01. American Society for Testing and Materials, Philadelphia, PA.

16.4 American Society for Testing and Materials. 1994. Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water, Designation D 2777-86, p. 31-44. In 1994 Annual Book of ASTM Standards, Section 11: Water and Environmental Technology, Volume 11.01. American Society for Testing and Materials, Philadelphia, PA.
16.5 Association of Official Analytical Chemists. 1989. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis. Journal of the Association of Official Analytical Chemists 72 (4): 694-704.
16.6 Bordner, R., J. Winter, and P. Scarpino (ed). 1978. Microbiological Methods for Monitoring the Environment: Water and Wastes. EPA-600/8-78-017, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.
16.7 Brenner, K.P., and C.C. Rankin. 1990. New Screening Test to Determine the Acceptability of 0.45-µm Membrane Filters for Analysis of Water. Applied and Environmental Microbiology 56: 54-64.
16.8 Brenner, K.P., and C.C. Rankin, Y.R. Roybal, G.N. Stelma, Jr., P.V. Scarpino, and A.P. Dufour. 1993. New Medium for the Simultaneous Detection of Total Coliforms and Escherichia coli in Water. Applied and Environmental Microbiology 59: 3534-3544.
16.9 Brenner, K.P., C.C. Rankin, and M. Sivaganesan. 1996. Interlaboratory Evaluation of MI Agar and the U.S. Environmental Protection Agency-Approved Membrane Filter Method for the Recovery of Total Coliforms and Escherichia coli from Drinking Water. Journal of Microbiological Methods 27: 111-119.
16.10 Brenner, K.P., C.C. Rankin, M. Sivaganesan, and P.V. Scarpino. 1996. Comparison of the Recoveries of Escherichia coli and Total Coliforms from Drinking Water by the MI Agar Method and the U.S. Environmental Protection Agency-Approved Membrane Filter Method. Applied and Environmental Microbiology 62 (1): 203-208.
16.11 Buntel, C.J. 1995. E. coli $-Glucuronidase (GUS) as a Marker for Recombinant Vaccinia Viruses. BioTechniques 19 (3); 352-353.
16.12 Federal Register. 1985. National Primary Drinking Water Regulations; Synthetic Organic Chemicals, Inorganic Chemicals and Microorganisms; Proposed Rule. Federal Register 50: 46936-47022.
16.13 Federal Register. 1994. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants; Final Rule. *Federal Register* 59: 62456-62471.
16.14 Federal Register. 1999. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule. *Federal Register* 64: 67450-67467.
16.15 U.S. Environmental Protection Agency. 1992. Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, Third Edition. EPA-814B-92-002, Office of Ground Water and Drinking Water, Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

17.0 Tables and Figures

Table 1. Statistical Summary of the Collaborative Study Results[1]

Target Organism	Sample Number	*E. coli* Count Category (Range)[2]	Initial n[3]	Final n[4]	S_r[5]	RSD_r[6] (%)	$\bar{\chi}$[7]	S_R[8]	RSD_R[9] (%)	$\frac{RSD_R}{RSD_r}$ Ratio
Escherichia coli	1	Low (≤ 10)	63	63	0.17	27.3	0.64	0.26	40.5	1.49
	2		63	63	0.21	25.0	0.84	0.33	39.0	1.56
	3	Medium (11-30)	63	63	0.10	7.9	1.27	0.15	12.1	1.52
	4		63	60	0.07	5.6	1.32	0.12	9.2	1.65
	5	High (> 30)	63	60	0.06	3.3	1.87	0.16	8.6	2.62
	6		63	63	0.09	4.3	1.99	0.25	12.6	2.91
Total Coliforms	1	Low (≤ 10)	63	63	0.10	4.3	2.35	0.62	26.4	6.11
	2		63	63	0.09	3.8	2.31	0.64	27.7	7.25
	3	Medium (11-30)	63	63	0.11	5.1	2.17	0.47	21.8	4.28
	4		63	57	0.10	3.3	3.07	0.21	6.9	2.08
	5	High (> 30)	63	63	0.15	4.8	3.10	0.43	14.0	2.96
	6		63	63	0.08	2.5	3.14	0.46	14.7	5.97

[1] The values are based on $\log_{10}$ transformed data (Reference 16.5).

[2] The samples were grouped by their *E. coli* count on MI agar into the following categories:
Low (# 10 *E. coli* / 100 mL, samples 1 and 2),
Medium (11-30 *E. coli* / 100 mL, samples 3 and 4), and
High (> 30 *E. coli* / 100 mL, samples 5 and 6).

[3] These values are based on triplicate analyses by each laboratory. The reference laboratory analyzed three sets of samples: the initial and final samples prepared and a sample shipped along with the other 18 lab samples.

[4] These values were obtained after removing outliers by the AOAC procedure (Reference 16.5).

[5] S_r, Single Operator Standard Deviation, a measure of repeatability.

[6] RSD_r, Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability.

[7] $\bar{\chi}$, The mean of the replicate analyses for all laboratories.

[8] S_R, Overall Standard Deviation, a measure of reproducibility.

[9] RSD_R, Overall Relative Standard Deviation (Coefficient of Variation), a measure of reproducibility.

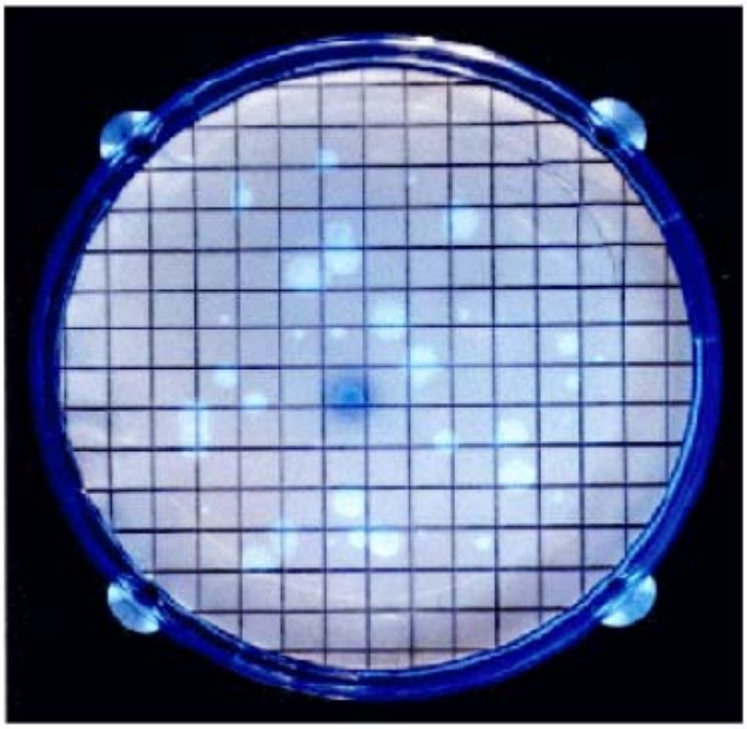


Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

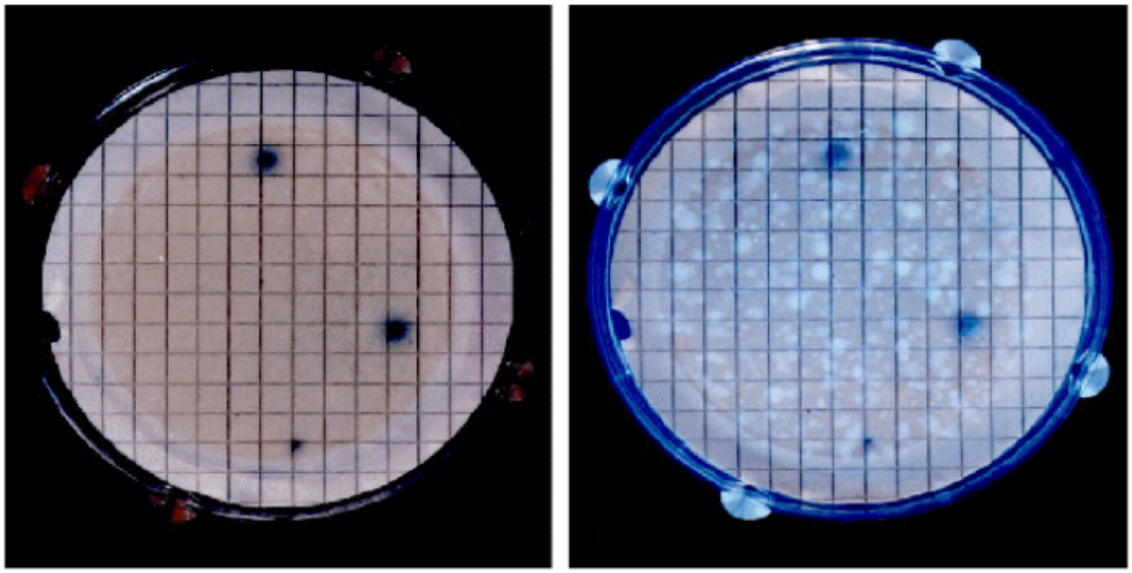


Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other than *E. coli* and non-coliforms are their natural color. Under long wave UV light, all total coliforms, including *E. coli*, are fluorescent, and noncoliforms are non-fluorescent (*i.e.*, they are not visible).

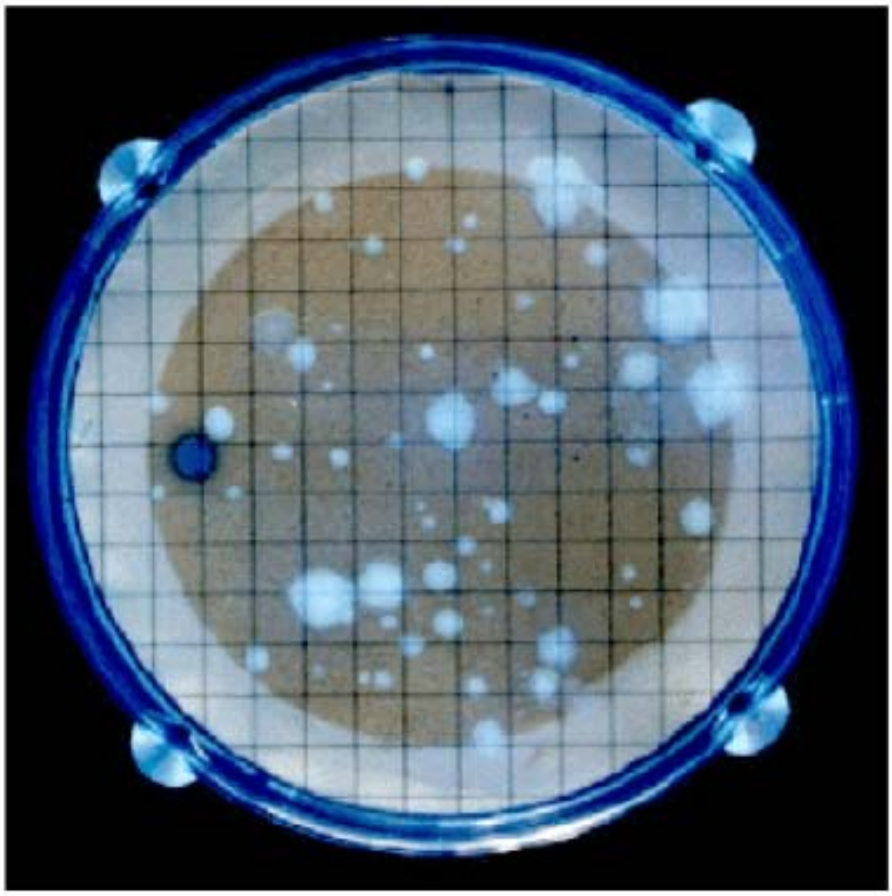


Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

Disclaimer

This method has been validated by the U.S. Environmental Protection Agency through an interlaboratory validation study, and will be proposed for use in drinking water monitoring in the *Federal Register*. This method is not an EPA-approved method until it is promulgated as an approved method in the *Federal Register*.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

Aeromonas is a common genus of bacteria indigenous to surface waters, and may be found in non-chlorinated or low-flow parts of chlorinated water distribution systems. Monitoring their presence in distribution systems is desirable because some aeromonads may be pathogenic and pose a potential human health risk. Method 1605 describes a membrane filtration technique for the detection and enumeration of *Aeromonas* species. This method uses a selective medium that partially inhibits the growth of non-target bacterial species while allowing most species of *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation and the presence of yellow colonies on ampicillin-dextrin agar medium with vancomycin (ADA-V). Yellow colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Safe Drinking Water Act.

Questions concerning this method or its application should be addressed to:

Mary Ann Feige
U.S. EPA Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
26 West Martin Luther King Drive
Cincinnati, OH 45268-1320

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Aeromonas* species in finished water samples. *Aeromonas* is a common genus of bacteria indigenous to surface waters. Its numbers are more likely to be greater during periods of warmer weather and when increased concentrations of organic nutrients are present. It is also more likely to be found in non-chlorinated water distribution systems or low-flow parts of chlorinated systems. Some *Aeromonas* species are opportunistic pathogens.

1.2 This method is adapted from Havelaar et al. (1987) for the enumeration of *Aeromonas* species in finished water by membrane filtration (Reference 15.1). It is a quantitative assay that uses a selective medium which partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation producing yellow colonies. Presumptively positive colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

1.3 This method is designed to meet the finished water monitoring requirements of the U.S. Environmental Protection Agency. *Aeromonas* was included on the Contaminant Candidate List (CCL) (Mar. 2, 1998, 63 *FR* 10274) and in the Revisions to the Unregulated Contaminant Monitoring Proposed Rule (UCMR) (September 17, 1999, 64 FR 50556). Contaminants listed in the UCMR are candidates for future regulation and may be included in a monitoring program for unregulated contaminants. Unregulated contaminant monitoring would be required for large systems and a representative sample of small and medium sized water distribution systems.

1.4 This method was subjected to an interlaboratory validation study involving 11 laboratories and 11 finished drinking water matrices. This method was not validated for other water types. Use of this method and appropriate validation for other water types is the responsibility of the user.

2.0 Summary of Method

2.1 The method provides a direct count of *Aeromonas* species in water based on the growth of yellow colonies on the surface of the membrane filter using a selective medium. A water sample is filtered through 0.45-Fm-pore-size membrane filter. The filter is placed on ampicillin-dextrin agar with vancomycin (ADA-V) and incubated at 35EC ± 0.5EC for 24 ± 2 hours. This medium uses ampicillin and vancomycin to inhibit non-*Aeromonas* species, while allowing most *Aeromonas* species to grow. The medium uses dextrin as a fermentable carbohydrate, and bromothymol blue as an indicator of acidity produced by the fermentation of dextrin. Presumptively identified yellow colonies are counted and confirmed by testing for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose and produce indole.

2.2 The membrane filtration procedure provides a direct count of culturable Aeromonas in water samples that is based on the growth of bacterial colonies on the surface of the membrane filter placed on a selective medium.

2.3 Aeromonas isolates may be archived for further analysis to determine species or hybridization group by inoculating a nutrient agar slant for short term use or shipping, or nutrient broth for freezing.

3.0 Definitions

3.1 Aeromonas are bacteria that are facultative anaerobes, Gram-negative, oxidase-positive, polarly flagellated, and rod shaped. They are classified as members of the family Aeromonadaceae. Demarta et al. (1999) reported 15 Aeromonas species based on 16S rDNA sequences though not all are officially recognized. Some species have been associated with human disease. In this method, Aeromonas are those bacteria that grow on ampicillin-dextrin agar with vancomycin (ADA-V), produce yellow colonies, are oxidase-positive, and have the ability to ferment trehalose and produce indole.

3.2 Definitions for other terms are provided in the glossary at the end of the method (Section 17.3).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material may clog the membrane filter and prevent filtration or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.2 Other ampicillin/vancomycin resistant bacteria that are not aeromonads may be able to grow on this medium. Some of these bacteria may also produce yellow colonies if they are able to produce acid byproducts from the fermentation of dextrin or some other media component, or if they produce a yellow pigment. Enterococcus are reported to produce pinpoint-size yellow colonies on ADA. Confirmation of presumptive Aeromonas colonies is necessary to mitigate false positives.

5.0 Safety

5.1 Some strains of Aeromonas are opportunistic pathogens. Sample containers and waste materials should be autoclaved prior to cleaning or disposal.

5.2 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and other materials.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Safety Data Sheet (formerly MSDS)s (SDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Equipment for collection and transport of samples to laboratory

- **6.1.1** Autoclavable sample container—Use sterile, non-toxic, glass or plastic containers with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis.
- **6.1.2** Ice chest
- **6.1.3** Ice packs

6.2 Autoclavable dilution bottles—125-mL marked at 99 mL or 90 mL; commercially produced dilution bottles may be used.

6.3 Rinse water bottles

6.4 Sterile plastic or autoclavable glass pipettes with a 2.5% tolerance—to deliver (TD), 1- and 10-mL

6.5 Pipette bulbs or automatic pipetter.

6.6 Autoclavable pipette container (if using glass pipettes).

6.7 Thermometer—with 0.5EC gradations checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.

6.8 Inoculating loop—Sterile metal, plastic, or wooden applicator sticks.

6.9 Burner—Flame or electric incinerator for sterilizing metal inoculating loops and forceps.

6.10 Colony counting device—Mechanical, electric or hand tally.

6.11 Hotplate stirrer

6.12 Magnetic stir bar

6.13 Graduated cylinders—100 mL, 500 mL and 1 L, sterile, polypropylene or glass

6.14 Balance—Capable of weighing samples up to 200 g, with a readability of 0.1 g

6.15 Weigh boats

6.16 pH meter

6.17 Turbidimeter (optional)

6.18 Equipment for membrane filter procedure

6.18.1 Incubator—Hot air or water-jacketed microbiological type to maintain a temperature of 35EC ± 0.5EC
6.18.2 Petri dishes—sterile, 50 × 9 mm or other appropriate size
6.18.3 Membrane filtration units (filter base and funnel made of glass, plastic, or stainless steel), wrapped with aluminum foil or Kraft paper, and sterilized by autoclaving.
6.18.4 Vacuum source
6.18.5 Flasks—1-L vacuum filter with appropriate tubing; a filter manifold to hold a number of units is optional
6.18.6 Side-arm flask to place between vacuum source and filtration devices or filter manifold
6.18.7 Membrane filters—Sterile, cellulose ester, white, gridded, 47-mm-diameter with 0.45-Fm pore size (Gelman E04WG04700 or equivalent)
6.18.8 Forceps—Sterile, straight or curved, with smooth tips to handle filters without causing damage
6.18.9 Ethanol or other alcohol in a container to sterilize forceps
6.18.10 Test tubes—125 × 16 mm sterile, screw-cap tube
6.19 Dissecting microscope—Low power (10X to 15X), binocular, illuminated
6.20 Autoclave—Capable of 121EC at 15 psi. Must meet requirements set forth in the Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition. (Reference 15.5)
6.21 Membrane filters (for sterilization purposes)—Sterile with 0.22-Fm pore size (Gelman Acrodisc No. 4192 or equivalent)

7.0 Reagents and Standards
7.1 Purity of reagents and culture media—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents and culture media shall conform to the specifications in Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9050 (Reference 15.2). The agar used in preparation of culture media must be of microbiological grade.
7.2 Purity of water—Reagent-grade water conforming to specifications in Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition (Reference 15.5) or Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9020 (Reference 15.2).

7.3 Phosphate buffered dilution water
7.3.1 Concentrated stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water. Adjust the pH to 7.2 ± 0.5 with 1N sodium hydroxide (NaOH) and dilute to 1 L with reagent-grade water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.
7.3.2 Magnesium chloride solution—Dissolve 81.1 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$) in reagent-grade water and dilute to 1 L. Autoclave or filter sterilize through a 0.22-Fm-pore-size filter.
7.3.3 Prepare phosphate buffered dilution water by adding 1.25 mL of concentrated stock phosphate buffer solution (Section 7.3.1) and 5.0 mL of magnesium chloride solution (Section 7.3.2) to a 1-L graduated cylinder and adjust final volume to 1 L with reagent-grade water. Prepare a portion of buffered dilution water in 1-L bottles for rinse water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.
7.3.4 Stored phosphate buffered dilution water should be free from turbidity.
7.4 Ampicillin-dextrin agar with vancomycin (ADA-V)
7.4.1 Preparation of dextrin agar—EPA highly recommends the use of commercial ADA (m-Aeromonas Selective Agar Base [Havelaar]), Section **7.4.1.1**. However, ADA may be prepared by the laboratory (Section 7.4.1.2)
7.4.1.1 Commercial dextrin agar—Tech Pac (distributor, tech@fuse.net), Cincinnati, Ohio; Biolife (www.biolifeit.com) Italiana Srl, 272 Viale Monza, Milan, Italy, Cat. No. 401019 or equivalent. Prepare 1-L of media, according to manufacturer's instructions. Cool to room temperature, and adjust

pH to 8.0 using 1N NaOH or 1N HCl. Autoclave for 15 min, cool to 50EC.

7.4.1.2 Laboratory-prepared dextrin agar.

7.4.1.2.1 5.0 g tryptose—Difco cat. no. 0124-17, or equivalent

7.4.1.2.2 11.4 g dextrin—Difco cat. no. 0161-17, or equivalent

7.4.1.2.3 2.0 g yeast extract—Difco cat. no. 0127-17, or equivalent

7.4.1.2.4 3.0 g sodium chloride (NaCl)—Baker cat. no. 3624, or equivalent

7.4.1.2.5 2.0 g potassium chloride (KCl)—Fisher cat. no. P217-500, or equivalent

7.4.1.2.6 0.1 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)—Fishercat. no. M63-500, or equivalent

7.4.1.2.7 0.06 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)—Sigma cat. no. F-2877, or equivalent

7.4.1.2.8 0.08 g bromothymol blue—Baker cat. no. D470, or equivalent

7.4.1.2.9 Sodium deoxycholate—Sigma cat. no. D-6750, or equivalent. Add 100 mg of sodium deoxycholate to 10 mL of reagent water.

7.4.1.2.10 13.0 g agar, bacteriological grade—Fisher cat. no. BP1423-500, or equivalent.

7.4.1.2.11 Add reagents in Sections 7.4.1.2.1 through 7.4.1.2.8 to 1-L of reagent-grade water, stir to dissolve and adjust pH to 8.0 using1N NaOH or 1N HCl. After the pH has been adjusted, add sodium deoxycholate (Section 7.4.1.2.9) and agar (Section7.4.1.2.10) and heat to dissolve. Autoclave for 15 min, cool to 50°C.

7.4.2 Ampicillin, sodium salt—Sigma cat. no. A0166, or equivalent. Add 10 mg of ampicillin, sodium salt to 10 mL reagent water. Prepare on the same day that medium is prepared and filter sterilize through a 0.22-Fm-pore-size filter. Alternatively, use Biolife cat. no. 4240012 prepared according to manufacturer's instructions, taking care to use an appropriate amount of ampicillin for the volume of media being prepared (for example, use two vials for a 1-L batch of ADA-V). Follow manufacturer's instructions
for appropriate storage temperature and shelf-life. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.3 Vancomycin hydrochloride—Sigma cat. no. V2002, or equivalent. Add 2 mg of vancomycin hydrochloride to 10 mL of reagent water. Filter sterilize through a 0.22-Fm-pore-size filter. Follow manufacturer's instructions for appropriate storage temperature and time. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.4 After dextrin agar (Section 7.4.1) has been autoclaved and cooled to 50EC, add the sterile ampicillin (Section 7.4.2) and sterile vancomycin hydrochloride solutions (Section 7.4.3).

7.4.5 Add approximately 5 mL of ADA-V per 50 × 9 mm Petri dish and allow to solidify. For larger plates, adjust volume appropriately. ADA-V plates should be stored in a tight fitting container (i.e. sealed plastic bag) at a temperature of 1EC to 5EC for no longer than 14 days.

7.5 Pentahydrate ACS Reagent grade sodium thiosulfate—Fisher cat. no. S445-500, or equivalent. Prepare a 3% stock solution by adding 3 g sodium thiosulfate to 100 mL reagent-grade water.

7.6 Disodium salt of ethylenediaminetetraacetic acid (EDTA)—Sigma cat. no. E 4884, or equivalent. EDTA should only be added to samples if metals in water samples exceed 1.0 mg/L. To prepare stock solution, add 12.4 g EDTA to 80 mL of reagent-grade water. Adjust pH to 8.0 using 10N NaOH. After the pH has been adjusted, bring the volume up to 100 mL with reagent-grade water.

7.7 Positive control culture—Aeromonas hydrophila ATCC #7966; obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, 20110-2209; http://www.atcc.org).

7.8 Negative culture control—Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-Aeromonas bacteria that may grow on ADA-V and to ensure that confirmation test results are appropriate. E. coli (ATCC #25922) is the negative culture control for oxidase, Pseudomonas aeruginosa (ATCC #27853) is the

negative culture control for trehalose fermentation, and Bacillus cereus (ATCC #11778) is the negative culture control for indole.

7.9 Nutrient agar—Difco cat. no. 0001-17 or equivalent. Prepare according to manufacturer's instructions.

7.10 Oxidase reagents—Dry Slide BBL cat. no. 231746 or equivalent.

7.11 0.5% Trehalose confirmation reagent

7.11.1 Add 5 g trehalose (Sigma cat. no. T0167, or equivalent) to 100 mL water and filter sterilize solution through a filter with 0.22-Fm-pore-size.

7.11.2 Prepare 900 mL purple broth base (Difco cat. no. 0222-17, or equivalent) according to manufacturer's instructions and autoclave.

7.11.3 Aseptically add 100 mL trehalose solution to the cooled 900 mL of purple broth base.

7.11.4 Dispense into 6 mL or larger size tubes and fill approximately half full. Store in refrigerator.

Note: *Alternatively, prepare purple broth base according to manufacturer's instructions, add 5 g trehalose per liter, and filter sterilize through a filter with 0.22-Fm-pore-size.*

7.12 Tryptone broth—Oxoid cat. no. CM0087B, or equivalent. Alternatively, the laboratory may prepare tryptone broth by adding 10 g of tryptone (Difco cat. no. 0123-17 or equivalent) and 5 g of NaCl to 1 L of reagent water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.13 Kovac's reagent—Biomeriuex cat. no. V7050, or equivalent

8.0 Sample Collection, Preservation, and Storage

8.1 Adherence to sample preservation procedures and holding time limits specified in *Standard Methods for the examination of Water and Wastewater* (Reference 15.2) is critical to the production of valid data. Sample results will be considered invalid if those conditions are not met.

8.2 Preparation of sample bottles and sample collection—Samples must be representative of the drinking water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

8.2.1 Use sterile, non-toxic, glass or plastic container (Section 6.1.1) with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis. Sampling procedures are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9060 (Reference 15.2).

8.2.2 Add 1 mL of 3% sodium thiosulfate stock (Section 7.5) per L of sample to sample bottles prior to autoclave sterilization. Alternatively, if using presterilized sample bottles, sodium thiosulfate should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size before adding to the sample bottles.

8.2.3 If metals in the sample exceed 1.0 mg/L, add 3 mL of EDTA stock solution (Section 7.6) per L of sample to sample bottles prior to autoclave sterilization. If using presterilized sample bottles, EDTA should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size.

8.2.4 Collect a minimum of 1-L of sample.

8.3 Sample preservation and handling

8.3.1 Immediately following sample collection, tighten the sample container lid(s) and place the sample container(s) upright in an insulated, plastic-lined storage cooler with ice packs or in a refrigerator to chill prior to packing the cooler for shipment. Do not freeze the sample.

8.3.2 Use enough solidly frozen ice packs to ensure that the samples will arrive at a temperature of 1°C to 10°C. Use a minimum of two ice packs per shipment and add extra ice packs for multiple samples. Place one or more ice packs on each side of the container to stabilize samples.

8.3.3 Samples must be maintained at a temperature of 1°C to 10°C during shipment. Samples must not be frozen.

Note: Sample temperature during shipment is critical. Ice packs must be frozen solid immediately prior to shipment.

8.4 Verify and record sample arrival temperature when received in the laboratory. Refrigerate samples at $1^{o}C$ to $5^{o}C$ upon receipt at the laboratory and analyze as soon as possible after collection. Samples must be analyzed within 30 hours of sample collection.

9.0 Quality Control

9.1 Each laboratory that uses Method 1605 is required to operate a formal quality assurance (QA) program. The minimum QA requirements consist of the initial demonstration of capability (IDC) test (Section 9.4), ongoing analysis of spiked reagent water (ODC test, Section 9.8) and spiked finished drinking water samples (MS/MSD, Section 9.7), and analysis of negative culture controls (Section 9.6), dilution/rinse water blanks (Section 9.5), and media sterility checks (Section 9.2.6) as tests of continued acceptable performance. Spiked sample results are compared to acceptance criteria for precision, which are based on data generated during the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished water matrices. The more stringent QA requirements in this method, relative to other, currently used methods for bacterial determination, are an effort to improve overall microbiological QA. The specifications contained in this method can be met if the analytical system is maintained under control. Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections 10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

9.2 General QC requirements—Specific quality control (QC) requirements for Method 1605 are provided below. QA and QC criteria for facilities, personnel, and laboratory equipment, instrumentation, and supplies used in microbiological analyses must be followed according to Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141, Reference 15.2) and the U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fourth Edition (March 1997) (Reference 15.5).

9.2.1 Initial demonstration of capability (IDC). The laboratory shall demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples. The procedure for performing the IDC is described in Section 9.4. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.2.2 Dilution/rinse water blanks. The laboratory shall analyze dilution/rinse water blanks to demonstrate freedom from contamination. The procedures for analysis of dilution/rinse water blanks are described in Section 9.5. At a minimum, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series.

9.2.3 Negative culture controls. The laboratory shall analyze negative culture controls (Section 9.6) to ensure that ADA-V and the confirmation procedures are performing properly. Negative culture controls should be run whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples.

9.2.4 Matrix spike/matix spike duplicate (MS/MSD). The laboratory shall analyze one set of MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples (Section 9.7). Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also

recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines. When possible, MS/MSD analyses should be conducted on the same day as ODC samples, using the same spiking procedure and volume.
9.2.4.1 Precision. MS/MSD sample results should meet the precision criteria set forth in Section 12, Table 1.
9.2.4.2 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the number of Aeromonas in the spike is unknown. However, each laboratory should control chart the mean number of Aeromonas per MS/MSD set (adjusted for background) and maintain a record of spike preparation procedures and spike volume. The laboratory should compare number of Aeromonas in MS/MSD samples to results of ODC samples (Section 9.2.5 and 9.8) spiked on the same day. This comparison should help the laboratory recognize when a matrix is interfering with method recovery. If the laboratory observes consistent ODC results from week to week, control charting the MS/MSD results by source may also help to recognize fluctuations in recovery from a particular source.

9.2.5 Ongoing demonstration of capability (ODC). The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate, Section 9.8).
9.2.5.1 Frequency. The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently. No more than one set of ODC samples is required per day, provided that the same equipment (i.e., incubators) are being used for all the samples.
9.2.5.2 Precision. ODC sample results must meet the precision criteria set forth in Section 12, Table 1.
9.2.5.3 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the initial spike dose for ODC samples is unknown.
As a result, each laboratory should control chart the mean number of Aeromonas per ODC sample set and maintain a record of spike preparation procedures and ODC spike volume. Maintaining this information will enable the laboratory to recognize when problems arise. Example: A laboratory that prepares spiking suspensions according to Section 9.3, spikes QC samples with 5 mL of dilution D2, and typically recovers approximately 50 Aeromonas per sample, and maintains a control chart of these counts. If the laboratory continues to prepare spiking suspensions the same way, but the number of Aeromonas counted declines noticeably (e.g. 20 Aeromonas per sample), then there may be a problem with the media, reagents, or the spiking suspension.
9.2.6 Media sterility checks. The laboratory shall test media sterility by incubating one unit (tube or plate) from each batch of medium (ADA-V, nutrient agar slant, nutrient agar, streak plate, trehalose, and tryptone) at 35°C ± 0.5°C for 24 ± 2 hours and observing for growth.
9.2.7 Analyst colony counting variability. If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one ODC sample per month (Section 9.9), at a minimum.
9.2.8 Record maintenance. The laboratory shall maintain records to define the quality of data that are generated. The laboratory shall maintain a record of the date and results of all QC sample analyses described in Section 9.2. A record of media sterility check, dilution/rinse water blank, analyst counting variability, IDC, ODC, and MS/MSD sample results must be maintained. Laboratories shall maintain reagent and material lot numbers along with samples analyzed using each of the lots. Laboratories shall also maintain media preparation records.
9.2.9 Performance studies. The laboratory should periodically analyze external QC samples, such as performance evaluation (PE) samples, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.
9.2.10 Autoclave sterilization verification. At a minimum, the laboratory shall verify autoclave sterilization according to the procedure in Section 9.10 on a monthly basis.
9.2.11 Culture maintenance. The laboratory should use 24 ± 2 hour-old nutrient agar slant cultures for preparation of IDC, ODC, and MS/MSD spiking suspension dilutions. The laboratory

should use 22 to 72 hour-old nutrient agar slant cultures to inoculate ADA-V streak plates for analysis of negative culture controls. With regard to the preparation of subcultures, it is recommended that a maximum of three passages be prepared to help avoid contamination. After three passages, start a new subculture from the frozen stock.

9.2.12 Method modification.

9.2.12.1 **Membrane filtration**. Because recovery criteria are not available for this method, laboratories are not permitted to modify the membrane filtration procedures (Section 10.1 through Section 10.10.) or ADA-V media.

9.2.12.2 **Confirmation procedures**. The confirmation procedures in Section 10.11 may be modified, provided that the laboratory demonstrates the ability to generate acceptable performance by performing an IDC test (Section 9.2.1) and the appropriate negative culture control test(s) (Section 9.2.3) before analyzing any field samples using the modified confirmation. 100% of the colonies submitted to confirmation from IDC and negative culture control samples must give the appropriate confirmation response. These tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2) and media sterility checks (Section 9.2.6).

9.3 Preparation of Aeromonas spiking suspension for use in spiking IDC, ODC, and MS/MSD samples—This dilution scheme is adapted from Standard Methods for the Examination of Water and Wastewater, 19th Edition, Section 9020 B (Reference 15.9). This entire process should be performed quickly to avoid loss of viable organisms. See Section 16, Flowchart 1, for an example of this dilution scheme. Please note: Provided that all QC acceptance criteria are met and the recommended target range of 20 - 60 CFU per plate are typically observed, laboratories may prepare QC spiking suspensions using commercial products or other procedures such as growing bacteria in a broth, measuring optical density, and spiking each test sample with an equivalent volume.

9.3.1 Inoculate Aeromonas hydrophila (ATCC #7966) onto the entire surface of several nutrient agar slants with a slope approximately 6.3 cm long in a 125 × 16 mm screw-cap tube. Incubate for 24 ± 2 hours at 35°C ± 0.5°C.

9.3.2 From the slant that has the best growth, prepare serial dilutions using four dilution bottles with 99 mL of sterile buffered dilution water (A, B, C and D below in Sections 9.3.3 and 9.3.4) and one dilution bottle containing 90-mL of sterile buffered dilution water (D2 below in Section 9.3.5).

9.3.3 Pipette 1 mL of buffered dilution water from bottle “A” to one of the slants. Emulsify the growth on the slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar. Pipette the suspension back into dilution bottle “A.” Repeat this procedure a second time to remove any remaining growth on the agar slant, without disturbing the agar.

9.3.4 Make serial dilutions as follows:

9.3.4.1 Shake bottle “A” vigorously and pipette 1 mL to bottle “B”

9.3.4.2 Shake bottle “B” vigorously and pipette 1 mL to bottle “C”

9.3.4.3 Shake bottle “C” vigorously and pipette 1 mL to bottle “D”

9.3.4.4 Shake bottle “D” vigorously and pipette 10 mL to bottle “D2”; this should result in a final dilution of approximately 10 CFU / mL.

9.3.5 Filter 1- to 5-mL portions in triplicate from bottles “D” and “D2" according to the procedure in Section 10 to determine the number of CFU in the dilutions. The recommended target dilution and spike volume is one that produces 20 to 60 colonies per ADA-V plate. (It may be difficult to count plates with more than 60 colonies due to crowding.) Dilutions should be stored at 1EC to 5EC and may be used throughout the day they are prepared. However, it should be noted that the QC acceptance criteria were established using dilutions that were prepared immediately prior to spiking samples.

9.3.6 Analysts may practice the dilution scheme by placing filters on nutrient agar plates instead of ADA-V plates. After a growth pattern is determined and the analyst

can accurately determine the target concentrations, dilutions from Section 9.3.5 may be used for spiking IDC, ODC, and MS/MSD samples. However, multiple dilutions should be analyzed in replicate when new cultures are received from an outside source to ensure that the analyst can accurately spike target concentrations.

Note: If it is more convenient for your laboratory, an acceptable alternative to the dilution scheme presented in Section 9.3, is to pipette 11 mL of dilution D into a dilution bottle D2, which contains 99 mL of dilution water. There should be approximately 10^{10} Aeromonas hydrophila CFU per slant. Therefore, dilution bottles "A" through "D2" should contain approximately 10^{10}, 10^{8}, 10^{6}, 10^{4}, and 10^{3} CFU per dilution bottle, respectively. Depending on the growing conditions, these numbers may vary. As a result, until experience has been gained, more dilutions may need to be filtered to determine the appropriate dilution.

9.4 Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.4.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.4.2 For each of the four IDC test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.4.3 Process IDC test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per IDC test sample to the confirmation procedures in Section 10.11.

9.4.4 Using all four IDC sample results, compute the relative standard deviation (RSD) of Aeromonas CFU per 100 mL. (See glossary for definition of RSD.) Compare the RSD with the corresponding limits for IDC (Section 12). If the RSD meets the acceptance criteria, the system performance is acceptable and analysis of samples may begin. If the RSD falls outside the range, system performance is unacceptable. In this event, identify and correct the problem and repeat the test.

9.5 Dilution/rinse water blanks—On an ongoing basis, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series. For example, if a laboratory plans to run 30 samples during a filtration series, a dilution/rinse water blank should be processed at the beginning, middle, and end of the filtration series.

9.5.1 Process 100-mL dilution/rinse water blanks according to the procedures in Section 10, as appropriate.

9.5.2 No growth should appear in dilution/rinse water blanks. If growth appears, prepare new dilution/rinse water and reanalyze a 100-mL dilution/rinse water blank. If colonies are present after analyzing the new dilution/rinse water, assess laboratory technique and reagents. If growth in dilution/rinse water blank(s) is presumptively positive, all associated sample results should be discarded and sources re-sampled immediately.

9.6 Negative culture controls— Negative controls should be run whenever a new batch of medium or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples. Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate. *E. coli* is (ATCC #25922) the negative culture control for oxidase, *Pseudomonas aeruginosa* (ATCC #27853) is the negative culture control for trehalose fermentation, and *Bacillus cereus* (ATCC #11778) is the negative culture control for indole.

9.6.1 Using pure cultures obtained from a qualified outside source (Sections 7.7 and 7.8), inoculate negative culture controls onto nutrient agar slants and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Alternatively, nutrient agar slants may be inoculated up to 72 hours in advance. If nutrient agar slants will be incubated for more than 24 ± 2 hours, consider incubation at room temperature to ensure that the slants do not dry out prior to use.

9.6.2 For each negative culture control, place a membrane filter on an ADA-V plate, streak onto the filter, taking care not to break the filter, and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Streaking on a filter will give the laboratory a more realistic example of the appearance of these organisms in field samples. Although not recommended, laboratories may streak directly onto the ADA-V (without the filter).

9.6.3 For each ADA-V negative culture control plate, pick a single colony, streak the colony onto a plate of nutrient agar medium (Section 7.9), and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies. *Please note: Bacillus cereus typically grows only at the point of inoculation on ADA-V or not at all. If Bacillus cereus did not grow on the ADA-V plate, inoculate the streak plate from the nutrient agar slant that was originally used to inoculate the ADA-V plate.*

9.6.4 Negative culture control confirmation procedures

9.6.4.1 Oxidase negative culture control—From the streak plate, submit a single *E. coli* colony to the oxidase confirmation procedure described in Section 10.11.

9.6.4.2 Trehalose negative culture control—From the streak plate, submit a single *Pseudomonas aeruginosa* colony to the trehalose confirmation procedure described in Section 10.11.

9.6.4.3 Indole negative culture control—From the streak plate, submit a single *Bacillus cereus* colony to the indole confirmation procedure described in Section 10.11.

9.6.5 If any of the negative culture controls result in a positive confirmation, prepare, check and/or replace the associated media, reagents, and/or respective control organism and reanalyze the appropriate negative culture control(s). All presumptively positive colonies that have been archived from field samples (10 per sample) should be confirmed using media/reagents that exhibit the appropriate negative culture control response.

9.7 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall analyze MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples. Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines.

9.7.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Sections 9.3.1 through 9.3.4.

9.7.2 For each of the 500-mL MS and MSD test samples, spike enough volume of the appropriate dilution to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.7.3 Process MS/MSD test samples and an unspiked finished drinking water sample according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. (If the filter clogs during filtration, follow the instructions in Section 10, making sure to filter the same volume for both the MS and MSD. The same QC acceptance criteria apply.) Submit 10 colonies per IDC test sample to the confirmation procedures in Section 10.11.

Note: If results exceed the optimum range because of "background" target colonies (as indicated by the results of the unspiked matrix sample), the MS/MSD should be repeated and a smaller volume of sample, for example 200-mL, should be spiked.

9.7.4 For the MS and MSD test samples, calculate the number of confirmed Aeromonas CFU per 100 mL according to Section 11 and adjust based on any background Aeromonas observed in the unspiked sample.

9.7.5 Calculate the relative percent difference (RPD) using the following equation:

$$RPD = 100 \frac{| X_{MS} - X_{MSD} |}{X_{mean}}$$

where **RPD** is the relative percent difference
$\mathbf{X_{MS}}$ is the number of confirmed Aeromonas per 100 mL in the MS sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{MSD}}$ is the number of confirmed Aeromonas per 100 mL in the MSD sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{mean}}$ is the mean number of confirmed Aeromonas per 100 mL in the MS and MSD

9.7.6 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of finished water samples from this source may continue. If the MS/MSD results are unacceptable and the ODC sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. If the MS/MSD results are unacceptable, all associated field data should be flagged.

9.8 Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate). The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently.

9.8.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.8.2 For each of the 500-mL positive control (PC) and positive control duplicate (PC/PCD) test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.8.3 Process PC/PCD test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per PC/PCD test sample to the confirmation procedures in Section 10.11.

9.8.4 Calculate the relative percent difference (RPD) using the following equation:

$$PRD = 100 \frac{| X - X_{PCD} |}{X_{mean}}$$

where
RPD is the relative percent difference
X_{PC} is the number of confirmed Aeromonas per 100 mL in the PC sample

X_{PCD} is the number of confirmed Aeromonas per 100 mL in the PCD sampleX_{mean} is the mean number of confirmed Aeromonas per 100 mL in the PC and PCD samples

9.8.5 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of samples may continue. If RPD falls outside the range, system performance is unacceptable. Identify and correct the problem and perform another ODC test before continuing with the analysis of field samples.

9.8.6 As part of the QA program for the laboratory, method precision for ODC samples should be charted and records retained.

9.9 Analyst colony counting variability—If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one positive field sample per month. Compare each analyst's count of the target colonies. Counts should fall within 10% between analysts. If counts fail to fall within 10% of each other, analysts should perform additional sets of counts, until the number of target colonies counted fall within 10% between analysts for at least three consecutive samples. If there are no positive samples, an MS, MSD, or ODC sample can be used for this determination (MS or MSD are preferable to ODC samples, since they may have other background growth).

9.10 Autoclave sterilization verification—Verify autoclave sterilization monthly by placing Bacillus stearothermophilus spore suspensions or strips inside glassware. Sterilize at 121^{o}C for 15 minutes. Place in trypticase soy broth tubes and incubate at 55^{o}C for 48 hours. Check for growth to verify that sterilization was adequate. If sterilization was inadequate, determine appropriate time for autoclave sterilization. Filter sterilization may be used provided that these same QC steps are instituted for the filtrate.

10.0 Procedure

10.1 The membrane filter (MF) procedure with ampicillin-dextrin agar with vancomycin (ADA-V) is used to enumerate Aeromonas in finished waters.

10.2 Label each Petri dish with sample identification, preparation date, and analysis start date/time.

10.3 Use a sterile MF unit assembly (Section 6.18.3) at the beginning of each filtration series. The laboratory must sanitize each MF unit between filtrations by using a UV sanitizer, flowing steam, or boiling water for 2 min. A filtration series ends when 30 minutes or more elapse between sample filtrations.

10.4 Sterilize forceps with alcohol. Flame off excess alcohol. Using sterile forceps, place the MF (grid side up) over the sterilized funnel. Carefully place the top half of the filtration unit over the funnel and lock it in place.

10.5 Shake the sample bottle vigorously approximately 25 times to distribute the bacteria uniformly. Using aseptic technique, transfer one, 500-mL aliquot of sample to a single funnel. Use a graduated cylinder with a "to deliver" tolerance of approximately 2.5%.

Note: Laboratories must filter the entire 500-mL sample volume unless the filter clogs. If the filter clogs, a minimum of 100 mL of sample must be filtered, which may require multiple filtrations. If less than 500 mL are filtered and analyzed due to filter clogging, measure the residual, unfiltered volume to determine the volume filtered, and adjust the reporting limit accordingly.

10.6 Filter each sample under partial vacuum through a sterile membrane filter. Rinse the funnel after each sample filtration by filtering three, 30-mL portions of sterile buffered dilution water, being sure to thoroughly rinse the sides of the funnel.

10.7 Upon completion of the final rinse, disengage the vacuum and remove the funnel.

10.8 Using sterile forceps, immediately remove the MF and place it grid-side-up on the ADA-V medium with a rolling motion to avoid trapping air under the filter. Reseat the

membrane filter if bubbles occur. Place the inverted Petri dishes in the 35°C ± 0.5°C incubator within 30 minutes of preparation. Sterilize forceps and sanitize the MF unit between the analysis of each sample.

10.9 After 24 ± 2 hours of incubation at 35°C ± 0.5°C, count and record yellow colonies under magnification using a dissecting microscope.

10.10 Isolation of a yellow colony on ampicillin-dextrin agar with vancomycin (ADA-V) should be considered presumptively positive for Aeromonas.

10.11 Confirmation—All presumptive colonies, up to ten per sample, must be submitted to confirmation. In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. If the result for any confirmation procedure is negative, no further confirmation steps are necessary. Slight variations in color and morphology may be present between different Aeromonas species grown on ADA-V medium. The colonies selected for confirmation should be representative of all yellow (presumptively positive) colony morphology types on ADA-V plate. For example, if 30 bright yellow colonies and 20 dull yellow colonies are observed, then 6 bright yellow and 4 dull yellow colonies should be submitted to confirmation.

Note: It is important to record the number of colonies of each presumptively positive morphological type so that the final density of Aeromonas can be reported based on percent confirmation of each morphological type. Also, the laboratory may submit more than ten presumptively positive colonies to the confirmation step.

10.11.1 Nutrient agar streak plate. To confirm as Aeromonas, pick a colony and streak the colony onto a plate of nutrient agar medium (Section 7.9) and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies.

10.11.2 Oxidase confirmation. Apply a very small amount of a discreet colony from the nutrient agar to the oxidase dry slide using a wooden or plastic applicator. Do not use iron or other reactive wire because it may cause false positive reactions. Also, do not transfer any medium with the culture material, as this could lead to inconsistent results. A blue/purple color reaction within 10 seconds is considered a positive oxidase test. For commercially-prepared reagent, adhere to manufacturer's expiration date. Freshly-made solutions should be used within one week. Please note: This method was validated using nutrient agar, if the oxidase reagent is to be dropped directly on colonies, use tryptic soy agar plates because nutrient agar plates give inconsistent results. The use of tryptic soy agar plates for streaking (Section 10.11.1) has not been validated and is considered a method modification and, as a result, the laboratory must demonstrate acceptable performance for the QC analyses described in Section 9.2.12.

Note: Timing of the color reaction is critical, as some Gram-positive bacteria may give false positives after 10 seconds. Also, it is important to put just a small amount of the colony on the oxidase dry slide or saturated pad, as too much bacteria can also cause a false positive oxidase test.

10.11.3 Trehalose confirmation. If the oxidase test is positive, then test for trehalose fermentation. Trehalose fermentation is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of 0.5% trehalose in purple broth base (Section 7.11) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. A change in color of the medium from purple to yellow is considered a positive for trehalose fermentation.

10.11.4 Indole confirmation. If the oxidase and trehalose tests are positive, then test for indole production. (If the laboratory prefers, the indole confirmation procedure may be started on the same day as the trehalose confirmation.) Indole production is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of tryptone broth (Section 7.12) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. After incubation, add 0.2 to 0.3 mL (4 to 6 drops) of Kovac's test reagent (Section 7.13) to each tube, let stand for approximately 10 minutes and observe results. A pink to red color in the surface layer constitutes a positive indole test. The original color of the Kovac's reagent indicates a negative indole test. An

orange color probably indicates the presence of skatole, a breakdown product of indole, and is considered a positive result.
10.11.5 If a colony is oxidase, trehalose, and indole positive, report as a confirmed Aeromonas and archive the colony for further identification.
Note: If samples are to be archived for further analysis to determine species or hybridization group, from the nutrient agar plate (Section 10.11.1), inoculate a nutrient agar slant for short term use or shipment to another laboratory.

11.0 Data Analysis and Calculations
11.1 See Standard Methods for the Examination of Water and Wastewater (Reference 15.2) for general counting rules. The density of Aeromonas determined by the membrane filter (MF) procedure is calculated by direct identification and enumeration of yellow colonies by a dissecting microscope (Section 6.19) followed by oxidase, trehalose, and indole confirmation. Bacterial density is recorded as presumptive Aeromonas colony forming units (CFU) per 100 mL of sample and confirmed Aeromonas CFU per 100 mL.
11.2 Counting colonies on ADA-V
11.2.1 Record the number of presumptive Aeromonas CFU/100mL. If there is more than one morphological type that is considered to be presumptively positive, record the number of presumptive positives for each morphological type, as well as the total number of presumptive positives.
11.2.2 If there are more than 200 colonies, including background colonies, report results as too numerous to count (TNTC) and resample. If the filter is TNTC with more background colonies than presumptive aeromonads, split the 500 mL resample between 3 or 4 filters in order to better differentiate the colony morphology types. If the filter is TNTC with mostly aeromonads, a minimum of three dilutions (e.g. 100 mL, 10 mL and 1 mL) should be analyzed.
11.2.3 If the colonies are not discrete and appear to be growing together, report results as confluent growth (CG) and resample.

11.3 Confirmation and calculation of Aeromonas density
11.3.1 In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. For the final density of confirmed Aeromonas, adjust the initial, presumptive count based on the positive confirmation percentage for each presumptively positive morphological type and report as confirmed CFU per 100 mL.
11.3.2 Calculate the number of positive confirmations for each presumptively positive morphological type from all filters of a given sample using the following equation:

$$\left(\frac{\text{Number positively confirmed}}{\text{Number submitted to confirmation}} \text{ X Number of presumptive positives}\right) = A$$

$$A \text{ X } \frac{100}{\text{mL filtered}} = \text{Confirmed Aeromonas /100 mL}$$

11.3.3 Record the number of confirmed Aeromonas per 100 mL for each colony morphology.
11.3.4 Sum the number of confirmed Aeromonas per 100 mL for all presumptively positive colony types (Section 11.3.2) and report as the density of confirmed Aeromonas per 100 mL.
11.3.5 Example 1: In this example, 500 mL of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 1 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Bright yellow, round, opaque	30	6	6	6
Type B: Dull yellow, oval, translucent	20	4	3	3
			Total number of confirmed *Aeromonas* per sample:	**9 per 100 mL**

Example 1 results in 9 confirmed *Aeromonas* / 100 mL.

11.3.6 Example 2: In this example, ***200 mL*** of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 2 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Dull yellow, round, opaque	40	5	5	20
Type B: Dull yellow, round, translucent	40	5	3	12
			Total number of confirmed *Aeromonas* per sample:	**32 per 100 mL**

Example 2 results in 32 confirmed *Aeromonas* / 100 mL.

11.3.7 If there were no presumptively positive colonies or if none of the presumptive colonies are confirmed, then report the results as less than the detection limit (DL) in CFU per 100 mL based on sample volume filtered. If less than 500 mL are filtered, then adjust the reporting limit per 100 mL accordingly. The DL may be calculated as follows:

DL per 100 mL = 100 / volume filtered CFU per 100mL

11.3.7.1 Example 3: If 500 mL of sample was filtered and there were no confirmed colonies, then report as <0.2 CFU/100 mL.

11.3.7.2 Example 4: If 100 mL of sample was filtered and there were no confirmed colonies, then report as <1.0 CFU/100 mL.

12.0 Method Performance
12.1 Specificity of media **12.1.1** Please refer to Section 16, Table 2, for results of *Aeromonas* growth after 24 hours on ADA at 30EC and 35EC and ADA-V at 35EC .
12.1.2 ADA-V was able to support the growth of the *Aeromonas* species (*hydrophila, caviae,* and *veronii/sobria*) most often associated with human disease.
12.1.3 Efforts continue to identify colonies which give a presumptive positive on the ADA-V media but do not confirm.
12.2 The QC acceptance criteria listed in Table 1, below are based on data generated through the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished drinking water matrices. Detailed method QC procedures applicable to these criteria are discussed in Section 9.

Table 1. QC Acceptance Criteria for Method 1605 QC specification

	Maximum acceptable precision
Initial demonstration of capability (IDC): This test will require the analysis of 4 spiked reagent water samples	RSD = 22%
Ongoing demonstration of capability (ODC): This test will require the analysis of 2 spiked reagent water samples	RPD = 37%
Matrix spike/matrix spike duplicate (MS/MSD) precision: This test will require the analysis of 2 spiked finished water (matrix) samples	RPD = 48%

13.0 Pollution Prevention
13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.
13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.
14.0 Waste Management
14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known or suspected of having bacterial contamination from this work must be sterilized prior to disposal.
14.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both of which are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.
15.0 References
15.1 Havelaar, A.H., M. During, and J.F.M. Versteegh. 1987. Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. Journal of Applied Microbiology. 62:279-287.
15.2 Standard Methods for the Examination of Water and Wastewater. 1998. 20th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.
15.3 Demarta, A., M. Tonolla, A. Caminada, N. Ruggeri, and R. Peduzzi. 1999. Signature region within the 16S rDNA sequences of *Aeromonas popoffii.* FEMS Microbiol. Letts. 172:239-246.
15.4 Annual Book of ASTM Standards, Vol. 11.01. American Society for Testing and Materials. Philadelphia, PA 19103.

15.5 Manual for the Certification of Laboratories Analyzing Drinking Water. 1997. 4th Edition. EPA-815-B-97-001. Office of Ground Water and Drinking Water. U.S. EPA.
15.6 Moyer, N. P. 1996. Isolation and enumeration of aeromonads. *In*: The Genus *Aeromonas*. Eds. B. Austin, M. Altwegg, P. Gosling, and S. Joseph. John Wiley and Sons publisher, Chichester, U.K.
15.7 Reagent Chemicals, American Chemical Society Specifications. American Chemical Society, Washington, D.C.
15.8 Handfield, M., P. Simard, and R. Letarte. 1996. Differential media for quantitative recovery of waterborne *Aeromonas hydrophila*. Applied Environmental Microbiology 62:3544-3547.
15.9 Standard Methods for the Examination of Water and Wastewater. 1995. 19th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.
15.10 Janda, J.M. and S.L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Journal of Clinical Infectious diseases. 27:332-344.

16.0 Tables and Flowcharts

Collection #	Hybridization group	*Aeromonas* species	ADA at 30oC	ADA at 35oC	ADA-V at 35oC
ATCC 7966	Group 1	*hydrophila*	+	+	+
ATCC 35654	Group 1	*hydrophila*	+	+	+
AMC 12723-W	Group 1	*hydrophila*	+	+	+
ATCC 51108	Group 2	*bestiarum*	+	+	+
AMC 14228-V	Group 2	*bestiarum*	+	+	+
ATCC 336581	Group 3	*salmonicida/salmonicida*	-	-	NA
AMC 15228-V	Group 3	*salmonicida*	+	+	+
ATCC 15468	Group 4	*caviae*	+	+	+
MML 1685-E	Group 4	*caviae*	+	+	+
ATCC 33907	Group 5	*media*	-	-	NA
AMC Leftwich	Group 5	*media*	-	-	NA
ATCC 233091	Group 6	*eucrenophila*	+	-	NA
ATCC 35993	Group 7	*sobria*	+	+	+
Muldoon SMHC	Group 7	*sobria*	+	+	+
ATCC 9071	Group 8	*veronii/sobria*	+	+	+
AMC 1123-W	Group 8	*veronii/sobria*	+	+	+
ATCC 43700	Group 12	*schubertii*	+2	+5	+5
AMC 1108-W	Group 12	*schubertii*	+	-	NA
ATCC 496573	unknown	*trota*	-	-	NA
NMRI 206	unknown	*trota*	-	-	NA
ATCC 51208	unknown	*allosaccharophila*	+	+	+
ATCC 49568	Group 9	*jandaei*	+	+	+
AS 14	Group 9	*jandaei*	+	+	+
ATCC 35622	Group 10	*veronii/veronii*	+	+	+
WR 4659	Group 10	*veronii/veronii*	+	+	+
CECT 4342	Group 11	*encheleia*	+	-	NA
LMG 175414	unknown	*popoffii*	+	+	+
AMC (ATCC 35941)	unknown	ornithine positive	-	-	NA
AMC (ATCC 43946)	unknown	Group 501	+	+	+

CDC 0434-84 Group 3 Motile Group 3 + + +
(1) Respective *Aeromonas* cultures grew on ADA medium when streaked, but not when filtered.

(2) Respective Aeromonas cultures grew when streaked on ADA medium at 30°C, however filtration was not performed with these cultures.(3) Respective Aeromonas cultures did not grow on ADA medium when streaked.(4) Respective Aeromonas cultures grew poorly on ADA medium at both temperatures and on ADA-V at 35°C. The same pattern of poor growth was also observed on non-selective media.(5) Respective Aeromonas cultures grew poorly on ADA and ADA-V medium at 35°C. The same pattern of poor growth was also observed on non-selective media. Results: A Based on ADA results, it was assumed that the culture would not grow on ADA-V at 35°C.
+ positive growth
-No growth

ATCC = American Type Culture Collection, Manassas, VA Other cultures were obtained from Amy Carnahan, University of Maryland. Serial dilutions representing approximately 10-200 CFU were filtered and the membrane placed on ADA or ADA-V medium as described in Section 10. Additional membranes representing the same dilution for each of the respective cultures were placed on brain heart infusion agar as a control.

17.3 Definitions
Confirmed colonies—Presumptively positive colonies that test positive for oxidase, ferment trehalose, and produce indole

Dilution/rinse water blank—A 100-mL aliquot of dilution/rinse water that is treated exactly as a sample and carried through all portions of the procedure until determined to be negative or positive. The Dilution/rinse water blank is used to determine if the sample has become contaminated by the introduction of a foreign microorganism through poor technique.

Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples.

Must—This action, activity, or procedural step is required.

Negative culture control—A non-*Aeromonas* bacteria processed to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate.

Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate).

Positive control—A 500-mL reagent water spiked with 20 - 80 CFU of Aeromonas. The positive control is analyzed exactly like a sample. Its purpose is to ensure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Presumptive positive colonies—Colonies that are yellow on ADA-V.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean.

Selective medium—A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of the target bacteria.

Should—This action, activity, or procedural step is suggested but not required.

Common Water Quality and Sampling Questions and Review

These statements will be more explained in the previous chapters.

1. What are the correct procedures to follow in collecting bacteriological samples?

Use a sterile plastic or glass bottle. Sodium thiosulfate should be added to neutralize the chorine residual. Refrigerate the sample to 4^{o} C. The regulations call for a minimum of five samples for the month from any system that has positive sample results. Small systems that take only one sample per month have to take four (4) repeats when they get a total coliform positive test result. If any system has to take repeat samples, it must also take a minimum of five (5) routine samples the following month. Small systems that normally take less than 5 samples/month will have to increase the number to 5 samples. They can return to normal sampling schedules the following month if no repeats are required.

2. What are the proper sampling techniques for microbiological sampling?

Proper sampling techniques are extremely important in obtaining accurate water quality information. An improperly taken coliform sample may indicate bacteriological
contamination of your water when the water is actually safe. You can avoid the cost of additional testing by using good sampling procedures. Carefully follow these steps in taking a sample for bacteriological testing:

1. Select the sampling point. The sampling point must be a faucet from which water is commonly taken for public use.

The sampling point should be a non-swivel faucet.

Remove any aerator or screen and flush.

It should not be a faucet that leaks, permitting water to run over the outside of the faucet. Leaking faucets can promote bacterial growth.

If an outside faucet must be used, disconnect any hoses or other attachments and be sure to flush the line thoroughly.

Do not use fire hydrants as sampling points. Do not dip the bottle in reservoirs, spring boxes or storage tanks in order to collect the sample.

3. What do the following abbreviations stand for and what do they mean: gpm, MGD, TTHM, psi, HAA, NTU, and mg/L.

Gallons per minute- Million Gallons a Day - Total Trihalomethanes – Pounds Per Square Inch –Haloacetic acids - Nephelometric turbidity unit -Milligrams Per Liter

4. What is the relationship between mg/L and ppm; ug/L and ppb?

Milligram per liter: Milligram per liter of substance and part per million are equals amounts in water. While you can easily convert between micrograms/liter and milligrams/liter, and between PPM and PPB, it's not so easy to convert between the different types of units such as milligrams/liter to PPM.

To convert micrograms per liter to milligrams per liter, divide by 1000.

To convert to PPM, you would first need to know the density of the substance, and the density of what the substance is in.

5. Ug/L: Represents the concentration of something in water or soil. One ppb represents one microgram of something per liter of water (ug/l), or one microgram of something per kilogram of soil (ug/kg).

Parts per million (ppm) or Milligrams per liter (mg/l) - one part per million corresponds to one minute in two years or a single penny in $10,000.

Parts per billion (ppb) or Micrograms per liter - one part per billion corresponds to one minute in 2,000 years, or a single penny in $10,000,000.

Parts per trillion (ppt) or Nanograms per liter (nanograms/l) - one part per trillion corresponds to one minute in 2,000,000 years, or a single penny in $10,000,000,000.

6. What do the following terms represent in reference to water quality.
Total coliform: The coliform family has been divided into two groups. Results may come back as either total coliform positive (TC positive) or fecal coliform positive, or (FC positive or *E. coli* positive.) Total coliform positive means that no human coliform are present.

7. Fecal Coliform: Fecal coliform positive indicates the presence of *E. coli*, which means there is a greater chance of pathogens being present. The laboratory tests for coliform include the MPN method, the Membrane Filter test, the Colilert test, and the presence-absence test.

8. Presence-absence Test: Presence-Absence Broth is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

9. Physical Characteristics of Water: A characteristic of water defined by the temperature, turbidity, color, taste, and odor of the water.

10. Point-of-entry sample (POE): A type of water sample taken after treatment and before reaching the first consumer.

11. Acute Health Effect: An immediate (i.e. within hours or days) effect that may result from exposure to certain drinking water contaminants (e.g., pathogens).

12. Non-acute violation: If the MCL is exceeded and none of the positive results indicated a presence of Fecal Coliform, a Tier 2 violation has occurred. This level of violation used to be called a non-acute violation.

13. Routine Sample: Samples collected on a routine basis to monitor for contamination. Collection should be in accordance with an approved sampling plan.

14. Repeat Sample: Short answer… Samples collected following a '***coliform present'*** routine sample. The number of repeat samples to be collected is based on the number of routine samples you normally collect. Long Answer. Anytime a microbiological sample result comes back positive, indicating the presence of total or fecal coliform/ E.coli, repeat samples must be taken. Three repeats are usually required. One must be taken at the site of the positive sample. The two samples must be taken upstream and downstream of the original site (within five service connections). These repeat samples must be taken within 24 hours of notification of positive results. They must be identified as a Repeat Sample on the sample form. Repeat samples may be required to be sealed with a red evidentiary seal tape. The tape must cover the cap and extend down the sides of the bottle. The sample forms must also include the reference number for the positive sample.

There is an important exception to the three repeat samples rule. The regulations also state that when repeats are taken the minimum number of samples is raised to five for the month. A system that collects just one sample a month must collect four repeat samples, when the sample is positive, in order to have five samples as required.

Whenever a system has to take repeat samples, a minimum of five routine samples must also be submitted the following month. This is only an issue for systems that normally turn in four or fewer samples each month. If the five samples are negative the system can return to its normal sampling schedule the next month.

Small systems that have fewer than four sampling sites have a problem complying with the "upstream and downstream" aspects of the repeat sampling requirements. In this case, samples should be taken at as many separate sites as possible and then wait a minimum of 2 hours before resampling enough sites to get the required number of samples. Repeat sample with red seal tape.

15. Treatment technique: An enforceable procedure or level of technical performance which public water systems must follow to ensure control of a contaminant.

16. Action level: The level of lead or copper which, if exceeded, triggers treatment or other requirements that a water system must follow.

17. What does the membrane filter test analyze with regards to bacteriological sampling?
Membrane Filter Technique: A standard test used for measuring coliform numbers (quantity) in water is the membrane filter technique. This technique involves filtering a known volume of water through a special sterile filter. These filters are made of nitrocellulose acetate and polycarbonate, are 150 µm thick, and have 0.45 µm diameter pores. A grid pattern is printed on these filter disks in order to facilitate colony counting. When the water sample is filtered, bacteria (larger than 0.45 µm) in the sample are trapped on the surface of the filter. The filter is then carefully removed, placed in a sterile petri plate on a pad saturated with a liquid medium, and incubated for 20-24 hours at 37°C.

One assumes that each bacterium trapped on the filter will then grow into a separate colony. By counting the colonies one can directly determine the number of bacteria in the water sample that was filtered. The broth medium usually employed in detecting total coliforms is M-Endo Broth MF. Total coliform colonies will be pink to dark red in color and will appear to have a golden green color.

18. What do the following terms mean in relation to drinking water quality: disinfection, pathogenic, toxic, pH, aesthetic, culinary and potable.
Disinfection: The chemical process of killing or inactivating most microorganisms in water. See also Sterilization.

19. Pathogenic: Organisms or bugs that cause disease. These include bacteria, viruses, cysts and anything capable of causing disease in humans, like cryptosporidiosis, typhoid, cholera and so on. There are other organisms that do not create disease, these are called non-pathogenic organisms.

20. Toxic: Stuff that will kill you. A substance which is poisonous to living organisms.

21. pH: A measure of the acidity of water. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. For example, the acidity of a sample with a pH of **5** is ten times greater than that of a sample with a pH of **6**. A difference of 2 units, from **6** to **4**, would mean that the acidity is one hundred times greater, and so on. Normal rain has a pH of **5.6** – slightly acidic because of the carbon dioxide picked up in the earth's atmosphere by the rain.

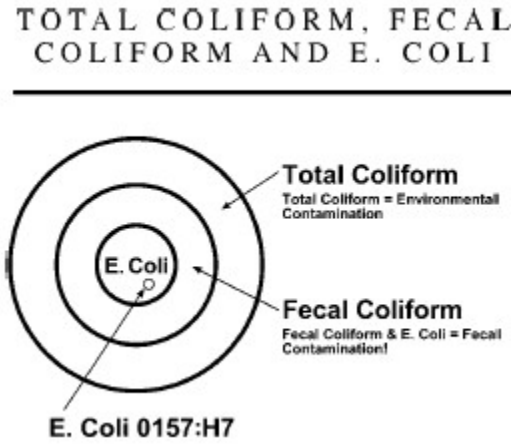
22. Aesthetic: Attractive or appealing water or things in water that will not make you sick but may appear to change the water's color or taste.

23. Culinary: Having to do with cooking food. Potable water is often called culinary water.

24. Potable: Water that is free of objectionable pollution, contamination, or infective agents. Generally speaking, we serve only potable water and not palatable water. Palatable is pleasant tasting water.

25. What is hardness in water and what chemicals cause it?
Hardness: Water that contains high amounts of dissolved minerals, specifically calcium and magnesium.
Ion Exchange: A method of water softening where hardness causing ions are exchanged with sodium ions; also effective in removing many inorganic contaminants such as nitrates, copper, and lead; and treating aesthetic water problems.

26. What is Escherichia Coliform and what does it indicate in relation to drinking water?
E. coli is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness. The presence of *E. coli* in a drinking water sample almost always indicates recent fecal contamination meaning there is a greater risk that pathogens are present.



Total coliform, fecal coliform, and *E. coli* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. *E. coli* is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or *E. coli*, depending on the lab testing method.

27. What problems are associated with Hydrogen Sulfide in the water?
Hydrogen sulfide is a gas which, when dissolved in water, gives it a “rotten egg” odor. Chlorination will remove this gas from the water but the effectiveness of the chlorine for disinfection is lessened.

28. When Hydrogen sulfide reacts with chlorine, it produces Sulfuric acid and elemental Sulfur: It is therefore recommended that aeration be applied prior to the addition of chlorine for the most effective disinfection.

29. Why is it important to know what the turbidity of the water is when using chlorine?
To be careful not to overdose with chlorine or properly dose with chlorine.

30. What is the log removal for Cryptosporidium?
The LT1ESWTR extends further this necessary protection from Cryptosporidium to communities of fewer than 10,000 persons. Today's rule for the first time establishes Cryptosporidium control requirements for systems serving less than 10,000 persons by requiring a minimum 2-log removal for Cryptosporidium. The rule also strengthens filter performance requirements to ensure 2-log Cryptosporidium removal, establishes individual filter monitoring to minimize poor performance in individual units, includes Cryptosporidium in the definition of GWUDI, and explicitly considers unfiltered system watershed control provisions. The rule also reflects a commitment to the importance of maintaining existing levels of microbial protection in public water systems as plants take steps to comply with newly applicable DBP standards.

31. What is the log removal?
This log-reduction terminology was developed by engineers as a way to express levels of decreased biological contamination in water by factors of 10 that could be easily converted to percent reduction. The most commonly used logarithmic base is 10 because it is compatible with our base-10 decimal system. The log of 10 in the base 10 logarithmic system is 1 and the log of 100 is 2, with the log of 1000 being 3, etc. A 1-log reduction is nine out of 10 and would be equivalent to a 90 percent reduction. A 2-log reduction would be 99 out of 100 or 99 percent reduction and a 3-log reduction would be 999 out of 1000 or 99.9 percent reduction. A 99.99 percent reduction would be called a 4-log reduction.

32. What are the turbidity requirements for Direct and Conventional filtration plants?
For conventional and direct filtration systems (including those systems utilizing in-line filtration), the turbidity level of representative samples of a system's filtered water (measured every four hours) must be less than or equal to ***0.3 NTU*** in at least 95 percent of the measurements taken each month. The turbidity level of representative samples of a system's filtered water must not exceed ***1 NTU*** at any time. Conventional filtration is defined as a series of processes including coagulation, flocculation, sedimentation, and filtration resulting in substantial particulate removal. Direct filtration is defined as a series of processes including coagulation and filtration but excluding sedimentation resulting in substantial particle removal.

33. What are chloramines, how are they formed, and do they have any beneficial use?
Chloramines: Ammonia and Chlorine are combined. Cl_2NH_3 Yes, limited use and this chemical will create less THMS than chlorine alone. Chloramine is a disinfectant used to treat drinking water. It is formed by mixing chlorine with ammonia. Although it is a weaker disinfectant than chlorine, it is more stable and extends disinfectant benefits throughout a water utility's distribution system (a system of pipes water is delivered to homes through). Some water systems use chloramine as a secondary disinfectant to maintain a disinfectant residual throughout the distribution system so that drinking water remains safe as it travels from the treatment facility to the customer. Chloramine has been used by water systems for almost 90 years, and its use is closely regulated. Since chloramine is not as reactive as chlorine, it forms fewer disinfection byproducts. Some disinfection byproducts, such as the trihalomethanes (THMs) and haloacetic acids (HAAs), may have adverse health effects and are closely regulated. Because a chloramine residual is more stable and longer lasting than free chlorine, it provides better protection against bacterial regrowth in systems with large storage tanks and dead-end water mains. Chloramine, like chlorine, is effective in controlling biofilm, which is a coating in the pipe caused by bacteria. Controlling biofilm also tends to reduce coliform bacteria concentrations and biofilm-induced corrosion of pipes.

Common Water Treatment and Distribution Chemicals

Chemical Name	Common Name	Chemical Formula
Aluminum hydroxide		Al(OH)3
Aluminum sulfate	Alum, liquid	AL2(SO4)3 . 14(H2O)
Ammonia		NH3
Ammonium		NH4
Bentonitic clay	Bentonite	
Calcium bicarbonate		Ca(HCO3)2
Calcium carbonate	Limestone	CaCO3
Calcium chloride		CaCl2
Calcium Hypochlorite	HTH	Ca(OCl)2 . 4H2O
Calcium hydroxide	Slaked Lime	Ca(OH)2
Calcium oxide	Unslaked (Quicklime)	CaO
Calcium sulfate	Gypsum	CaSO4
Carbon	Activated Carbon	C
Carbon dioxide		CO2
Carbonic acid		H2CO3
Chlorine gas		Cl2
Chlorine Dioxide		ClO2
Copper sulfate	Blue vitriol	CuSO4 . 5H2O
Dichloramine		NHCl2
Ferric chloride	Iron chloride	FeCl3
Ferric hydroxide		Fe(OH)3
Ferric sulfate	Iron sulfate	Fe2(SO4)3
Ferrous bicarbonate		Fe(HCO3)2
Ferrous hydroxide		Fe(OH)3
Ferrous sulfate	Copperas	FeSO4.7H20
Hydrofluorsilicic acid		H2SiF6
Hydrochloric acid	Muriatic acid	HCl
Hydrogen sulfide		H2S
Hypochlorus acid		HOCL
Magnesium bicarbonate		Mg(HCO3)2
Magnesium carbonate		MgCO3
Magnesium chloride		MgCl2
Magnesium hydroxide		Mg(OH)2
Magnesium dioxide		MgO2
Manganous bicarbonate		Mn(HCO3)2
Manganous sulfate		MnSO4
Monochloramine		NH2Cl
Potassium bicarbonate		KHCO3
Potassium permanganate		KMnO3

Chemical Name	Common Name	Chemical Formula
Sodium carbonate	Soda ash	Na2CO3
Sodium chloride	Salt	NaCl
Sodium chlorite		NaClO2
Sodium fluoride		NaF
Sodium fluorsilicate		Na2SiF6
Sodium hydroxide	Lye	NaOH
Sodium hypochlorite		NaOCl
Sodium Metaphosphate	Hexametaphosphate	NaPO3
Sodium phosphate	Disodium phosphate	Na3PO4
Sodium sulfate		Na2SO4
Sulfuric acid		H2SO4



When should employees read the label for a hazardous chemical? (Before starting the job every time that chemical is used—its hazards or protections could change or an employee could forget or confuse it with other chemicals.)

Element Information

Number	Element	Valence
1	Hydrogen	(-1), +1
2	Helium	0
3	Lithium	+1
4	Beryllium	+2
5	Boron	-3, +3
6	Carbon	(+2), +4
7	Nitrogen	-3, -2, -1, (+1), +2, +3, +4, +5
8	Oxygen	-2
9	Fluorine	-1, (+1)
10	Neon	0
11	Sodium	+1
12	Magnesium	+2
13	Aluminum	+3
14	Silicon	-4, (+2), +4
15	Phosphorus	-3, +1, +3, +5
16	Sulfur	-2, +2, +4, +6
17	Chlorine	-1, +1, (+2), +3, (+4), +5, +7
18	Argon	0
19	Potassium	+1
20	Calcium	+2
21	Scandium	+3
22	Titanium	+2, +3, +4
23	Vanadium	+2, +3, +4, +5
24	Chromium	+2, +3, +6
25	Manganese	+2, (+3), +4, (+6), +7
26	Iron	+2, +3, (+4), (+6)
27	Cobalt	+2, +3, (+4)
28	Nickel	(+1), +2, (+3), (+4)
29	Copper	+1, +2, (+3)
30	Zinc	+2
31	Gallium	(+2). +3
32	Germanium	-4, +2, +4
33	Arsenic	-3, (+2), +3, +5
34	Selenium	-2, (+2), +4, +6
35	Bromine	-1, +1, (+3), (+4), +5
36	Krypton	0
37	Rubidium	+1
38	Strontium	+2
39	Yttrium	+3
40	Zirconium	(+2), (+3), +4
41	Niobium	(+2), +3, (+4), +5
42	Molybdenum	(+2), +3, (+4), (+5), +6
43	Technetium	+6
44	Rubidium	(+2), +3, +4, (+6), (+7), +8
45	Rhodium	(+2), (+3), +4, (+6)
46	Palladium	+2, +4, (+6)
47	Silver	+1, (+2), (+3)
48	Cadmium	(+1), +2
49	Indium	(+1), (+2), +3
50	Tin	+2, +4

51	Antimony	-3, +3, (+4), +5
52	Tellurium	-2, (+2), +4, +6
53	Iodine	-1, +1, (+3), (+4), +5, +7
54	Xenon	0
55	Cesium	+1
56	Barium	+2
57	Lanthanum	+3
58	Cerium	+3, +4
59	Praseodymium	+3
60	Neodymium	+3, +4
61	Promethium	+3
62	Samarium	(+2), +3
63	Europium	(+2), +3
64	Gadolinium	+3
65	Terbium	+3, +4
66	Dysprosium	+3
67	Holmium	+3
68	Erbium	+3
69	Thulium	(+2), +3
70	Ytterbium	(+2), +3
71	Lutetium	+3
72	Hafnium	+4
73	Tantalum	(+3), (+4), +5
74	Tungsten	(+2), (+3), (+4), (+5), +6
75	Rhenium	(-1), (+1), +2, (+3), +4, (+5), +6, +7
76	Osmium	(+2), +3, +4, +6, +8
77	Iridium	(+1), (+2), +3, +4, +6
78	Platinum	(+1), +2, (+3), +4, +6
79	Gold	+1, (+2), +3
80	Mercury	+1, +2
81	Thallium	+1, (+2), +3
82	Lead	+2, +4
83	Bismuth	(-3), (+2), +3, (+4), (+5)
84	Polonium	(-2), +2, +4, (+6)
85	Astatine	?
86	Radon	0
87	Francium	?
88	Radium	+2
89	Actinium	+3
90	Thorium	+4
91	Protactinium	+5
92	Uranium	(+2), +3, +4, (+5), +6

Reference: *Lange's Handbook of Chemistry, 8th Ed.*, Norbert A. Lange (Ed.), Handbook Publishers, Inc. 1952.

Common Used Products	Chemical Name
acetone	dimethyl ketone
acid of sugar	oxalic acid
alcohol, grain	ethyl alcohol
alcohol, wood	methyl alcohol
alum	aluminum potassium sulfate
alumina	aluminum oxide
antichlor	sodium thiosulfate
aqua ammonia	aqueous solution of ammonium hydroxide
aqua regia	nitrohydrochloric acid
aqua fortis	nitric acid
aromatic spirit of ammonia	ammonia in alcohol
asbestos	magnesium silicate
aspirin	acetylsalicylic acid
baking soda	sodium bicarbonate
banana oil (artificial)	isoamyl acetate
benzol	benzene
bichloride of mercury	mercuric chloride
black copper oxide	cupric oxide
black lead	graphite (carbon)
bleaching powder	chlorinated lime
blue vitriol	copper sulfate
bluestone	copper sulfate
borax	sodium borate
brimstone	sulfur
brine	aqueous sodium chloride solution
butter of antimony	antimony trichloride
butter of tin	anhydrous stannic chloride
calomel	mercury chloride
carbolic acid	phenol
carbonic acid gas	carbon dioxide
caustic potash	potassium hydroxide
caustic soda	sodium hydroxide
chalk	calcium carbonate
Chile saltpeter	sodium nitrate
chrome, alum	chromic potassium sulfate
chrome, yellow	lead (VI) chromate
copperas	ferrous sulfate
cream of tartar	potassium bitartrate
crocus powder	ferric oxide
emery powder	impure aluminum oxide
Epsom salts	magnesium sulfate
ethanol	ethyl alcohol
fluorspar	natural calcium fluoride
formalin	aqueous formaldehyde solution
French chalk	natural magnesium silicate
galena	natural lead sulfide
Glauber's salt	sodium sulfate
gypsum	natural calcium sulfate
hydrocyanic acid	hydrogen cynanide
hypo (photography)	sodium thiosulfate solution
lime	calcium oxide

Common Used Products	Chemical Name
limewater	aqueous solution of calcium hydroxide
lunar caustic	silver nitrate
magnesia	magnesium oxide
mercury oxide, black	mercurous oxide
methanol	methyl alcohol
methylated spirits	methyl alcohol
muriatic acid	hydrochloric acid
oil of vitriol	sulfuric acid
oil of wintergreen (artificial)	methyl salicylate
Paris green	copper acetoarsenite
Paris white	powdered calcium carbonate
pear oil (artificial)	isoamyl acetate
pearl ash	potassium carbonate
plaster of Paris	calcium sulfate
plumbago	graphite
potash	potassium carbonate
potassa	potassium hydroxide
Prussic acid	hydrogen cyanide
pyro	tetrasodium pyrophosphate
quicklime	calcium oxide
quicksilver	mercury
red lead	lead tetraoxide
Rochelle salt	potassium sodium tartrate
rouge, jeweler's	ferric oxide
rubbing alcohol	isopropyl alcohol
sal ammoniac	ammonium chloride
sal soda	sodium carbonate
salt, table	sodium chloride
salt of lemon	potassium binoxalate
salt of tartar	potassium carbonate
saltpeter	potassium nitrate
silica	silicon dioxide
soda ash	sodium carbonate
soda lye	sodium hydroxide
soluble glass	sodium silicate
spirit of hartshorn	ammonium hydroxide solution
sugar, table	sucrose
talc or talcum	magnesium silicate
vinegar	impure dilute acetic acid
vitamin C	ascorbic acid
washing soda	sodium carbonate
water glass	sodium silicate

Math Conversion Factors

1 PSI = 2.31 Feet of Water
1 Foot of Water = .433 PSI
1.13 Feet of Water = 1 Inch of Mercury
454 Grams = 1Pound
1 Gallon of Water = 8.34 lbs/gallon
1 mg/L = 1 PPM
17.1 mg/L = 1 Grain/Gallon
1% = 10,000 mg/L
694 Gallons per Minute = MGD
1.55 Cubic Feet per Second = 1 MGD
60 Seconds = 1 Minute
1440 Minutes = 1 Day
.746 kW = 1 Horsepower

LENGTH
12 Inches = 1 Foot
3 Feet = 1 Yard
5,280 Feet = 1 Mile

AREA
144 Square Inches = 1 Square Foot
43,560 Square Feet = 1 Acre

VOLUME
1000 Milliliters = 1 Liter
3.785 Liters = 1Gallon
231 Cubic Inches = 1 Gallon
7.48 Gallons = 1 Cubic Foot of Water
62.38 Pounds = 1 Cubic Foot of Water

Dimensions

SQUARE: Area (sq. ft.) = Length X Width
Volume (cu.ft) = Length (ft) X Width (ft) X Height (ft)

CIRCLE: Area (sq. ft.) = 3.14 X Radius (ft) X Radius (ft)

CYLINDER: Volume (Cu. Ft.) = 3.14 X Radius (ft) X Radius (ft) X Depth (ft)

SPHERE: $\frac{(3.14)\ (\text{Diameter})^3}{(6)}$ Circumference = 3.14 X Diameter

General

POUNDS PER DAY AKA SOLIDS APPLIED =
Concentration (mg/L) X Flow (MG) X 8.34

Percent Efficiency = $\frac{\text{In} - \text{Out}}{\text{In}} \times 100$

TEMPERATURE: $^0F = (^0C \times 9/5) + 32$
$^0C = (^0F - 32) \times 5/9$

CONCENTRATION: Conc. (A) X Volume (A) = Conc. (B) X Volume (B)

FLOW RATE (Q): Q = A X V (Quantity = Area X Velocity)

FLOW RATE (gpm): Flow Rate (gpm) = $\frac{2.83\ (\text{Diameter, in})^2\ (\text{Distance, in})}{\text{Height, in}}$

ACTUAL LEAKAGE = $\frac{\text{Leak Rate (GPD)}}{\text{Length (mi.) X Diameter (in)}}$

VELOCITY = $\frac{\text{Distance (ft)}}{\text{Time (Sec)}}$

WATER HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960}$

BRAKE HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Efficiency}}$

MOTOR HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Eff. X Motor Eff.}}$

MEAN OR AVERAGE = $\frac{\text{Sum of the Values}}{\text{Number of Values}}$

TOTAL HEAD (ft) = Suction Lift (ft) X Discharge Head (ft)

SURFACE LOADING RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Surface Area (sq. ft)}}$
(gal/min/sq.ft)

MIXTURE STRENGTH (%) = $\frac{\text{(Volume 1, gal) (Strength 1, \%) + (Volume 2, gal) (Strength 2,\%)}}{\text{(Volume 1, gal) + (Volume 2, gal)}}$

FLUORIDE ION PURITY (%) = $\frac{\text{(Molecular weight of Fluoride) (100\%)}}{\text{Molecular weight of Chemical}}$

INJURY FREQUENCY RATE = $\frac{\text{(Number of Injuries) 1,000,000}}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = $\frac{\text{Volume of Basin (gals) X 24 hrs}}{\text{Flow (GPD)}}$

BY-PASS WATER (gpd) = $\frac{\text{Total Flow (GPD) X Plant Effluent Hardness (gpg)}}{\text{Filtered Hardness (gpg)}}$

Hardness

HARDNESS (mg/L as $CaCO_3$) = $\frac{\text{A (mls of titrant) X 1000}}{\text{Mls of Sample}}$

Ca HARDNESS as mg/L $CaCo_3$ = 2.5 X (Ca, mg/L)

Mg HARDNESS as mg/L $CaCo_3$ = 4.12 (Mg, mg/L)

ALKALINITY-TOTAL = $\frac{\text{Mls of Titrant X Normality X 50,000}}{\text{Mls of Sample}}$
(mg/L)

EXCHANGE CAPACITY (grains) = Resin Volume (cu. ft.) X Removal Capacity

HARDNESS TO GRAIN/GALLON = $\frac{\text{Hardness (mg/L) X gr./gal}}{\text{17.1 mg/L}}$

LANGELIER INDEX = pH - pH_s

Chemical Addition

CHEMICAL FEED RATE = $\frac{\text{Chemical Feed (ml/min)}}{\text{3785 ml/gal}}$
(gpm)

CHLORINE DOSE (mg/L) = Chlorine Demand (mg/L) + Chlorine Residual (mg/L)

POLYMER % = $\frac{\text{Dry Polymer (lbs.)}}{\text{Dry Polymer (lbs.) + Water (lbs.)}}$

DESIRED PAC = $\frac{\text{Volume (MG) X Dose (mg/L) X 8.34}}{\text{1 MG}}$
(lbs./MG)

PAC (lbs./gal) = $\frac{\text{PAC (mg/L) X 3.785 (1/gallon)}}{\text{1000 (mg/g) X 454 (g/lb.)}}$

Filtration

FILTRATION RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Surface Area (sq. ft.)}}$
(gpm/sq. ft)

BACKWASH PUMPING RATE = Filter Area (sq. ft) X Backwash Rate (gpm/sq. ft)
(gpm)

FILTRATION RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Filter Area (sq. ft.)}}$
(gpm/sq. ft)

C Factor

Slope = $\frac{\text{Energy Loss, ft}}{\text{Distance, ft}}$

$$\frac{\text{Flow, GPM}}{193.75\ (\text{Diameter, ft})^{2.63}\ (\text{Slope})^{0.54}}$$

Chlorine Pre-Quiz Answers

1. Use a new, approved gasket on the connector
2. 1/4 turn to unseat the valve, then open one complete turn
3. The cylinder may rupture
4. The ratio of the density of the liquid to the density of water at 4 degrees C
5. Gold, Platinum, and Tantalum
6. Gas chlorine
7. Secure each cylinder in an upright position. Attach the protective bonnet over the valve. Firmly secure each cylinder.
8. Open chlorine metering orifice slightly. Inspect vacuum lines. Start injector water supply
9. In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate.
10. Chlorine gas forms a mixture of hydrochloric and hypochlorous acids
11. Because it is too easy to roll
12. A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber colored liquid, a noncombustible gas, and a strong oxidizer. Chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air.
13. Notify local emergency response team. Warn and evacuate people in adjacent areas. Be sure that no one enters the leak area without adequate self-contained breathing equipment.
14. Burning of eyes, nose, and mouth; lacrimation and rhinorrhea; Coughing, sneezing, choking, nausea and vomiting, headaches and dizziness, fatal pulmonary edema, pneumonia, conjunctivitis, keratitis, pharyngitis, burning chest pain, dyspnea, hemoptysis, hypoxemia, dermatitis; and skin blisters.
15. 646 mg/L
16. Get out of the area of the leak, proceeding upwind, and 2) take only very short breaths through the mouth
17. 0.195
18. True
19. True
20. True
21. HOCl and OCl-; free available chlorine
22. $Cl_2 + H_2O \rightarrow H^+ + Cl^- + HOCl$
23. $HOCl \leftrightarrow H^+ + OCl^-$
24. Hypochlorous acid

Glossary

ABANDONED WELL: Wells that have been or need to be sealed by an approved method.

ABSENCE OF OXYGEN: The complete absence of oxygen in water described as Anaerobic.

ACCURACY: How closely an instrument measures the true or actual value.

ACID AND BASE ARE MIXED: When an acid and a base are mixed, an explosive reaction occurs and decomposition products are created under certain conditions.

ACID: Slowly add the acid to water while stirring. An operator should not mix acid and water or acid to a strong base.

ACID RAIN: A result of airborne pollutants.

ACTIVATED CHARCOAL (GAC or PAC): Granular Activated Charcoal or Powered Activated Charcoal. Used for taste and odor removal. A treatment technique that is not included in the grading of a water facility.

ACTIVATED CARBON FILTRATION: Can remove organic chemicals that produce off-taste and odor. These compounds are not dangerous to health but can make the water unpleasant to drink. Carbon filtration comes in several forms, from small filters that attach to sink faucets to large tanks that contain removable cartridges. Activated carbon filters require regular maintenance or they can become a health hazard.

ADSORPTION: *Not to be confused with absorption.* Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term sorption encompasses both processes, while desorption is the reverse process. Adsorption is present in many natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification.

ADSORPTION CLARIFIERS: The concept of the adsorption clarifier package plant was developed in the early 1980s. This technology uses an up-flow clarifier with low-density plastic bead media, usually held in place by a screen. This adsorption media is designed to enhance the sedimentation/clarification process by combining flocculation and sedimentation into one step. In this step, turbidity is reduced by adsorption of the coagulated and flocculated solids onto the adsorption media and onto the solids already adsorbed onto the media. Air scouring cleans adsorption clarifiers followed by water flushing. Cleaning of this type of clarifier is initiated more often than filter backwashing because the clarifier removes more solids. As with the tube-settler type of package plant, the sedimentation/ clarification process is followed by mixed-media filtration and disinfection to complete the water treatment.

AIR GAP SEPARATION: A physical separation space that is present between the discharge vessel and the receiving vessel; for an example, a kitchen faucet.

AIR HAMMER: A pneumatic cylindrical hammering device containing a piston used on air rotary rigs. The air hammer's heavy piston moves up and down by the introduction of compressed air creating a hammering action on the bit.

AIR HOOD: The most suitable protection when working with a chemical that produces dangerous fumes.

AIR ENTRAINMENT: The dissolution or inclusion of air bubbles into water.

AIRLIFT: The lifting of water and/or cuttings to the surface by the injection of high pressure bursts of air. Airlift occurs continuously when drilling with air rotary and can be used for well development with a surging technique.

AIR PUMPING: Continuous airlifting to remove water from the well.

AIR ROTARY: A method of rotary well drilling that utilizes compressed air as the primary drilling fluid.

AGGLOMERATION: A jumbled cluster or mass of varied parts. The act or process of agglomerating.

ALKALINITY: Alkalinity or AT is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate. Alkalinity is closely related to the acid neutralizing capacity (ANC) of a solution and ANC is often incorrectly used to refer to alkalinity. However, the acid neutralizing capacity refers to the combination of the solution and solids present (e.g., suspended matter, or aquifer solids), and the contribution of solids can dominate the ANC (see carbonate minerals below).

ALTERNATIVE DISINFECTANTS: Disinfectants - other than chlorination (halogens) - used to treat water, e.g. ozone, ultraviolet radiation, chlorine dioxide, and chloramine. There is limited experience and scientific knowledge about the by-products and risks associated with the use of alternatives.

ALGAE: Microscopic plants that are free-living and usually live in water. They occur as single cells floating in water, or as multicellular plants like seaweed or strands of algae that attach to rocks.

ALPHA AND BETA RADIOACTIVITY: Represent two common forms of radioactive decay. Radioactive elements have atomic nuclei so heavy that the nucleus will break apart, or disintegrate spontaneously. When decay occurs, high-energy particles are released. These high-energy particles are called radioactivity. Although radioactivity from refined radioactive elements can be dangerous, it is rare to find dangerous levels of radioactivity in natural waters. An alpha particle is a doubly-charged helium nucleus comprised of two protons, two neutrons, and no electrons. A beta particle is a high-speed electron. Alpha particles do not penetrate matter easily, and are stopped by a piece of paper. Beta particles are much more penetrating and can pass through a millimeter of lead.

ALUMINUM SULFATE: The chemical name for Alum. The molecular formula of Alum is $Al_2(SO_4)3{\sim}14H_2O$. It is a cationic polymer.

AMOEBA: Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.

AMMONIA: NH_3 A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water. Most ammonia in water is present as the ammonium ion rather than as ammonia.

AMMONIATOR: A control device which meters gaseous ammonia directly into water under positive pressure.

ANAEROBIC: An abnormal condition in which color and odor problems are most likely to occur.

ANAEROBIC CONDITIONS: When anaerobic conditions exist in either the metalimnion or hypolimnion of a stratified lake or reservoir, water quality problems may make the water unappealing for domestic use without costly water treatment procedures. Most of these problems are associated with Reduction in the stratified waters.

ANEROID: Using no fluid, as in aneroid barometer.

ASEPTIC: Free from the living germs of disease, fermentation, or putrefaction.

ANNULAR SPACE: The space between the borehole wall and either drill piping or casing within a well.

ANNULUS: See Annular Space.

AMMONIA: A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water.

AQUICLUDE: A layer or layers of soils or formations which water cannot pass through (ex - solid bedrock or very stiff clay). The opposite of aquifer.

AQUIFER: A saturated layer or layers of soils or formations which water can pass through and be provided in usable quantities to supply wells or springs (ex – saturated semi consolidated sediment or saturated fractured bedrock.) An underground geologic formation capable of storing significant amounts of water.

AQUIFER PARAMETERS: Referring to such attributes as specific capacity, aquifer storage, transmissivity, hydraulic conductivity, gradient, and water levels. Refers to all of the components of Darcy's Law and related parameters.

ARTESIAN AQUIFER: A confined aquifer in which the pressure head results in a water elevation higher than the land surface.

ARTESIAN WELL: A well constructed within an artesian aquifer. When an artesian well is opened it will flow naturally.

As: The chemical symbol of Arsenic.

AS NITROGEN: An expression that tells how the concentration of a chemical is expressed mathematically. The chemical formula for the nitrate ion is NO_3 , with a mass of 62. The concentration of nitrate can be expressed either in terms of the nitrate ion or in terms of the principal element, nitrogen. The mass of the nitrogen atom is 14. The ratio of the nitrate ion mass to the nitrogen atom mass is 4.43. Thus a concentration of 10 mg/L nitrate expressed as nitrogen would be equivalent to a concentration of 44.3 mg/L nitrate expressed as nitrate ion. When dealing with nitrate numbers it is very important to know how numeric values are expressed.

ASYNCHRONOUS: Not occurring at the same time.

AUGER RIG: A drilling rig, which drives a rotating spiral flange to drill into the earth.

ATOM: The general definition of an ion is an atom with a positive or negative charge. Electron is the name of a negatively charged atomic particle.

BACKFLOW PREVENTION: To stop or prevent the occurrence of, the unnatural act of reversing the normal direction of the flow of liquid, gases, or solid substances back in to the public potable (drinking) water supply. See Cross-connection control.

BACKFLOW: To reverse the natural and normal directional flow of a liquid, gases, or solid substances back in to the public potable (drinking) water supply. This is normally an undesirable effect.

BACKSIPHONAGE: A liquid substance that is carried over a higher point. It is the method by which the liquid substance may be forced by excess pressure over or into a higher point.

BACTERIA: Small, one-celled animals too small to be seen by the naked eye. Bacteria are found everywhere, including on and in the human body. Humans would be unable to live without the bacteria that inhabit the intestines and assist in digesting food. Only a small percentage of bacteria cause disease in normal, healthy humans. Other bacteria can cause infections if they get into a cut or wound. Bacteria are the principal concern in evaluating the microbiological quality of drinking water, because some of the bacteria-caused diseases that can be transmitted by drinking water are potentially life-threatening.

BACTERIOPHAGE: Any of a group of viruses that infect specific bacteria, usually causing their disintegration or dissolution. A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage. Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

BAILER: A device used to withdrawal water or sediment from a well utilizing a check valve type mechanism.

BARITE: Processed barium sulfate, often used to increase drilling fluid densities in mud rotary.

BATTERY: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

BENTONITE: High quality clay composed primarily of montmorillonite. Used to thicken drilling mud in mud rotary drilling and used to form seals in well construction or abandonment.

BEST AVAILABLE TECHNOLOGY ECONOMICALLY ACHIEVABLE (BAT): A level of technology based on the best existing control and treatment measures that are economically achievable within the given industrial category or subcategory.

BEST MANAGEMENT PRACTICES (BMPs): Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the U.S. BMPs also include treatment requirements, operating procedures and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

BEST PRACTICABLE CONTROL TECHNOLOGY CURRENTLY AVAILABLE (BPT): A level of technology represented by the average of the best existing wastewater treatment performance levels within an industrial category or subcategory.

BEST PROFESSIONAL JUDGMENT (BPJ): The method used by a permit writer to develop technology-based limitations on a case-by-case basis using all reasonably available and relevant data.

BIT: The primary cutting edge of a drill string.

BLANK CASING: A section of well casing that is solid.

BLOWDOWN: The discharge of water with high concentrations of accumulated solids from boilers to prevent plugging of the boiler tubes and/or steam lines. In cooling towers, blowdown is discharged to reduce the concentration of dissolved salts in the recirculating cooling water.

BOREHOLE DEVIATION: A boreholes' orientation deviates from vertical while drilling.

BOREHOLE GEOPHYSICS: A surveying technique of utilizing specialized tools to measure various physical parameters of the aquifer, formation, and well.

BOREHOLE: The hole that is formed when drilling into the earth.

BOULDER: An individual rock or solid mass of rock larger than 10 inches in diameter.

BREAK POINT CHLORINATION: The process of chlorinating the water with significant quantities of chlorine to oxidize all contaminants and organic wastes and leave all remaining chlorine as free chlorine.

BRIDGING: The tendency of sediment, filter, or seal media to create an obstruction if installed in too small an annulus or to rapidly. Also can occur within filter packs requiring development.

BROMINE: Chemical disinfectant (HALOGEN) that kills bacteria and algae. This chemical disinfectant has been used only on a very limited scale for water treatment because of its handling difficulties. This chemical causes skin burns on contact, and a residual is difficult to obtain.

BUCKET AUGER: A single cylindrical type of auger flight consisting of offset cutting blades at the bottom. A bucket auger rig rotates the bucket and its blades cut into the earth and fill the bucket with cuttings, which are dumped on the surface as needed.

BUFFER: Chemical that resists pH change, e.g. sodium bicarbonate

BUTTON BIT: A bit that is constructed with raised (typically carbide) buttons that strengthen the bit and aid in crushing and grinding efficiency. A button bit may be of a roller, hammer, or percussion type.

CABLE TOOL: (Also called Percussion Drilling) A method of drilling which utilizes the consecutive lifting and dropping of a heavy drill string via a system of cables.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

Ca: The chemical symbol for calcium.

CADMIUM: A contaminant that is usually not found naturally in water or in very small amounts.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

CALCIUM ION: Is divalent because it has a valence of +2.

CALCIUM, MAGNESIUM AND IRON: The three elements that cause hardness in water.

$CaOCl_2.4H_2O$: The molecular formula of Calcium hypochlorite.

CAPILLARY ACTION: The occurrence of an upward movement of fluid into previously unsaturated soil due to adhesion and surface tension which develops between the fluid and soil particles.

CAPILLARY FRINGE: The uppermost portion of an aquifer where the vadose zone ends. The capillary action of soils permits moisture to extend upwards into the vadose zone within the capillary fringe.

CARBON DIOXIDE GAS: The pH will decrease and alkalinity will change as measured by the Langelier index after pumping carbon dioxide gas into water.

CARBONATE HARDNESS: Carbonate hardness is the measure of Calcium and Magnesium and other hard ions associated with carbonate (CO_32^-) and bicarbonate (HCO_3^-) ions contained in a solution, usually water. It is usually expressed either as parts per million (ppm or mg/L), or in degrees (KH - from the German "Karbonathärte"). One German degree of carbonate hardness is equivalent to about 17.8575 mg/L. Both measurements (mg/L or KH) are usually expressed "as $CaCO_3$" – meaning the amount of hardness expressed as if calcium carbonate was the sole source of hardness. Every bicarbonate ion only counts for half as much carbonate hardness as a carbonate ion does. If a solution contained 1 liter of water and 50 mg $NaHCO_3$ (baking soda), it would have a carbonate hardness of about 18 mg/L as $CaCO_3$. If you had a liter of water containing 50 mg of Na_2CO_3, it would have a carbonate hardness of about 29 mg/L as $CaCO_3$.

CARBONATE, BICARBONATE AND HYDROXIDE: Chemicals that are responsible for the alkalinity of water.

CARBONATE ROCK: Rock that is composed primarily of calcium carbonate.

CASING DRIVER: A percussion or hammering device used to force casing into the subsurface.

CASING: A column of specially designed pipe of metal or plastic material installed in wells in order to keep a borehole open to permit serviceability of and/or construction and completion of a well within it.

CATHEAD: A specially designed auxiliary reel that normally utilizes heavy rope rather than steel cable. Often used on cable tool or percussion drilling rigs for the operation of drive blocks.

CATHODIC PROTECTION: An operator should protect against corrosion of the anode and/or the cathode by painting the copper cathode. Cathodic protection interrupts corrosion by supplying an electrical current to overcome the corrosion-producing mechanism. Guards against stray current corrosion.

CAUSTIC: NaOH (also called Sodium Hydroxide) is a strong chemical used in the treatment process to neutralize acidity, increase alkalinity or raise the pH value.

CAUSTIC SODA: Also known as sodium hydroxide and is used to raise pH.

CAVERN: Large open spaces (>5ft.) encountered while drilling. More often associated with limestone formations in a karst environment.

CEILING AREA: The specific gravity of ammonia gas is 0.60. If released, this gas will accumulate first at the ceiling area. Cl_2 gas will settle on the floor.

CEMENT GROUT: Cement of fine consistency, capable of being pumped. Used to seal in and around wells.

CENTRALIZER: Stand offs attached to well casing and screen to maintain annular space. In drilling, it has the same meaning as stabilizer or drill collar.

CENTRIFUGAL FORCE: That force when a ball is whirled on a string that pulls the ball outward. On a centrifugal pump, it is that force which throws water from a spinning impeller.

CENTRIFUGAL PUMP: A pump consisting of an impeller fixed on a rotating shaft and enclosed in a casing, having an inlet and a discharge connection. The rotating impeller creates pressure in the liquid by the velocity derived from centrifugal force.

CESIUM (also Caesium)**:** Symbol Cs- A soft, silvery-white ductile metal, liquid at room temperature, the most electropositive and alkaline of the elements, used in photoelectric cells and to catalyze hydrogenation of some organic compounds.

CHAIN OF CUSTODY (COC): A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHAIN OF CUSTODY (COC)**:** A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHECK VALVE: Allows water to flow in only one direction.

CHELATION: A chemical process used to control scale formation in which a chelating agent "captures" scale-causing ions and holds them in solution.

CHEMICAL FEED RATE: Chemicals are added to the water in order to improve the subsequent treatment processes. These may include pH adjusters and coagulants. Coagulants are chemicals, such as alum, that neutralize positive or negative charges on small particles, allowing them to stick together and form larger particles that are more easily removed by sedimentation (settling) or filtration. A variety of devices, such as baffles, static mixers, impellers and in-line sprays, can be used to mix the water and distribute the chemicals evenly.

CHEMICAL OXIDIZER: KMnO4 or Potassium Permanganate is used for taste and odor control because it is a strong oxidizer which eliminates many organic compounds.

CHEMICAL REATION RATE: In general, when the temperature decreases, the chemical reaction rate also decreases. The opposite is true for when the temperature increases.

CHEMISORPTION: (or chemical adsorption) Is adsorption in which the forces involved are valence forces of the same kind as those operating in the formation of chemical compounds.

CHLORAMINATION: Treating drinking water by applying chlorine before or after ammonia. This creates a persistent disinfectant residual called chloramines.

CHLORAMINES: A group of chlorine ammonia compounds formed when chlorine combines with organic wastes in the water. Chloramines are not effective as disinfectants and are responsible for eye and skin irritation as well as strong chlorine odors.

CHLORINATION: The process in water treatment of adding chlorine (gas or solid hypochlorite) for purposes of disinfection.

CHLORINE: A chemical used to disinfect water. Chlorine is extremely reactive, and when it comes in contact with microorganisms in water it kills them. Chlorine is added to swimming pools to keep the water safe for swimming. Chlorine is available as solid tablets for swimming pools. Some public water system's drinking water treatment plants use chlorine in a gas form because of the large volumes required. Chlorine is very effective against algae, bacteria and viruses. Protozoa are resistant to chlorine because they have thick coats; protozoa are removed from drinking water by filtration.

CHLORINE DEMAND: Amount of chlorine required to react on various water impurities before a residual is obtained. Also, means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

CHLORINE FEED: Chlorine may be delivered by vacuum-controlled solution feed chlorinators. The chlorine gas is controlled, metered, introduced into a stream of injector water and then conducted as a solution to the point of application.

CHLORINE, FREE: Chlorine available to kill bacteria or algae. The amount of chlorine available for sanitization after the chlorine demand has been met. Also known as chlorine residual.

CIRCULATION: The continual flow of drilling fluid from injection to recovery and recirculation at the surface.

CLEAR WELL: A large underground storage facility sometimes made of concrete. A clear well or a plant storage reservoir is usually filled when demand is low. The final step in the conventional filtration process, the clearwell provides temporary storage for the treated water. The two main purposes for this storage are to have filtered water available for backwashing the filter and to provide detention time (or contact time) for the chlorine (or other disinfectant) to kill any microorganisms that may remain in the water.

ClO_2: The molecular formula of Chlorine dioxide.

COAGULATION: The best pH range for coagulation is between 5 and 7. Mixing is an important part of the coagulation process you want to complete the coagulation process as quickly as possible.

COBBLES: A rock smaller than a boulder but larger than a pebble. A cobble is greater than 2.5 inches in diameter and smaller than 10 inches in diameter.

COLIFORM: Bacteria normally found in the intestines of warm-blooded animals. Coliform bacteria are present in high numbers in animal feces. They are an indicator of potential contamination of water. Adequate and appropriate disinfection effectively destroys coliform bacteria. Public water systems are required to deliver safe and reliable drinking water to their customers 24 hours a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill. Fortunately, public water systems take many steps to ensure that the public has safe, reliable drinking water. One of the most important steps is to regularly test the water for coliform bacteria. Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans. Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, water system operators work to find the source of contamination and restore safe drinking water. There are three different groups of coliform bacteria; each has a different level of risk.

COLIFORM TESTING: The effectiveness of disinfection is usually determined by Coliform bacteria testing. A positive sample is a bad thing and indicates that you have bacteria contamination.

COLLOIDAL SUSPENSIONS: Because both iron and manganese react with dissolved oxygen to form insoluble compounds, they are not found in high concentrations in waters containing dissolved oxygen except as colloidal suspensions of the oxide.

COLORIMETRIC MEASUREMENT: A means of measuring an unknown chemical concentration in water by measuring a sample's color intensity.

COMMUTATOR: A device for reversing the direction of a current. (in a DC motor or generator) a cylindrical ring or disk assembly of conducting members, individually insulated in a supporting structure with an exposed surface for contact with current-collecting brushes and mounted on the armature shaft, for changing the frequency or direction of the current in the armature windings.

CHRONIC: A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

COMBINED CHLORINE: The reaction product of chlorine with ammonia or other pollutants, also known as chloramines.

COMMUNITY WATER SYSTEM: A water system which supplies drinking water to 25 or more of the same people year-round in their residences.

COMPLIANCE CYCLE: A 9-calendar year time-frame during which a public water system is required to monitor. Each compliance cycle consists of 3 compliance periods.

COMPLAINCE PERIOD: A 3-calendar year time-frame within a compliance cycle.

COMPLETION (WELL COMPLETION): Refers to the final construction of the well including the installation of pumping equipment.

COMPOSITE SAMPLE: A water sample that is a combination of a group of samples collected at various intervals during the day.

CONDENSATION: The process that changes water vapor to tiny droplets or ice crystals.

CONE OF DEPRESSION: That portion of the water table or potentiometric surface that experiences drawdown from a pumped well.

CONFINED AQUIFER: An aquifer that is isolated by confining layers on both its top and bottom. Pressures within a confined aquifer are normally greater than atmospheric pressure resulting in a potentiometric head.

CONFINING LAYER: An extensive layer of soil or formation that resists the movement of water from an aquifer below or above it. Confining layers isolate aquifers thereby confining them. May or may not be an aquiclude. (ex – Clay or silt rich layer)

CONSOLIDATED: Soil, sediment, or formation that is solidified or cemented together as a unit.

CONTACT TIME, pH and LOW TURBIDITY: Factors which are important in providing good disinfection using chlorine.

CONTACT TIME: If the water temperature decreases from 70°F (21°C) to 40°F (4°C). The operator needs to increase the detention time to maintain good disinfection of the water.

CONTAINS THE ELEMENT CARBON: A simple definition of an organic compound.

CONTAMINANT: Any natural or man-made physical, chemical, biological, or radiological substance or matter in water, which is at a level that may have an adverse effect on public health, and which is known or anticipated to occur in public water systems.

CONTAMINATE: tr.v. con·tam·i·nated, con·tam·i·nat·ing, con·tam·i·nates
1. To make impure or unclean by contact or mixture.
2. To expose to or permeate with radioactivity.

CONTAMINATION: A degradation in the quality of groundwater in result of the it's becoming polluted with unnatural or previously non-existent constituents.

CONTINUOUS SLOT SCREEN: A wire wrapped or plastic slotted screen in which the slot openings completely encircle the inner ribs of the screen.

CONTROL TASTE AND ODOR PROBLEMS: $KMnO_4$ Potassium permanganate is a strong oxidizer commonly used to control taste and odor problems.

CONVENTIONAL: A standard or common procedure to a group of more complex methods. (ex – Direct Rotary *conventional* vs. Reverse *non-conventional*)

COPPER: The chemical name for the symbol Cu.

CORROSION: The removal of metal from copper, other metal surfaces and concrete surfaces in a destructive manner. Corrosion is caused by improperly balanced water or excessive water velocity through piping or heat exchangers.

CORROSIVITY: The Langelier Index measures corrosivity.

COUPON: A coupon placed to measure corrosion damage in the water mains.

CROSS-CONNECTION: A physical connection between a public water system and any source of water or other substance that may lead to contamination of the water provided by the public water system through backflow. Might be the source of an organic substance causing taste and odor problems in a water distribution system.

CROSS-CONTAMINATION: The mixing of two unlike qualities of water. For example, the mixing of good water with a polluting substance like a chemical.

CUTTING HEAD (CUTTER HEAD): The bit portion of auger flighting that serves as the primary cutting edge of the auger.

CUTTING SHOE: A hardened steel sleeve with a wedged or armored cutting edge that is installed on well casing that is to be driven into the earth.

CUTTINGS: Crushed rock, soil, or formation material generated by the drilling action of a bit.

CRYPTOSPORIDIUM: A disease-causing parasite, resistant to chlorine disinfection. It may be found in fecal matter or contaminated drinking water. Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

CYANURIC ACID: White, crystalline, water-soluble solid, $C_3H_3O_3N_3 \cdot 2H_2O$, used chiefly in organic synthesis. Chemical used to prevent the decomposition of chlorine by ultraviolet (UV) light.

CYANOBACTERIA: Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

DAILY MAXIMUM LIMITATIONS: The maximum allowable discharge of pollutants during a 24 hour period. Where daily maximum limitations are expressed in units of mass, the daily discharge is the total mass discharged over the course of the day. Where daily maximum limitations are expressed in terms of a concentration, the daily discharge is the arithmetic average measurement of the pollutant concentration derived from all measurements taken that day.

DANGEROUS CHEMICALS: The most suitable protection when working with a chemical that produces dangerous fumes is to work under an air hood or fume hood.

DARCY'S LAW: (Q=KIA) A fundamental equation used in the groundwater sciences to determine aquifer characteristics, where Q=Flux, K=Hydraulic Conductivity (Permeability), I = Hydraulic Gradient (change in head), and A = Cross Sectional Area of flow.

DECIBELS: The unit of measurement for sound.

DECOMPOSE: To decay or rot.

DECOMPOSTION OF ORGANIC MATERIAL: The decomposition of organic material in water produces taste and odors.

DEMINERALIZATION PROCESS: Mineral concentration of the feed water is the most important consideration in the selection of a demineralization process. Acid feed is the most common method of scale control in a membrane demineralization treatment system.

DENTAL CARIES PREVENTION IN CHILDREN: The main reason that fluoride is added to a water supply.

DEPOLARIZATION: The removal of hydrogen from a cathode.

DESICCANT: When shutting down equipment which may be damaged by moisture, the unit may be protected by sealing it in a tight container. This container should contain a desiccant.

DESORPTION: Desorption is a phenomenon whereby a substance is released from or through a surface. The process is the opposite of sorption (that is, adsorption and absorption). This occurs in a system being in the state of sorption equilibrium between bulk phase (fluid, i.e. gas or liquid solution) and an adsorbing surface (solid or boundary separating two fluids). When the concentration (or pressure) of substance in the bulk phase is lowered, some of the sorbed substance changes to the bulk state. In chemistry, especially chromatography, desorption is the ability for a chemical to move with the mobile phase. The more a chemical desorbs, the less likely it will adsorb, thus instead of sticking to the stationary phase, the chemical moves up with the solvent front. In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid-vapor interface.

DEVELOPMENT: The cleaning of the well and bore once construction is complete.

DETENTION LAG: Is the period of time between the moment of change in a chlorinator control system and the moment when the change is sensed by the chlorine residual indicator.

DETENTION LAG TIME: The minimum detention time range recommended for flocculation is 5 – 20 minutes for direct filtration and up to 30 minutes for conventional filtration.

DIATOMACEOUS EARTH: A fine silica material containing the skeletal remains of algae.

DIRECT CURRENT: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

DIRECT ROTARY: The conventional method of rotary drilling involving the rotation of a drill string and standard use of drilling fluid to penetrate the earth.

DISCHARGE HEAD: See Total Dynamic Head.

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISINFECTION BY-PRODUCTS (DBPs): The products created due to the reaction of chlorine with organic materials (e.g. leaves, soil) present in raw water during the water treatment process. The EPA has determined that these DBPs can cause cancer. Chlorine is added to drinking water to kill or inactivate harmful organisms that cause various diseases. This process is called disinfection. However, chlorine is a very active substance and it reacts with naturally occurring substances to form compounds known as disinfection byproducts (DBPs). The most common DBPs formed when chlorine is used are trihalomethanes (THMs), and haloacetic acids (HAAs).

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLUTION : The chemical and physical process of dissolving rock. Typically, limestone or carbonate rocks can be dissolved via the percolation or movement of groundwater that, in its infancy, is slightly acidic. As time goes on, the rock may also be physically worn away by the rapid movement of groundwater through the interconnected open spaces created by the initial chemical dissolving process.

DISTILLATION, REVERSE OSMOSIS AND FREEZING: Processes that can be used to remove minerals from the water.

DRAG BIT: A style of drill bit used in rotary drilling when soil or formation conditions are loosely consolidated and are comprised of fine-grained sediments.

DRAWDOWN: The change in water level from static to pumping level.

DRILL COLLAR: A section of the drill string that provides sufficient mass and diameter to maintain vertical borehole alignment and consistent borehole diameter.

DRILL FOAM: Surfactant used in air rotary drilling and well development.

DRILL PIPE: Sections of the drill string that are connected one to another in order to achieve a desired length while also providing a pathway for the circulation of drilling fluid.

DRILL STEM: The complete drill string or, in cable drilling, the equivalent of a drill collar.

DRILL STRING: The complete drilling assembly in rotary drilling including drill pipe, subs, collars, and bit.

DRILLER: A specially trained individual that operates the drilling rig.

DRILLING FLUID: Fluid circulated through the borehole in rotary drilling methods used to lift cuttings to the surface, provide borehole stability, and cool the bit. Drilling Fluid may consist of mud, water, air, foam, or other additives.

DRILLING PERMIT: A certificate of approval to drill and construct a well often required by the state or local regulating authority.

DRILLING PRESSURE: The pressure exerted within the borehole during drilling. The pressure required to circulate drilling fluid to the surface.

DRIVE BLOCK: A heavy collar that attaches over the drill pipe and is dropped successively to advance casing into the earth. Used primarily in cable tool or percussion drilling methods.

DRIVE CLAMP: A fitting that is attached to the top of a drill string or stem serving as a striking surface for driving casing into the earth.

DRIVE UNIT: The portion of a rotary rig that provides the rotation to the drill string. (ex – top drive or table drive unit). Also may be called the drive head.

DRIVING: The installation of a well or casing via forcing of it into the earth by repeated striking.

DRY ACID: A granular chemical used to lower pH and or total alkalinity.

E. COLI, Escherichia coli: A bacterium commonly found in the human intestine. For water quality analyses purposes, it is considered an indicator organism. These are considered evidence of water contamination. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

EFFECTIVENESS OF CHLORINE: The factors which influence the effectiveness of chlorination the most are pH, turbidity and temperature. Effectiveness of Chlorine decreases occurs during disinfection in source water with excessive turbidity.

ELECTRON: The name of a negatively charged atomic particle.

ELEMENTARY BUSINESS PLAN: Technical Capacity, Managerial Capacity, and Financial Capacity make up the elementary business plan. To become a new public water system, an owner shall file an elementary business plan for review and approval by state environmental agency.

EMERGENCY RESPONSE TEAM: A local team that is thoroughly trained and equipped to deal with emergencies, e.g. chlorine gas leak. In case of a chlorine gas leak, get out of the area and notify your local emergency response team in case of a large uncontrolled chlorine leak.

ENHANCED COAGULATION: The process of joining together particles in water to help remove organic matter.

ENTAMOEBA HISTOLYTICA: Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

ENTEROVIRUS: A virus whose presence may indicate contaminated water; a virus that may infect the gastrointestinal tract of humans.

EUGLENA: Euglena are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features. Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food.

EVOLUTION: Any process of formation or growth; development: the evolution of a language; the evolution of the airplane. A product of such development; something evolved: The exploration of space is the evolution of decades of research.

Biology. Change in the gene pool of a population from generation to generation by such processes as mutation, natural selection, and genetic drift. A process of gradual, peaceful, progressive change or development, as in social or economic structure or institutions, a motion incomplete in itself, but combining with coordinated motions to produce a single action, as in a machine. A pattern formed by or as if by a series of movements: the evolutions of a figure skater.

An evolving or giving off of gas, heat, etc. **evolutional**, adjective ev·o·lu·tion·al·ly, adverb Synonyms 1. unfolding, change, progression, metamorphosis. Antonyms 1. stasis, inactivity, changelessness.

F: The chemical symbol of Fluorine.

FAUCET WITH AN AERATOR: When collecting a water sample from a distribution system, a faucet with an aerator should not be used as a sample location.

FAULT: A break in the earth's crust where movement has occurred.

FAULTING: A geological process involving the breaking and displacement of rock or formation through movements within the earth's crust along a fault.

FECAL COLIFORM: A group of bacteria that may indicate the presence of human or animal fecal matter in water. Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

FILTRATION: The process of passing water through materials with very small holes to strain out particles. Most conventional water treatment plants used filters composed of gravel, sand, and anthracite. These materials settle into a compact mass that forms very small holes. Particles are filtered out as treated water passes through these holes. These holes are small enough to remove microorganisms including algae, bacteria, and protozoans, but not viruses. Viruses are eliminated from drinking water through the process of disinfection using chlorine. A series of processes that physically removes particles from water. A water treatment step used to remove turbidity, dissolved organics, odor, taste and color.

FILTER CLOGGING: An inability to meet demand may occur when filters are clogging.

FILTRATION METHODS: The conventional type of water treatment filtration method includes coagulation, flocculation, sedimentation, and filtration. Direct filtration method is similar to conventional except that the sedimentation step is omitted. Slow sand filtration process does not require pretreatment, has a flow of 0.1 gallons per minute per square foot of filter surface area, and is simple to operate and maintain. The Diatomaceous earth method uses a thin layer of fine siliceous material on a porous plate. This type of filtration medium is only used for water with low turbidity. Sedimentation, adsorption, and biological action treatment methods are filtration processes that involve a number of interrelated removal mechanisms. Demineralization is primarily used to remove total dissolved solids from industrial wastewater, municipal water, and seawater.

FINISHED WATER: Treated drinking water that meets minimum state and federal drinking water regulations.

FLIGHTING: The spiral flanged drill pipe used in auger drilling.

FLOATING SUB: A collapsible section of drill pipe shorter than primary drill pipe. Used to provide a cushion between the drive unit and the drill string.

FLOCCULATION: The process of bringing together destabilized or coagulated particles to form larger masses that can be settled and/or filtered out of the water being treated. Conventional coagulation–flocculation-sedimentation practices are essential pretreatments for many water purification systems—especially filtration treatments. These processes agglomerate suspended solids together into larger bodies so that physical filtration processes can more easily remove them. Particulate removal by these methods makes later filtering processes far more effective. The process is often followed by gravity separation (sedimentation or flotation) and is always followed by filtration. A chemical coagulant, such as iron salts, aluminum salts, or polymers, is added to source water to facilitate bonding among particulates. Coagulants work by creating a chemical reaction and eliminating the negative charges that cause particles to repel each other. The coagulant-source water mixture is then slowly stirred in a process known as flocculation. This water churning induces particles to collide and clump together into larger and more easily removable clots, or "flocs." The process requires chemical knowledge of source water characteristics to ensure that an effective coagulant mix is employed. Improper coagulants make these treatment methods ineffective. The ultimate effectiveness of coagulation/flocculation is also determined by the efficiency of the filtering process with which it is paired.

FLOCCULANTS: Flocculants, or flocculating agents, are chemicals that promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc. Flocculants are used in water treatment processes to improve the sedimentation or filterability of small particles. For example, a flocculant may be used in swimming pool or drinking water filtration to aid removal of microscopic particles which would otherwise cause the water to be cloudy and which would be difficult or impossible to remove by filtration alone. Many flocculants are multivalent cations such as aluminum, iron, calcium or magnesium. These positively charged molecules interact with negatively charged particles and molecules to reduce the barriers to aggregation. In addition, many of these chemicals, under appropriate pH and other conditions such as temperature and salinity, react with water to form insoluble hydroxides which, upon precipitating, link together to form long chains or meshes, physically trapping small particles into the larger floc.

Long-chain polymer flocculants, such as modified polyacrylamides, are manufactured and sold by the flocculant producing business. These can be supplied in dry or liquid form for use in the flocculation process. The most common liquid polyacrylamide is supplied as an emulsion with 10-40 % actives and the rest is a carrier fluid, surfactants and latex. Emulsion polymers require activation to invert the emulsion and allow the electrolyte groups to be exposed.

FLOC SHEARING: Likely to happen to large floc particles when they reach the flocculation process.

FLOCCULATION BASIN: A compartmentalized basin with a reduction of speed in each compartment. This set-up or basin will give the best overall results.

FLOOD RIM: The point of an object where the water would run over the edge of something and begin to cause a flood.

FLOW MUST BE MEASURED: A recorder that measures flow is most likely to be located in a central location.

FLUORIDE: High levels of fluoride may stain the teeth of humans. This is called Mottling. This chemical must not be overfed due to a possible exposure to a high concentration of the chemical. The most important safety considerations to know about fluoride chemicals are that all fluoride chemicals are extremely corrosive**.** These are the substances most commonly used to furnish fluoride ions to water: Sodium fluoride, Sodium silicofluoride and Hydrofluosilicic acid.

FLUORIDE FEEDING: Always review fluoride feeding system designs and specifications to determine whether locations for monitoring readouts and dosage controls are convenient to the operation center and easy to read and correct.

FLUX: The term flux describes the rate of water flow through a semipermeable membrane. When the water flux decreases through a semipermeable membrane, it means that the mineral concentration of the water is increasing.

FORMATION: A series of layers, deposits, or bodies of rock, which are geologically similar and related in depositional environment or origin. A formation can be clearly distinguished relative to bounding deposits or formations due to its particular characteristics and composition.

FORMATION OF TUBERCLES: This condition is of the most concern regarding corrosive water effects on a water system. It is the creation of mounds of rust inside the water lines.

FRACTURE: A discrete break in a rock or formation.

FRACTURED AQUIFER: An aquifer within and otherwise massive block that has been made permeable due to the concentrated presence of fractures typically resultant of faulting or concentrated joints.

FREE CHLORINE: In disinfection, chlorine is used in the form of free chlorine or as hypochlorite ion.

FREE CHLORINE RESIDUAL: Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination. The reason for chlorinating past the breakpoint is to provide protection in case of backflow.

GATE VALVE: The most common type of valve used in isolating a small or medium sized section of a distribution system and is the only linear valve used in water distribution. All the other valves are in the rotary classification.

GIARDIA LAMLIA: Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

GIARDIASAS, HEPATITIS OR TYHOID: Diseases that may be transmitted through the contamination of a water supply but not AIDS.

GIS – GRAPHIC INFORMATION SYSTEM: Detailed information about the physical locations of structures such as pipes, valves, and manholes within geographic areas with the use of satellites.

GEOTECHNICAL: Characteristics of soil, rock, or formation such as grain size, shear strength, porosity, and compressibility, etc. Of particular concern to a geologist or engineer relative to soil or aquifer characteristics.

GLOBE VAVLVE: The main difference between a globe valve and a gate valve is that a globe valve is designed as a controlling device.

GOOD CONTACT TIME, pH and LOW TURBIDITY: These are factors that are important in providing good disinfection when using chlorine.

GPM: Gallons per minute.

GRAB SAMPLE: A sample which is taken from a water or wastestream on a one-time basis with no regard to the flow of the water or wastestream and without consideration of time. A single grab sample should be taken over a period of time not to exceed 15 minutes.

GRAINSIZE: The dimension of particle classifications such as gravel, sand, silt, and clay. Often based on the unified soil classification system.

GROUNDWATER: Water that percolates through and exists within saturated portions of the earth's crust and is replenished by the hydrologic cycle.

GROUT: A type of cement that is normally fine grained and used to effectively construct well seals and used in well abandonment. Grout may also be used to stabilize otherwise unstable boreholes, permitting continued drilling.

GT: Represents (Detention time) x (mixing intensity) in flocculation.

H_2SO_4: The molecular formula of Sulfuric acid.

HALIDES: A halide is a binary compound, of which one part is a halogen atom and the other part is an element or radical that is less electronegative than the halogen, to make a fluoride, chloride, bromide, iodide, or astatide compound. Many salts are halides. All Group 1 metals form halides with the halogens and they are white solids. A halide ion is a halogen atom bearing a negative charge. The halide anions are fluoride (F), chloride (Cl), bromide (Br), iodide (I) and astatide (At). Such ions are present in all ionic halide salts.

HALL EFFECT: Refers to the potential difference (Hall voltage) on the opposite sides of an electrical conductor through which an electric current is flowing, created by a magnetic field applied perpendicular to the current. Edwin Hall discovered this effect in 1879.

HALOACETIC ACIDS: Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$).

HAMMER BIT: The bit driven by the hammer to cut into rock or formation.

HAMMER: See Air Hammer

HARD ROCK: Consolidated formation or solid rock.

HARD WATER: Hard water causes a buildup of scale in household hot water heaters. Hard water is a type of water that has high mineral content (in contrast with soft water). Hard water primarily consists of calcium (Ca2+), and magnesium (Mg2+) metal cations, and sometimes other dissolved compounds such as bicarbonates and sulfates. Calcium usually enters the water as either calcium carbonate ($CaCO_3$), in the form of limestone and chalk, or calcium sulfate ($CaSO_4$), in the form of other mineral deposits. The predominant source of magnesium is dolomite ($CaMg(CO_3)2$). Hard water is generally not harmful. The simplest way to determine the hardness of water is the lather/froth test: soap or toothpaste, when agitated, lathers easily in soft water but not in hard water. More exact measurements of hardness can be obtained through a wet titration. The total water 'hardness' (including both Ca2+ and Mg2+ ions) is read as parts per million or weight/volume (mg/L) of calcium carbonate (CaCO3) in the water. Although water hardness usually only measures the total concentrations of calcium and magnesium (the two most prevalent, divalent metal ions), iron, aluminum, and manganese may also be present at elevated levels in some geographical locations.

HARDNESS: A measure of the amount of calcium and magnesium salts in water. More calcium and magnesium lead to greater hardness. The term "hardness" comes from the fact that it is hard to get soap suds from soap or detergents in hard water. This happens because calcium and magnesium react strongly with negatively-charged chemicals like soap to form insoluble compounds.

HARTSHORN: The antler of a hart, formerly used as a source of ammonia. Ammonium carbonate.

HAZARDS OF POLYMERS: Slippery and difficult to clean-up are the most common hazards associated with the use of polymers in a water treatment plant.

HEAD: The measure of the pressure of water expressed in feet of height of water. 1 PSI = 2.31 feet of water or 1 foot of head equals about a half a pound of pressure or .433 PSI. There are various types of heads of water depending upon what is being measured. Static (water at rest) and Residual (water at flow conditions).

HEADWORKS: The facility at the "**head**" of the water source where water is first treated and routed into the distribution system.

HEALTH ADVISORY: An EPA document that provides guidance and information on contaminants that can affect human health and that may occur in drinking water, but which the EPA does not currently regulate in drinking water.

HERTZ: The term used to describe the frequency of cycles in an alternating current (AC) circuit.

HETEROTROPHIC PLATE COUNT: A test performed on drinking water to determine the total number of all types of bacteria in the water.

HF: The molecular formula of Hydrofluoric acid.

HIGH TURBIDITY CAUSING INCREASED CHLORINE DEMAND: May occur or be caused by the inadequate disinfection of water.

HOLLOW STEM (AUGER): An auger form of drilling in which the flighting is hollow.

HOLLOW STEM FLIGHT: The hollow spiral flanged drill pipe used on hollow stem auger rigs.

HOMOPOLAR: Of uniform polarity; not separated or changed into ions; not polar in activity. Electricity. unipolar.

HYDRAULIC CONDUCTIVITY: A primary factor in Darcy's Law, the measure of a soil or formations ability to transmit water, measured in gallons per day (gpd) See also Permeability and Darcy's Law.

HYDRIDES: Hydride is the name given to the negative ion of hydrogen, H. Although this ion does not exist except in extraordinary conditions, the term hydride is widely applied to describe compounds of hydrogen with other elements, particularly those of groups 1–16. The variety of compounds formed by hydrogen is vast, arguably greater than that of any other element. Various metal hydrides are currently being studied for use as a means of hydrogen storage in fuel cell-powered electric cars and batteries. They also have important uses in organic chemistry as powerful reducing agents, and many promising uses in hydrogen economy.

HYDROCHLORIC AND HYPOCHLOROUS ACIDS: HCL and HOCL The compounds that are formed in water when chlorine gas is introduced.

HYDROFLUOSILIC ACID: (H_2SiF_6) a clear, fuming corrosive liquid with a pH ranging from 1 to 1.5. Used in water treatment to fluoridate drinking water.

HYDROGEN SULFIDE OR CHLORINE GAS: These chemicals can cause olfactory fatigue.

HYDROLOGIC CYCLE: (Water Cycle) The continual process of precipitation (rain and snowfall), evaporation (primarily from the oceans), peculation (recharge to groundwater), runoff (surface water), and transpiration (plants) constituting the renew ability and recycling of each component.

HYDROPHOBIC: Does not mix readily with water.

HYGROSCOPIC: Absorbing or attracting moisture from the air.

HYPOCHLORITE (OCL-) AND ORGANIC MATERIALS: Heat and possibly fire may occur when hypochlorite is brought into contact with an organic material.

HYPOLIMNION: The layer of water in a thermally stratified lake that lies below the thermocline, is noncirculating, and remains perpetually cold.

IMPELLERS: The semi-open or closed props or blades of a turbine pump that when rotated generate the pumping force.

IMPERVIOUS: Not allowing, or allowing only with great difficulty, the movement of water.

IN SERIES: Several components being connected one to the other without a bypass, requiring each component to work dependent on the one before it.

INFILTRATION: The percolation of fluid into soil or formation. See also percolation.

INFECTIOUS PATHOGENS/MICROBES/GERMS: Are considered disease-producing bacteria, viruses and other microorganisms.

INFLATABLE PACKER: A rubber or fiber bladder device that is inflated to seal against either casing or borehole walls.

INFORMATION COLLECTION RULE: ICR EPA collected data required by the Information Collection Rule (May 14, 1996) to support future regulation of microbial contaminants, disinfectants, and disinfection byproducts. The rule was intended to provide EPA with information on chemical byproducts that form when disinfectants used for microbial control react with chemicals already present in source water (disinfection byproducts (DBPs)); disease-causing microorganisms (pathogens), including Cryptosporidium; and engineering data to control these contaminants.

INITIAL MONITORING YEAR: An initial monitoring year is the calendar year designated by the Department within a compliance period in which a public water system conducts initial monitoring at a point of entry.

INORGANIC CONTAMINANTS: Mineral-based compounds such as metals, nitrates, and asbestos. These contaminants are naturally-occurring in some water, but can also get into water through farming, chemical manufacturing, and other human activities. EPA has set legal limits on 15 inorganic contaminants.

INORGANIC IONS: Present in all waters. Inorganic ions are essential for human health in small quantities, but in larger quantities they can cause unpleasant taste and odor or even illness. Most community water systems will commonly test for the concentrations of seven inorganic ions: nitrate, nitrite, fluoride, phosphate, sulfate, chloride, and bromide. Nitrate and nitrite can cause an illness in infants called methemoglobinemia. Fluoride is actually added to the drinking water in some public water systems to promote dental health. Phosphate, sulfate, chloride, and bromide have little direct effect on health, but high concentrations of inorganic ions can give water a salty or briny taste.

INSOLUBLE COMPOUNDS: Are types of compounds cannot be dissolved. When iron or manganese reacts with dissolved oxygen (DO) insoluble compound are formed.

INTAKE FACILITIES: One of the more important considerations in the construction of intake facilities is the ease of operation and maintenance over the expected lifetime of the facility. Every intake structure must be constructed with consideration for operator safety and for cathodic protection.

ION EXCHANGE: An effective treatment process used to remove iron and manganese in a water supply. The hardness of the source water affects the amount of water an ion exchange softener may treat before the bed requires regeneration.

IRON: Fe The elements iron and manganese are undesirable in water because they cause stains and promote the growth of iron bacteria.

IRON AND MANGANESE: Fe and Mn In water they can usually be detected by observing the color of the inside walls of filters and the filter media. If the raw water is pre-chlorinated, there will be black stains on the walls below the water level and a black coating over the top portion of the sand filter bed. When significant levels of dissolved oxygen are present, iron and manganese exist in an oxidized state and normally precipitate into the reservoir bottom sediments. The presence of iron and manganese in water promote the growth of Iron bacteria. Only when a water sample has been acidified then you can perform the analysis beyond the 48 hour holding time. Iron and Manganese in water may be detected by observing the color of the of the filter media. Maintaining a free chlorine residual and regular flushing of water mains may control the growth of iron bacteria in a water distribution system.

IRON BACTERIA: Perhaps the most troublesome consequence of iron and manganese in the water is they promote the growth of a group of microorganism known as Iron Bacteria.

IRON FOULING: You should look for an orange color on the resin and backwash water when checking an ion exchange unit for iron fouling

JARS (DRILLING JARS): Metal sections of a drill string that when released provide a jarring force or action to aid in removing drill string. Used primarily in cable tool or percussion drilling methods.

JETTING: The process of injecting high velocity streams of water and/or air through a system of nozzles or jets into the well screen and filter pack for well development.

KARST TOPOGRAPHY: The visual presence of karst on the surface.

KARST: The presence of caverns, voids, sink holes as characteristic features of a weathered limestone or other carbonate formation on or beneath the surface.

KELLY: A multi-faceted section of drill pipe driven by a kelly drive (table or top drive).

KILL = C X T: Where other factors are constant, the disinfecting action may be represented by: Kill=C x T. C= Chlorine T= Contact time.

KINETIC ENERGY: The ability of an object to do work by virtue of its motion. The energy terms that are used to describe the operation of a pump are pressure and head.

LACRIMATION: The secretion of tears, esp. in abnormal abundance Also, lachrymation, lachrimation.

LANGELIER INDEX: A measurement of Corrosivity. The water is becoming corrosive in the distribution system causing rusty water if the Langelier index indicates that the pH has decreased from the equilibrium point. Mathematically derived factor obtained from the values of calcium hardness, total alkalinity, and pH at a given temperature. A Langelier index of zero indicates perfect water balance (i.e., neither corroding nor scaling). The Langelier Saturation Index (sometimes Langelier Stability Index) is a calculated number used to predict the calcium carbonate stability of water. It indicates whether the water will precipitate, dissolve, or be in equilibrium with calcium carbonate. Langelier developed a method for predicting the pH at which water is saturated in calcium carbonate (called pHs). The LSI is expressed as the difference between the actual system pH and the saturation pH.
LSI = pH - pHs

LEACHING: A chemical reaction between water and metals that allows for removal of soluble materials.

LEAD AND COPPER: Initial tap water monitoring for lead and copper must be conducted during 2 consecutive 6-month periods.

LIME: Is a chemical that may be added to water to reduce the corrosivity. When an operator adds lime to water, Calcium and magnesium become less soluble.

LIME SODA SOFTENING: In a lime soda softening process, to the pH of the water is raised to 11.0. In a lime softening process, excess lime is frequently added to remove Calcium and Magnesium Bicarbonate. The minimum hardness which can be achieved by the lime-soda ash process is 30 to 40 mg/L as calcium carbonate. The hardness due to noncarbonate hardness is most likely to determine the choice between lime softening and ion exchange to remove hardness.

LIME SOFTENING: Lime softening is primarily used to “soften” water—that is to remove calcium and magnesium mineral salts. But it also removes harmful toxins like radon and arsenic. Though there is no consensus, some studies have even suggested that lime softening is effective at removal of Giardia. Hard water is a common condition responsible for numerous problems. Users often recognize hard water because it prevents their soap from lathering properly. However, it can also cause buildup (“scale”) in hot water heaters, boilers, and hot water pipes. Because of these inconveniences, many treatment facilities use lime softening to soften hard water for consumer use. Before lime softening can be used, managers must determine the softening chemistry required. This is a relatively easy task for groundwater sources, which remain more constant in their composition. Surface waters, however, fluctuate widely in quality and may require frequent changes to the softening chemical mix. In lime softening, lime and sometimes sodium carbonate are added to the water as it enters a combination solids contact clarifier. This raises the pH (i.e., increases alkalinity) and leads to the precipitation of calcium carbonate. Later, the pH of the effluent from the clarifier is reduced again, and the water is then filtered through a granular media filter. The water chemistry requirements of these systems require knowledgeable operators, which may make lime softening an economic challenge for some very small systems.

LINE SHAFT TURBINE: See vertical turbine.

LOGGED (LOGGING): The assessment and documentation of geological and water production data obtained while drilling progresses or following drilling through the use of borehole geophysical logging tools.

L.O.T.O.: Lock Out, Tag Out. If a piece of equipment is locked out, the key to the lock-out device the key should be held by the person who is working on the equipment. The tag is an identification device and the lock is a physical restraint.

M-ENDO BROTH: The coliform group is used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Broth is used for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. m-Endo Broth is prepared according to the formula of Fifield and Schaufus.1 It is recommended by the American Public Health Association in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.2,3 The US EPA specifies using m-Endo Broth in the total coliform methods for testing water using single-step, two-step, and delayed incubation membrane filtration methods.

MAGNESIUM HARDNESS: Measure of the magnesium salts dissolved in water – it is not a factor in water balance.

MAGNETIC STARTER: Is a type of motor starter should be used in an integrated circuit to control flow automatically.

MARBLE AND LANGELIER TESTS: Are used to measure or determine the corrosiveness of a water source.

MAXIMUM CONTAMINANT LEVEL (MCLs): The maximum allowable level of a contaminant that federal or state regulations allow in a public water system. If the MCL is exceeded, the water system must treat the water so that it meets the MCL.

MAXIMUM CONTAMINANT LEVEL GOAL (MCLG): The level of a contaminant at which there would be no risk to human health. This goal is not always economically or technologically feasible, and the goal is not legally enforceable.

MCL for TURBIDITY: Turbidity is undesirable because it causes health hazards. An MCL for turbidity was established by the EPA because turbidity does not allow for proper disinfection.

MEASURE CORROSION DAMAGE: A coupon such as a strip of metal and is placed to measure corrosion damage in the distribution system in a water main.

MECHANICAL SEAL: A mechanical device used to control leakage from the stuffing box of a pump. Usually made of two flat surfaces, one of which rotates on the shaft. The two flat surfaces are of such tolerances as to prevent the passage of water between them. Held in place with spring pressure.

MEDIUM WATER SYSTEM: More than 3,300 persons and 50,000 or fewer persons.

MEGGER: Is a portable instrument used to measure insulation resistance. The megger consists of a hand-driven DC generator and a direct reading ohm meter. Used to test the insulation resistance on a motor.

M-ENDO BROTH: The media shall be brought to the boiling point when preparing M-Endo broth to be used in the membrane filter test for total coliform.

METALIMNION: Thermocline, middle layer of a thermally stratified lake which is characterized by a rapid decrease in temperature in proportion to depth.

METALLOID: Metalloid is a term used in chemistry when classifying the chemical elements. On the basis of their general physical and chemical properties, nearly every element in the periodic table can be termed either a metal or a nonmetal. A few elements with intermediate properties are, however, referred to as metalloids. (In Greek metallon = metal and eidos = sort)

METHANE: Methane is a chemical compound with the molecular formula CH_4. It is the simplest alkane, and the principal component of natural gas. Methane's bond angles are 109.5 degrees. Burning methane in the presence of oxygen produces carbon dioxide and water. The relative abundance of methane and its clean burning process makes it a very attractive fuel. However, because it is a gas at normal temperature and pressure, methane is difficult to transport from its source. In its natural gas form, it is generally transported in bulk by pipeline or LNG carriers; few countries still transport it by truck.

MILLILITER: One one-thousandth of a liter; A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

Mg/L: Stands for "**milligrams per liter**." A common unit of chemical concentration. It expresses the mass of a chemical that is present in a given volume of water. A milligram (one one-thousandth of a gram) is equivalent to about 18 grains of table salt. A liter is equivalent to about one quart.

MICROBIOLOGICAL: Is a type of analysis in which a composite sample unacceptable.

MICROBE OR MICROBIAL: Any minute, simple, single-celled form of life, especially one that causes disease.

MICROBIAL CONTAMINANTS: Microscopic organisms present in untreated water that can cause waterborne diseases.

MICROORGANISMS: Very small animals and plants that are too small to be seen by the naked eye and must be observed using a microscope. Microorganisms in water include algae, bacteria, viruses, and protozoa. Algae growing in surface waters can cause off-taste and odor by producing the chemicals MIB and geosmin. Certain types of bacteria, viruses, and protozoa can cause disease in humans. Bacteria are the most common microorganisms found in treated drinking water. The great majority of bacteria are not harmful. In fact, humans would not be able to live without the bacteria that inhabit the intestines. However, certain types of bacteria called coliform bacteria can signal the presence of possible drinking water contamination.

MILLILITER: One one-thousandth of a liter. A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

MOISTURE: If a material is hygroscopic, it must it be protected from water.

MOISTURE AND POTASSIUM PERMANGANATE: The combination of moisture and potassium permanganate produces heat.

MOLECULAR WEIGHT: The molecular mass (abbreviated Mr) of a substance, formerly also called molecular weight and abbreviated as MW, is the mass of one molecule of that substance, relative to the unified atomic mass unit u (equal to 1/12 the mass of one atom of carbon-12). This is distinct from the relative molecular mass of a molecule, which is the ratio of the mass of that molecule to 1/12 of the mass of carbon 12 and is a dimensionless number. Relative molecular mass is abbreviated to Mr.

MOTTLING: High levels of fluoride may stain the teeth of humans.

M.S.D.S.: Safety Data Sheet (formerly MSDS). A safety document must an employer provide to an operator upon request.

MUD BALLS IN FILTER MEDIA: Is a possible result of an ineffective or inadequate filter backwash.

MUD CAKE: A film of mud drilling fluid that builds up on borehole walls adding to borehole stability and limits the groundwater's ability to enter the borehole while drilling.

MUD CAKING: The process of building up the mud cake.

MUD ENGINEER: A specially trained individual who's responsible for maintaining proper drilling fluid densities and viscosity.

MUD PIT: Single or multiple subsurface or surface containment system used for settling cuttings out of drilling fluid and for recirculation of drilling fluid.

MUD PUMP: A specially designed pump that can pass particles of mud and cuttings (drilling fluid) at variable pressures, serving as the primary component in a mud rotary drilling system (similar to a grout or cement pump).

MUD ROTARY: The method of rotary drilling with mud circulation as the drilling fluid.

MURIATIC ACID: An acid used to reduce pH and alkalinity. Also used to remove stain and scale.

MYCOTOXIN: A toxin produced by a fungus.

NaOCl: Is the molecular formula of Sodium hypochlorite.

NaOH: Is the molecular formula of Sodium hydroxide.

NASCENT: Coming into existence; emerging.

NATURAL GRAVEL PACK (NATURALLY PACKED): Refers to a well that has no gravel pack installed but is simply allowed to develop a filter pack composed of the aquifer particles itself. Usually coarse grained and hard rock aquifers are naturally packed.

NH_3: The molecular formula of Ammonia.

NH_4+: The molecular formula of the Ammonium ion.

NITRATES: A dissolved form of nitrogen found in fertilizers and sewage by-products that may leach into groundwater and other water sources. Nitrates may also occur naturally in some waters. Over time, nitrates can accumulate in aquifers and contaminate groundwater.

NITROGEN: Nitrogen is a nonmetal, with an electronegativity of 3.0. It has five electrons in its outer shell and is therefore trivalent in most compounds. The triple bond in molecular nitrogen (N_2) is one of the strongest in nature. The resulting difficulty of converting (N_2) into other compounds, and the ease (and associated high energy release) of converting nitrogen compounds into elemental N_2, have dominated the role of nitrogen in both nature and human economic activities.

NITROGEN AND PHOSPHORUS: Pairs of elements and major plant nutrients that cause algae to grow.

NO_3^-: The molecular formula of the Nitrate ion.

NON-CARBONATE HARDNESS: The portion of the total hardness in excess of the alkalinity.

NON-CARBONATE IONS: Water contains non-carbonate ions if it cannot be softened to a desired level through the use of lime only.

NON-POINT SOURCE POLLUTION: Air pollution may leave contaminants on highway surfaces. This non-point source pollution adversely impacts reservoir water and groundwater quality.

NON-TRANSIENT, NON-COMMUNITY WATER SYSTEM: A water system which supplies water to 25 or more of the same people at least six months per year in places other than their residences. Some examples are schools, factories, office buildings, and hospitals which have their own water systems.

NORMALITY: It is the number of equivalent weights of solute per liter of solution. Normality highlights the chemical nature of salts: in solution, salts dissociate into distinct reactive species (ions such as H^+, Fe_3^+, or Cl^-). Normality accounts for any discrepancy between the concentrations of the various ionic species in a solution. For example, in a salt such as $MgCl_2$, there are two moles of Cl^- for every mole of Mg_2^+, so the concentration of Cl- as well as of Mg_2^+ is said to be 2 N (read: "two normal"). Further examples are given below. A normal is one gram equivalent of a solute per liter of solution. The definition of a gram equivalent varies depending on the type of chemical reaction that is discussed - it can refer to acids, bases, redox species, and ions that will precipitate. It is critical to note that normality measures a single ion which takes part in an overall solute. For example, one could determine the normality of hydroxide or sodium in an aqueous solution of sodium hydroxide, but the normality of sodium hydroxide itself has no meaning. Nevertheless it is often used to describe solutions of acids or bases, in those cases it is implied that the normality refers to the H+ or OH- ion. For example, 2 Normal sulfuric acid (H_2SO_4), means that the normality of H+ ions is 2, or that the molarity of the sulfuric acid is 1. Similarly for 1 Molar H_3PO_4 the normality is 3 as it contains three H+ ions.

NTNCWS: Non-transient non-community water system.

NTU (Nephelometric turbidity unit)**:** A measure of the clarity or cloudiness of water.

O_3: The molecular formula of ozone.

OIL TUBE: A tubular enclosure that houses the line shaft and bearings of a vertical turbine pump. Oil is allowed to pass through the oil tube in order to lubricate the pumps drive shaft and bearings.

OLIGOTROPHIC: A reservoir that is nutrient-poor and contains little plant or animal life. An oligotrophic ecosystem or environment is one that offers little to sustain life. The term is commonly utilized to describe bodies of water or soils with very low nutrient levels. It derives etymologically from the Greek oligo (small, little, few) and trophe (nutrients, food). Oligotrophic environments are of special interest for the alternative energy sources and survival strategies upon which life could rely.

ORGANIC PRESURSORS: Natural or man-made compounds with chemical structures based upon carbon that, upon combination with chlorine, leading to trihalomethane formation.

OSMOSIS: Osmosis is the process by which water moves across a semi permeable membrane from a low concentration solute to a high concentration solute to satisfy the pressure differences caused by the solute.

OVERBURDEN: Normally a thin loosely consolidated or unconsolidated sediment overlying competent formation.

OVER-RANGE PROTECTION DEVICES: Mechanical dampers, snubbers and an air cushion chamber are examples of surging and overrange protection devices.

OXIDE: An oxide is a chemical compound containing at least one oxygen atom as well as at least one other element. Most of the Earth's crust consists of oxides. Oxides result when elements are oxidized by oxygen in air. Combustion of hydrocarbons affords the two principal oxides of carbon, carbon monoxide and carbon dioxide. Even materials that are considered to be pure elements often contain a coating of oxides. For example, aluminum foil has a thin skin of Al_2O_3 that protects the foil from further corrosion.

OXIDIZED:
1. to convert (an element) into an oxide; combine with oxygen.
2. to cover with a coating of oxide or rust.
3. to take away hydrogen, as by the action of oxygen; add oxygen or any nonmetal.
4. to remove electrons from (an atom or molecule), thereby increasing the valence. Compare REDUCE (def. 12).

–verb (used without object)

5. to become oxidized.

OXIDIZING: The process of breaking down organic wastes into simpler elemental forms or by products. Also used to separate combined chlorine and convert it into free chlorine.

OXYGEN DEFICIENT ENVIRONMENT: One of the most dangerous threats to an operator upon entering a manhole.

OZONE: Ozone or trioxygen (O_3) is a triatomic molecule, consisting of three oxygen atoms. It is an allotrope of oxygen that is much less stable than the diatomic O_2. Ground-level ozone is an air pollutant with harmful effects on the respiratory systems of animals. Ozone in the upper atmosphere filters potentially damaging ultraviolet light from reaching the Earth's surface. It is present in low concentrations throughout the Earth's atmosphere. It has many industrial and consumer applications. Ozone, the first allotrope of a chemical element to be recognized by science, was proposed as a distinct chemical compound by Christian Friedrich Schönbein in 1840, who named it after the Greek word for smell (ozein), from the peculiar odor in lightning storms. The formula for ozone, O_3, was not determined until 1865 by Jacques-Louis Soret and confirmed by Schönbein in 1867. Ozone is a powerful oxidizing agent, far better than dioxygen. It is also unstable at high concentrations, decaying to ordinary diatomic oxygen (in about half an hour in atmospheric conditions):

$2\ O_3 = 3\ O_2$

This reaction proceeds more rapidly with increasing temperature and decreasing pressure. Deflagration of ozone can be triggered by a spark, and can occur in ozone concentrations of 10 wt% or higher.

OZONE DOES NOT PROVIDE A RESIDUAL: One of the major drawbacks to using ozone as a disinfectant.

OZONE, CHLORINE DIOXIDE, UV, CHLORAMINES: These chemicals may be used as alternative disinfectants.

PAC: A disadvantage of using PAC is it is very abrasive and requires careful maintenance of equipment. One precaution that should be taken in storing PAC is that bags of carbon should not be stored near bags of HTH. Removes tastes and odors by adsorption only. Powered activated carbon frequently used for taste and odor control because PAC is non-specific and removes a broad range of compounds. Jar tests and threshold odor number testing determines the application rate for powdered activated carbon. Powdered activated carbon, or PAC, commonly used for in a water treatment plant for taste and odor control. Powdered activated carbon may be used with some success in removing the precursors of THMs

PACKING: Material, usually of woven fiber, placed in rings around the shaft of a pump and used to control the leakage from the stuffing box.

PARAMECIUM: Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).

PATHOGENS: Disease-causing pathogens; waterborne pathogens A pathogen may contaminate water and cause waterborne disease.

Pb: The chemical symbol of Lead.

PCE: Perchloroethylene. Known also as perc or tetrachloroethylene, perchloroethylene is a clear, colorless liquid with a distinctive, somewhat ether-like odor. It is non-flammable, having no measurable flashpoint or flammable limits in air. Effective over a wide range of applications, perchloroethylene is supported by closed loop transfer systems, stabilizers and employee exposure monitoring.

PEAK DEMAND: The maximum momentary load placed on a water treatment plant, pumping station or distribution system.

PERCOLATION: The process of fluid penetrating or slowly flowing through soil, rock, or formation. See also infiltration.

PERCUSSION RIG: See Cable Tool.

PERFORATED SCREEN: Well screen that has openings mechanically cut into it.

PERFORMANCE CURVE: A graphical representation of a pumps efficiency relative to gpm and feet of head.

PEPTIDOGLYCAN: A polymer found in the cell walls of prokaryotes that consists of polysaccharide and peptide chains in a strong molecular network. Also called *mucopeptide*, *murein*.

PERMEATE: The term for water which has passed through the membrane of a reverse osmosis unit.

PERMEABILITY: A measure of a soil or formation's capacity to transmit water, typically in volume per time units. Equivalent to Darcy's hydraulic conductivity.

PERMEABLE: Soil or formation of which water can pass through.

pH: A unit of measure which describes the degree of acidity or alkalinity of a solution. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. The term pH is derived from "p", the mathematical symbol of the negative logarithm, and "H", the chemical symbol of Hydrogen. The definition of pH is the negative logarithm of the Hydrogen ion activity. $pH=-\log[H^+]$.

pH OF SATURATION: The ideal pH for perfect water balance in relation to a particular total alkalinity level and a particular calcium hardness level, at a particular temperature. The pH where the Langelier Index equals zero.

PHENOLPHTHALEIN/TOTAL ALKALINITY: The relationship between the alkalinity constituent's bicarbonate, carbonate, and hydroxide can be based on the P and T alkalinity measurement.

PHENOL RED: Chemical reagent used for testing pH in the range of 6.8 - 8.4.

PHOSPHATE, NITRATE AND ORGANIC NITROGEN: Nutrients in a domestic water supply reservoir may cause water quality problems if they occur in moderate or large quantities.

PHYSISORPTION: (Or physical adsorption) Is adsorption in which the forces involved are intermolecular forces (van der Waals forces) of the same kind as those responsible for the imperfection of real gases and the condensation of vapors, and which do not involve a significant change in the electronic orbital patterns of the species involved. The term van der Waals adsorption is synonymous with physical adsorption, but its use is not recommended.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

pCi/L: Picocuries per liter A curie is the amount of radiation released by a set amount of a certain compound. A picocurie is one quadrillionth of a curie.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

PIEZOMETRIC SURFACE: See potentiometric surface.

PILOT BIT: A bit used on auger rigs to cut a pilot hole ahead of the cutter head when drilling into more resistant formations.

PIPELINE APPURTENANCE: Pressure reducers, bends, valves, regulators (which are a type of valve), etc.

PITLESS ADAPTER: A fitting installed on a section of column pipe and well casing permitting piping from the well to be installed below grade. (Often requires a special permit for construction)

PLANKTON: The aggregate of passively floating, drifting, or somewhat motile organisms occurring in a body of water, primarily comprising microscopic algae and protozoa.

PLATFORM: The portion of the drilling rig where a driller and crew operate the drill rig.

PLUG: A removable cap installed behind the pilot and cutter bits on hollow stem auger flighting.

PLUNGER: See Surge-block.

POINT OF ENTRY: POE.

POLLUTION: To make something unclean or impure. See Contaminated.

POLYPHOSPHATES: Chemicals that may be added to remove low levels of iron and manganese.

POLYMER: A type of chemical when combined with other types of coagulants aid in binding small suspended particles to larger particles to help in the settling and filtering processes.

PORE SPACE: The interstitial space between sediments and fractures that is capable of storing and transmitting water.

POROSITY: A factor representing a rock, soil, or formations percentage of open space available for the percolation and storage of groundwater.

POST-CHLORINE: Where the water is chlorinated to make sure it holds a residual in the distribution system.

POTABLE: Good water which is safe for drinking or cooking purposes. Non-Potable: A liquid or water that is not approved for drinking.

POTENTIAL ENERGY: The energy that a body has by virtue of its position or state enabling it to do work.

POTENTIOMETRIC SURFACE: An imaginary surface representing the height a column of water will reach at any location within a confined aquifer. The measured surface of a confined aquifer related to the aquifer's pressure head.

PPM: Abbreviation for parts per million.

PRE-CHLORINE: Where the raw water is dosed with a large concentration of chlorine.

PRE-CHLORINATION: The addition of chlorine before the filtration process will help:
> Control algae and slime growth
> Control mud ball formation
> Improve coagulation
> Precipate iron
The addition of chlorine to the water prior to any other plant treatment processes.

PERKINESIS: The aggregation resulting from random thermal motion of fluid molecules.

PRESSURE: Pressure is defined as force per unit area. It is usually more convenient to use pressure rather than force to describe the influences upon fluid behavior. The standard unit for pressure is the Pascal, which is a Newton per square meter. For an object sitting on a surface, the force pressing on the surface is the weight of the object, but in different orientations it might have a different area in contact with the surface and therefore exert a different pressure.

PRESSURE HEAD: The height of a column of water capable of being maintained by pressure. See also Total Head, Total Dynamic Head.

PRESSURE MEASUREMENT: Bourdon tube, Bellows gauge and Diaphragm are commonly used to measure pressure in waterworks systems. A Bellows-type sensor reacts to a change in pressure.

PREVENTION: To take action; stop something before it happens.

PROTON, NEUTRON AND ELECTRON: Are the 3 fundamental particles of an atom.

PRODUCING ZONE: A specific productive interval.

PRODUCTIVE INTERVAL: The portion or portions of an aquifer in which significant water production is obtained within the well.

PROTIST: Any of a group of eukaryotic organisms belonging to the kingdom Protista according to some widely used modern taxonomic systems. The protists include a variety of unicellular, coenocytic, colonial, and multicellular organisms, such as the protozoans, slime molds, brown algae, and red algae. A unicellular protoctist in taxonomic systems in which the protoctists are considered to form a kingdom.

PROTOCTIST: Any of various unicellular eukaryotic organisms and their multicellular, coenocytic, or colonial descendants that belong to the kingdom Protoctista according to some taxonomic systems. The protoctists include the protozoans, slime molds, various algae, and other groups. In many new classification systems, all protoctists are considered to be protists.

PROTOZOA: Microscopic animals that occur as single cells. Some protozoa can cause disease in humans. Protozoa form cysts, which are specialized cells like eggs that are very resistant to chlorine. Cysts can survive the disinfection process, then "hatch" into normal cells that can cause disease. Protozoa must be removed from drinking water by filtration, because they cannot be effectively killed by chlorine.

PUBLIC NOTIFICATION: An advisory that EPA requires a water system to distribute to affected consumers when the system has violated MCLs or other regulations. The notice advises consumers what precautions, if any, they should take to protect their health.

PUBLIC WATER SYSTEM (PWS): Any water system which provides water to at least 25 people for at least 60 days annually. There are more than 170,000 PWSs providing water from wells, rivers and other sources to about 250 million Americans. The others drink water from private wells. There are differing standards for PWSs of different sizes and types.

PUMP SURGING: A process of well development whereby water is pumped nearly to the surface and then is allowed to fall back into the well. The process creates a backwashing action that cleans the well and nearby formation.

PUMPING LIFT: The height to which water must be pumped or lifted to, feet of head.

PWS: 3 types of public water systems. Community water system, non-transient non-community water system, transient non community water system.

RADIOCHEMICALS: (Or radioactive chemicals) Occur in natural waters. Naturally radioactive ores are particularly common in the Southwestern United States, and some streams and wells can have dangerously high levels of radioactivity. Total alpha and beta radioactivity and isotopes of radium and strontium are the major tests performed for radiochemicals. The federal drinking water standard for gross alpha radioactivity is set at 5 picocuries per liter.

RADIUS OF INFLUENCE: The distance away from a pumping well that water levels are affected by a wells cone of depression.

RAWHIDING: See Pump Surging.

RAW TURBIDITY: The turbidity of the water coming to the treatment plant from the raw water source.

RAW WATER: Water that has not been treated in any way; it is generally considered to be unsafe to drink.

REAGENT: A substance used in a chemical reaction to measure, detect, examine, or produce other substances.

REAM: The process of enlarging a borehole.

REAMER BIT: A special bit designed to ream existing boreholes.

RECHARGE: The infiltration component of the hydrologic cycle. Often used in the context of referring to: The infiltration of water back into an aquifer, resulting in the restoration of lost storage and water levels which had been decreased due to pumping and/or natural discharges from the aquifer.

RECIRCULATING SYSTEM: A system of constructed or surface mud pits that settle out cuttings from drilling fluid to be circulated back down the borehole.

RECORDER, FLOW: A flow recorder that measures flow is most likely to be located anywhere in the plant where a flow must be measured and in a central location.

RED WATER AND SLIME: Iron bacteria are undesirable in a water distribution system because of red water and slime complaints.

REDOX POTENTIAL: Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. In aqueous solutions, the reduction potential is the tendency of the solution to either gain or lose electrons when it is subject to change by introduction of a new species. A solution with a higher (more positive) reduction potential than the new species will have a tendency to gain electrons from the new species (i.e. to be reduced by oxidizing the new species) and a solution with a lower (more negative) reduction potential will have a tendency to lose electrons to the new species (i.e. to be oxidized by reducing the new species).

RELAY LOGIC: The name of a popular method of automatically controlling a pump, valve, chemical feeder, and other devices.

RESERVOIR: An impoundment used to store water.

RESIDUAL DISINFECTION PROTECTION: A required level of disinfectant that remains in treated water to ensure disinfection protection and prevent recontamination throughout the distribution system (i.e., pipes).

REVERSE MUD ROTARY: A non-conventional drilling method in which drilling fluid is injected through the borehole annulus downward through the bit and circulated back to the surface through the drill string.

REVERSE OSMOSIS: Forces water through membranes that contain holes so small that even salts cannot pass through. Reverse osmosis removes microorganisms, organic chemicals, and inorganic chemicals, producing very pure water. For some people, drinking highly purified water exclusively can upset the natural balance of salts in the body. Reverse osmosis units require regular maintenance or they can become a health hazard.

RIBBED STABILIZER: A stabilizer or drill collar that has cutting ribs attached to its side. Ribs are normally installed in vertical or spiral arrangements.

ROLLER BIT: A rotary drill bit having rotating cutting heads.

ROTAMETER: The name of transparent tube with a tapered bore containing a ball is often used to measure the rate of flow of a gas or liquid.

ROTARY RIG: A conventional rotary drill rig. Can be either an air or mud rotary rig.

ROTIFER: Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

RUNOFF: Surface water sources such as a river or lake are primarily the result of natural processes of runoff.

SAFE YIELD: A possible consequence when the “safe yield” of a well is exceeded and water continues to be pumped from a well, is land subsidence around the well will occur. Safe yield refers to a long-term balance between the water that is naturally and artificially recharged to an aquifer and the groundwater that is pumped out. When more water is removed than is recharged, the aquifer is described as being out of safe yield. When the water level in the aquifer then drops, we are said to be mining groundwater.

SALTS ARE ABSENT: Is a strange characteristic that is unique to water vapor in the atmosphere.

SAMPLE: The water that is analyzed for the presence of EPA-regulated drinking water contaminants. Depending on the regulation, EPA requires water systems and states to take samples from source water, from water leaving the treatment facility, or from the taps of selected consumers.

SAMPLING LOCATION: A location where soil or cuttings samples may be readily and accurately collected.

SAND, ANTHRACITE AND GARNET: Mixed media filters are composed of these three materials.

SANITARY SURVEY: Persons trained in public health engineering and the epidemiology of waterborne diseases should conduct the sanitary survey. The importance of a detailed sanitary survey of a new water source cannot be overemphasized. An on-site review of the water sources, facilities, equipment, operation, and maintenance of a public water systems for the purpose of evaluating the adequacy of the facilities for producing and distributing safe drinking water. The purpose of a non-regulatory sanitary survey is to identify possible biological and chemical pollutants which might affect a water supply.

SANITIZER: A disinfectant or chemical which disinfects (kills bacteria), kills algae and oxidizes organic matter.

SATURATION INDEX: See Langelier's Index.

SATURATOR: A device which produces a fluoride solution for the fluoride process. Crystal-grade types of sodium fluoride should be fed with a saturator. Overfeeding must be prevented to protect public health when using a fluoridation system.

SATURATED ZONE: Where an unconfined aquifer becomes saturated beneath the capillary fringe.

SCADA: A remote method of monitoring pumps and equipment. 130 degrees F is the maximum temperature that transmitting equipment is able to with stand. If the level controller may be set with too close a tolerance 45 could be the cause of a control system that is frequently turning a pump on and off.

SCALE: Crust of calcium carbonate, the result of unbalanced water. Hard insoluble minerals deposited (usually calcium bicarbonate) which forms on pool and spa surfaces and clog filters, heaters and pumps. Scale is caused by high calcium hardness and/or high pH. The regular use of stain prevention chemicals can prevent scale.

SCHMUTZDECKE: German, "grime or filth cover", sometimes spelt schmutzedecke) is a complex biological layer formed on the surface of a slow sand filter. The schmutzdecke is the layer that provides the effective purification in potable water treatment, the underlying sand providing the support medium for this biological treatment layer. The composition of any particular schmutzdecke varies, but will typically consist of a gelatinous biofilm matrix of bacteria, fungi, protozoa, rotifera and a range of aquatic insect larvae. As a schmutzdecke ages, more algae tend to develop, and larger aquatic organisms may be present including some bryozoa, snails and annelid worms.

SCROLL AND BASKET: The two basic types of centrifuges used in water treatment.

SEAL: For wells: to abandon a well by filling up the well with approved seal material including cementing with grout from a required depth to the land surface.

SECONDARY DRINKING WATER STANDARDS: Non-enforceable federal guidelines regarding cosmetic effects (such as tooth or skin discoloration) or aesthetic effects (such as taste, odor, or color) of drinking water.

SECTIONAL MAP: The name of a map that provides detailed drawings of the distribution system's zones. Sometimes we call these quarter-sections.

SEDIMENTATION BASIN: Where the thickest and greatest concentration of sludge will be found. Twice a year sedimentation tanks should be drained and cleaned if the sludge buildup interferes with the treatment process.

SEDIMENTATION: The process of suspended solid particles settling out (going to the bottom of the vessel) in water.

SEDIMENT: Grains of soil, sand, gravel, or rock deposited by and generated by water movement.

SENSOR: A float and cable system are commonly found instruments that may be used as a sensor to control the level of liquid in a tank or basin.

SESSILE: *Botany*. attached by the base, or without any distinct projecting support, as a leaf issuing directly from the stem. *Zoology*. permanently attached; not freely moving.

SETTLED SOLIDS: Solids that have been removed from the raw water by the coagulation and settling processes.

SHAKER: A device used in mud containment systems that vibrates various sized screens as drilling fluid passes through it, thereby separating cuttings from drilling fluid and providing a good sampling location.

SHOCK: Also known as superchlorination or break point chlorination. Ridding a water of organic waste through oxidization by the addition of significant quantities of a halogen.

SHORT-CIRCUITING: Short Circuiting is a condition that occurs in tanks or basins when some of the water travels faster than the rest of the flowing water. This is usually undesirable since it may result in shorter contact, reaction or settling times in comparison with the presumed detention times.

SHROUD: A baffle or piece of pipe installed over a pump to force water to pass the pumps motor.

SIEVE ANALYSIS: The process of sifting soil or formation samples through a series of screens to determine percentages of particle sizes.

SINGLE PHASE POWER: The type of power used for lighting systems, small motors, appliances, portable power tools and in homes.

SINUSOID: A curve described by the equation y = a sin x, the ordinate being proportional to the sine of the abscissa.

SINUSOIDAL: Mathematics. Of or pertaining to a sinusoid. Having a magnitude that varies as the sine of an independent variable: a sinusoidal current.

SLUDGE BASINS: After cleaning sludge basins and before returning the tanks into service the tanks should be inspected, repaired if necessary, and disinfected.

SLUDGE REDUCTION: Organic polymers are used to reduce the quantity of sludge. If a plant produces a large volume of sludge, the sludge could be dewatered, thickened, or conditioned to decrease the volume of sludge. Turbidity of source water, dosage, and type of coagulant used are the most important factors which determine the amount of sludge produced in a treatment of water.

SLURRY: A mixture of crushed rock and water.

SMALL WATER SYSTEM: 3,300 or fewer persons.

SOC: Synthetic organic chemical. A common way for a synthetic organic chemical such as dioxin to be introduced to a surface water supply is from an industrial discharge, agricultural drainage, or a spill.

SODA ASH: Chemical used to raise pH and total alkalinity (sodium carbonate)

SODIUM BICARBONATE: Commonly used to increase alkalinity of water and stabilize pH.

SODIUM BISULFATE: Chemical used to lower pH and total alkalinity (dry acid).

SODIUM HYDROXIDE: Also known as caustic soda, a by-product chlorine generation and often used to raise pH.

SOIL MOISTURE: A relative consideration of the degree to which a soil is saturated.

SOFTENING WATER: When the water has a low alkalinity it is advantageous to use soda ash instead of caustic soda for softening water.

SOFTENING: The process that removes the ions which cause hardness in water.

SOLAR DRYING BEDS OR LAGOONS: Are shallow, small-volume storage pond where sludge is concentrated and stored for an extended periods.

SOLAR DRYING BEDS, CENTRIFUGES AND FILTER PRESSES: Are procedures used in the dewatering of sludge.

SOLID, LIQUID AND VAPOR: 3 forms of matter.

SOLDER: A fusible alloy used to join metallic parts.

SOLID STEM (AUGER): An auger that is constructed of solid stem drill flights.

SPADNS: The lab reagent called SPADNS solution is used in performing the Fluoride test.

SPECIFIC CAPACITY (Sc): A measure of a well's pumping performance in gallons per minute per foot of drawdown.

SPIDER: A bearing or flange used in vertical turbine pumps to stabilize the drive shaft or shaft tube and seal column joints.

SPIRAL FLANGE: A continuous blade that wraps spirally around auger flighting.

SPIRIT OF HARTSHORN: A colorless, pungent, suffocating, aqueous solution of about 28.5 percent ammonia gas: used chiefly as a detergent, for removing stains and extracting certain vegetable coloring agents, and in the manufacture of ammonium salts.

SPLIT SPOON: A sampling device that is driven into the earth and operated by a wire line for the retrieval of soil or formation samples.

SPLIT FLOW CONTROL SYSTEM: This type of control system is to control the flow to each filter influent which is divided by a weir.

SPRAY BOTTLE OF AMMONIA: An operator should use ammonia to test for a chlorine leak around a valve or pipe. You will see white smoke if there is a leak.

SPRING PRESSURE: Is what maintains contact between the two surfaces of a mechanical seal.

STABILE: Reference to formation, soil, or sediments of sufficient strength to remain in place under its own weight and existing pressures.

STABILIZE: Actions taken to enhance borehole stability or vertical rotational when drilling.

STABILIZER: The portion of a drill string used to stabilize rotation.

STANDPIPE: A water tank that is taller than it is wide. Should not be found in low point.

STERILIZED GLASSWARE: The only type of glassware that should be used in testing for coliform bacteria.

STORAGE TANKS: Three types of water usage that determine the volume of a storage tank are fire suppression storage, equalization storage, and emergency storage. Equalization storage is the volume of water needed to supply the system for periods when demand exceeds supply. Generally, a water storage tank's interior coating (paint) protects the interior about 3-5 years.

S.T.P.: Standard temperature and pressure standard temperature and pressure the temperature of 0°C and pressure of 1 atmosphere, usually taken as the conditions when stating properties of gases.

STRATIFIED: Layered.

STUFFING BOX: That portion of the pump that houses the packing or mechanical seal.

SUB: A small section of drill pipe used to connect larger sections.

SUBMERSIBLE PUMP: A turbine pump that has the motor attached directly to it and therefore is operated while submerged.

SULFATE: Will readily dissolve in water to form an anion. Sulfate is a substance that occurs naturally in drinking water. Health concerns regarding sulfate in drinking water have been raised because of reports that diarrhea may be associated with the ingestion of water containing high levels of sulfate. Of particular concern are groups within the general population that may be at greater risk from the laxative effects of sulfate when they experience an abrupt change from drinking water with low sulfate concentrations to drinking water with high sulfate concentrations.

Sulfate in drinking water currently has a secondary maximum contaminant level (SMCL) of 250 milligrams per liter (mg/L), based on aesthetic effects (i.e., taste and odor). This regulation is not a federally enforceable standard, but is provided as a guideline for States and public water systems. EPA estimates that about 3% of the public drinking water systems in the country may have sulfate levels of 250 mg/L or greater. The Safe Drinking Water Act (SDWA), as amended in 1996, directs the U.S. Environmental Protection Agency (EPA) and the Centers for Disease Control and Prevention (CDC) to jointly conduct a study to establish a reliable dose-response relationship for the adverse human health effects from exposure to sulfate in drinking water, including the health effects that may be experienced by sensitive subpopulations (infants and travelers). SDWA specifies that the study be based on the best available peer-reviewed science and supporting studies, conducted in consultation with interested States, and completed in February 1999.

SULFIDE: The term sulfide refers to several types of chemical compounds containing sulfur in its lowest oxidation number of -2. Formally, "sulfide" is the dianion, S_2^-, which exists in strongly alkaline aqueous solutions formed from H_2S or alkali metal salts such as Li_2S, Na_2S, and K_2S. Sulfide is exceptionally basic and, with a pKa > 14, it does not exist in appreciable concentrations even in highly alkaline water, being undetectable at pH < ~15 (8 M NaOH). Instead, sulfide combines with electrons in hydrogen to form HS, which is variously called hydrogen sulfide ion, hydrosulfide ion, sulfhydryl ion, or bisulfide ion. At still lower pH's (<7), HS- converts to H_2S, hydrogen sulfide. Thus, the exact sulfur species obtained upon dissolving sulfide salts depends on the pH of the final solution. Aqueous solutions of transition metals cations react with sulfide sources (H_2S, NaSH, Na_2S) to precipitate solid sulfides. Such inorganic sulfides typically have very low solubility in water and many are related to minerals. One famous example is the bright yellow species CdS or "cadmium yellow". The black tarnish formed on sterling silver is Ag_2S. Such species are sometimes referred to as salts. In fact, the bonding in transition metal sulfides is highly covalent, which gives rise to their semiconductor properties, which in turn is related to the practical applications of many sulfide materials.

SUPERNATANT: The liquid layer which forms above the sludge in a settling basin.

SURFACE SEAL: The upper portion of a wells construction where surface contaminants are adequately prevented from entering the well, normally consisting of surface casing and neat cement grout.

SURFACE WATER SOURCES: Surface water sources such as a river or lake are primarily the result of Runoff.

SURFACE WATER: Water that is open to the atmosphere and subject to surface runoff; generally, lakes, streams, rivers.

SURFACTANT: Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Examples of such aggregates are vesicles and micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge).

SUSCEPTIBILITY WAIVER: A waiver that is granted based upon the results of a vulnerability assessment.

SURGE-BLOCK: A disc shaped device that fits tightly into a well and is moved up and down to agitate the water column in order to develop a well.

SURGING: The process of purging a well rapidly for well development.

SWAB: See Surge-block.

SWING ARM: A large moveable arm on a bucket auger rig that pulls the bucket auger out away from the drilling rig for dumping.

SYNCHRONY: Simultaneous occurrence; synchronism.

TABLE DRIVE: A drilling rig that uses a rotating table within the platform to turn a kelly driven drill string.

TABLE: The back portion of a drill rig where the drill pipe is inserted (or driven if a table drive), adjacent to or within the driller's platform.

TAPPING VALVE: The name of the valve that is specifically designed for connecting a new water main to an existing main that is under pressure.

TARGET DEPTH: The proposed construction depth of a well prior to drilling.

TASTE AND ODORS: The primary purpose to use potassium permanganate in water treatment is to control taste and odors. Anaerobic water undesirable for drinking water purposes because of color and odor problems are more likely to occur under these conditions. Taste and odor problems in the water may happen if sludge and other debris are allowed to accumulate in a water treatment plant.

TCE, *trichloroethylene*: A solvent and degreaser used for many purposes; for example dry cleaning, it is a common groundwater contaminant. Trichloroethylene is a colorless liquid which is used as a solvent for cleaning metal parts. Drinking or breathing high levels of trichloroethylene may cause nervous system effects, liver and lung damage, abnormal heartbeat, coma, and possibly death. Trichloroethylene has been found in at least 852 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency (EPA).

TDS-TOTAL DISSOLVED SOLIDS: An expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended form. Generally, the operational definition is that the solids (often abbreviated TDS) must be small enough to survive filtration through a sieve size of two micrometers. Total dissolved solids are normally only discussed for freshwater systems, since salinity comprises some of the ions constituting the definition of TDS. The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is generally considered not as a primary pollutant (e.g. it is not deemed to be associated with health effects), but it is rather used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of presence of a broad array of chemical contaminants. Ion exchange is an effective treatment process used to remove iron and manganese in a water supply. This process is ideal as long as the water does not contain a large amount of TDS. When determining the total dissolved solids, a sample should be filtered before being poured into an evaporating dish and dried. Demineralization may be necessary in a treatment process if the water has a very high value Total Dissolved Solids.

TELEMETERING: The use of a transmission line with remote signaling to monitor a pumping station or motors. Can be used to accomplish accurate and reliable remote monitoring and control over a long distribution system.

TEMPERATURE SAMPLE: This test should be performed immediately in the field, this is a grab sample.

TELESCOPING KELLY: A kelly with successively smaller sized pipe within itself that drops out as a borehole is drilled. This permits that drilling may proceed without adding drill pipe. Normally found on bucket auger rigs.

TELESCOPING: The successive decrease in borehole size with depth.

THE RATE DECREASES: In general, when the temperature decreases, the chemical reaction rate decreases also.

THICKENING, CONDITIONING AND DEWATERING: Common processes that are utilized to reduce the volume of sludge.

TIME FOR TURBIDITY BREAKTHROUGH AND MAXIMUM HEADLOSS: Are the two factors which determine whether or not a change in filter media size should be made.

TITRATION: A method of testing by adding a reagent of known strength to a water sample until a specific color change indicates the completion of the reaction.

TITRIMETRIC: Chemistry. Using or obtained by titration. Titrimetrically, adverb.

TOP DRIVE: A rotary type drill head that moves freely up and down the rigs mast while driving the drill string.

TOROID: A surface generated by the revolution of any closed plane curve or contour about an axis lying in its plane. The solid enclosed by such a surface.

TOTAL ALKALINITY: A measure of the acid-neutralizing capacity of water which indicates its buffering ability, i.e. measure of its resistance to a change in pH. Generally, the higher the total alkalinity, the greater the resistance to pH change.

TOTAL COLIFORM: Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

TOTAL DISSOLVED SOLIDS (TDS): The accumulated total of all solids that might be dissolved in water.

TOTAL DYNAMIC HEAD: The pressure (psi) or equivalent feet of water, required for a pump to lift water to its point of storage overcoming elevation head, friction loss, line pressure, drawdown and pumping lift.

TRANSIENT, NON-COMMUNITY WATER SYSTEM: TNCWS A water system which provides water in a place such as a gas station or campground where people do not remain for long periods of time. These systems do not have to test or treat their water for contaminants which pose long-term health risks because fewer than 25 people drink the water over a long period. They still must test their water for microbes and several chemicals. A Transient Non-community Water System: Is not required to sample for VOC's.

TREATED WATER: Disinfected and/or filtered water served to water system customers. It must meet or surpass all drinking water standards to be considered safe to drink.

TRIHALOMETHANES (THM): Four separate compounds including chloroform, dichlorobromomethane, dibromochloromethane, and bromoform. The most common class of disinfection by-products created when chemical disinfectants react with organic matter in water during the disinfection process. See Disinfectant Byproducts.

TRICONE BIT: A roller bit with three independent rolling bits with teeth or buttons that intermesh for efficient rock crushing and cutting.

TUBE SETTLERS: This modification of the conventional process contains many metal tubes that are placed in the sedimentation basin, or clarifier. These tubes are approximately 1 inch deep and 36 inches long, split-hexagonal shape and installed at an angle of 60 degrees or less. These tubes provide for a very large surface area upon which particles may settle as the water flows upward. The slope of the tubes facilitates gravity settling of the solids to the bottom of the basin, where they can be collected and removed. The large surface settling area also means that adequate clarification can be obtained with detention times of 15 minutes or less. As with conventional treatment, this sedimentation step is followed by filtration through mixed media.

TUBERCLES: The creation of this condition is of the most concern regarding corrosive water effects on a water system. Tubercles are formed due to joining dissimilar metals, causing electro-chemical reactions. Like iron to copper pipe. We have all seen these little rust mounds inside cast iron pipe.

TURBIDIMETER: Monitoring the filter effluent turbidity on a continuous basis with an in-line instrument is a recommended practice. Turbidimeter is best suited to perform this measurement.

TURBIDITY: A measure of the cloudiness of water caused by suspended particles.

TURBINE PUMP: A pump that utilizes rotating impellers on a shaft that generate centrifugal force for pumping water.

UNCONFINED AQUIFER: An aquifer that exists under atmospheric pressure and is not confined.

UNCONSOLIDATED: Sediment that is not cemented or is loosely arranged.

UNDER-REAM: The process of reaming, from within the borehole, a section of an existing smaller borehole area.

UNSATURATED ZONE: That portion of the subsurface, including the capillary fringe that is not saturated but may contain water in both vapor and liquid form. See also Vadose Zone.

UNSTABLE: Sediment or other material that cannot exit without rapidly decomposing or collapsing in on itself. (ex. unconsolidated sediment)

U.S. ENVIRONMENTAL PROTECTION AGENCY: In the United States, this agency responsible for setting drinking water standards and for ensuring their enforcement. This agency sets federal regulations which all state and local agencies must enforce.

UNDER PRESSURE IN STEEL CONTAINERS: After chlorine gas is manufactured, it is primarily transported in steel containers.

UNIT FILTER RUN VOLUME (UFRV): One of the most popular ways to compare filter runs. This technique is the best way to compare water treatment filter runs.

VADOSE ZONE: A portion of the subsurface above the water table that is not saturated but contains water in both vapor and liquid form. The portion of the subsurface where water percolates through to the saturated zone. See also Unsaturated Zone.

VANE: That portion of an impeller that throws the water toward the volute.

VARIABLE DISPLACEMENT PUMP: A pump that will produce different volumes of water dependent on the pressure head against it.

VELOCITY HEAD: The vertical distance a liquid must fall to acquire the velocity with which it flows through the piping system. For a given quantity of flow, the velocity head will vary indirectly as the pipe diameter varies.

VENTURI: If water flows through a pipeline at a high velocity, the pressure in the pipeline is reduced. Velocities can be increased to a point that a partial vacuum is created.

VERTICAL TURBINE: A type of variable displacement pump in which the motor or drive head is mounted on the wellhead and rotates a drive shaft connected to the pump impellers.

VIRION: A complete viral particle, consisting of RNA or DNA surrounded by a protein shell and constituting the infective form of a virus.

VIRUSES: Very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VITRIFICATION: Vitrification is a process of converting a material into a glass-like amorphous solid that is free from any crystalline structure, either by the quick removal or addition of heat, or by mixing with an additive. Solidification of a vitreous solid occurs at the glass transition temperature (which is lower than melting temperature, Tm, due to supercooling). When the starting material is solid, vitrification usually involves heating the substances to very high temperatures. Many ceramics are produced in such a manner. Vitrification may also occur naturally when lightning strikes sand, where the extreme and immediate heat can create hollow, branching rootlike structures of glass, called fulgurite. When applied to whiteware ceramics, vitreous means the material has an extremely low permeability to liquids, often but not always water, when determined by a specified test regime. The microstructure of whiteware ceramics frequently contain both amorphous and crystalline phases.

VOC WAIVER: The longest term VOC waiver that a public water system using groundwater could receive is 9 years.

VOLATILE ORGANIC COMPOUNDS: (**VOCs**) Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents. Volatile organic compounds (VOCs) are organic chemical compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere. A wide range of carbon-based molecules, such as aldehydes, ketones, and other light hydrocarbons are VOCs. The term often is used in a legal or regulatory context and in such cases the precise definition is a matter of law. These definitions can be contradictory and may contain "loopholes"; e.g. exceptions, exemptions, and exclusions. The United States Environmental Protection Agency defines a VOC as any organic compound that participates in a photoreaction; others believe this definition is very broad and vague as organics that are not volatile in the sense that they vaporize under normal conditions can be considered volatile by this EPA definition. The term may refer both to well characterized organic compounds and to mixtures of variable composition.

VOID: An opening, gap, or space within rock or sedimentary formations formed at the time of origin or deposition.

VOLTAGE: Voltage (sometimes also called electric or electrical tension) is the difference of electrical potential between two points of an electrical or electronic circuit, expressed in volts. It measures the potential energy of an electric field to cause an electric current in an electrical conductor. Depending on the difference of electrical potential it is called extra low voltage, low voltage, high voltage or extra high voltage. Specifically Voltage is equal to energy per unit charge.

VOLUTE: The spiral-shaped casing surrounding a pump impeller that collects the liquid discharge by the impeller.

VORTEX: The helical swirling of water moving towards a pump.

VIRUSES: Are very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VOLATILE ORGANIC COMPOUNDS: (**VOCs**) Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents.

VULNERABILITY ASSESSMENT: An evaluation of drinking water source quality and its vulnerability to contamination by pathogens and toxic chemicals.

WAIVERS: Monitoring waivers for nitrate and nitrite are prohibited.

WASHOUT: The rapid erosion of aquifer material from the borehole walls while a well is being drilled, which often results in a loss of circulation.

WATER COURSE: An opening within a cable tool drill string that allows fluid to flow in and out of the drill string thereby minimizing friction loss to the slurry.

WATER HAMMER: A surge in a pipeline resulting from the rapid increase or decrease in water flow. Water hammer exerts tremendous force on a system and can be highly destructive.

WATER PURVEYOR: The individuals or organization responsible to help provide, supply, and furnish quality water to a community.

WATER QUALITY: The 4 broad categories of water quality are: Physical, chemical, biological, radiological. Pathogens are disease causing organisms such as bacteria and viruses. A positive bacteriological sample indicates the presence of bacteriological contamination. Source water monitoring for lead and copper be performed when a public water system exceeds an action level for lead of copper.

WATER QUALITY CRITERIA: Comprised of both numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

WATER QUALITY STANDARD: A statute or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.

WATER TABLE: The measured water level surface of an unconfined aquifer.

WATER VAPOR: A characteristic that is unique to water vapor in the atmosphere is that water does not contain any salts.

WATERBORNE DISEASE: A disease, caused by a virus, bacterium, protozoan, or other microorganism, capable of being transmitted by water (e.g., typhoid fever, cholera, amoebic dysentery, gastroenteritis).

WATERSHED: An area that drains all of its water to a particular water course or body of water. The land area from which water drains into a stream, river, or reservoir.

WEATHERED: The existence of rock or formation in a chemically or physically broken down or decomposed state. Weathered material is in an unstable state.

WELL ABANDONMENT: The process of sealing a well by approved means. The filling of a well to the surface with cement grout.

WELL HEAD: The upper portion of the well that is constructed on the land surface, including the well manifold. Also a term used to refer to the area near the well that is subject to wellhead protection.

WELL HEAD PROTECTION: Programs designed to maintain the quality of groundwater used as public drinking water sources, by managing the land uses around the well field. A government program that encourages the limitation and elimination of activities, near and within a wells recharge area, which present a potential risk to the wells water supply.

WELL MANIFOLD: The piping, valves, and metering equipment used to connect the well to the distribution system, installed on the wellhead.

WELL SCREEN: A section of well casing that contains openings which permit water to enter the well but limit or prevent sediment from entering the well while pumping.

WELL SEAL: The watertight cap or seal installed within and between the well casing and pumping equipment. The metal or plastic plug or seal, which the pumping column rests on the top of casing.

WHOLE EFFLUENT TOXICITY: The total toxic effect of an effluent measured directly with a toxicity test.

YIELD: The volume of water measured in flow rates that are produced from the well.

ZONE OF AERATION: See Saturated Zone or Vadose Zone.

ZONE OF SATURATION: See Saturated Zone.

Course Credits

Benenson, Abram S., editor. 1990. *Control of Communicable Diseases in Man.* 15th ed. Baltimore: Victor Graphics, Inc.
Bick, H. 1972. Ciliated protozoa. An illustrated guide to the species used as biological indicators in freshwater biology. World Health Organization, Geneva. 198 pp.
Bickford, T.M., Lindsey, B.D., and Beaver, M.R., 1996, Bacteriological quality of ground water used for
Bisson, J.W. and Cabelli, V.J., 1980, *Clostridium perfringens* as a water pollution indicator: Journal of the Water Pollution Control Federation, v. 52, no. 2, p. 241-248.
Born, Stephen M., Douglas A. Yanggen, and Alexander Zaporozec. *A Guide to Groundwater Quality Planning and Management for Local Governments*. Wisconsin Geological and Natural History Survey, Madison, WI, 1987.
Brenner, K.P., Rankin, C.C., Roybal, Y.R., Stelma, G.R., Scarpino, P.V., and Dufour, A.P., 1993, New medium for simultaneous detection of total coliforms and *Escherichia coli* in water: Applied and Environmental Microbiology, v. 59, no. 11, p. 3534-3544.
Britton, L.J., and Greeson, P.E., ed., 1989, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.
Brooks, D., and Cech, I., 1979, Nitrates and bacterial distribution in rural domestic water supplies: Water
Butterworth, B.E., Kedderis, G.L., and Conolly, R.B. (1998) The chloroform risk assessment: A mirror of scientific understanding. CIIT Activities,18 no.4.
Cabelli, V.J., 1981, Health effects criteria for marine recreational waters: Cincinnati, Ohio, U.S. Environmental Protection Agency, EPA-600/1-80-031.
Cairns, J., and J.A. Ruthven. 1972. A test of the cosmopolitan distribution of fresh-water protozoans. Hydrobiologia 39:405-427.
Cairns, J., and W.H. Yongue. 1977. Factors affecting the number of species of freshwater protozoan communities. Pages 257-303 in J. Cairns, ed. Aquatic microbial communities. Garland, New York.
Cairns, J., G.R. Lanza, and B.C. Parker. 1972. Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. Proceedings of the National Academy of Sciences 124:79-127.
Christian, M.S., York, R.G., Hoberman, A.M., Diener, R.M., and Fisher, L.C. (2001b). Oral (drinking water) developmental toxicity studies of bromodichloromethane (BDCM) in rats and rabbits. International Journal of Toxicology, 20, 225-237.
Christian, M.S., York, R.G., Hoberman, A.M., Diener, R.M., Fisher, L.C., and Gates, G.A. (2001a). Biodisposition of dibromoacetic acid (DBA) and bromodichloromethane (BDCM) administered to rats and rabbits in drinking water during range-finding reproduction and developmental toxicity studies. International Journal of Toxicology, 20, 239-253.
Christian, M.S., York, R.G., Hoberman, A.M., Fisher, L.C., and Brown, W.R. (2002a). Oral (drinking water) two-generation reproductive toxicity study of bromodichloromethane (BDCM) in rats. International Journal of Toxicology, 21, 115-146.
Christian, M.S., York, R.G., Hoberman, A.M., Frazee, J., Fisher, L.C., Brown, W.R., and Creasy, D.M. (2002b). Oral (drinking water) two-generation reproductive toxicity study of dibromoacetic acid (DBA) in rats. International Journal of Toxicology, 21, 1-40.
Concern, Inc. *Groundwater: A Community Action Guide*. Washington, D.C., 1989.
Connell, G.F. (1996). The chlorination/chloramination handbook. Denver: American Water Works Association.
Coulston, F., and Kolbye, A. (Eds.) (1994). Regulatory Toxicology and Pharmacology, vol. 20, no. 1, part 2.
Craun, G.F., 1992, Waterborne disease outbreaks in the United States of America—Causes and prevention:World Health Statistician Quarterly, v. 45.
Craun, G.F., and Calderon, R., 1996, Microbial risks in groundwater systems–Epidemiology of waterborne outbreaks, *in* Under the microscope–Examining microbes in groundwater, Proceedings of the Groundwater Foundation's 12th Annual Fall Symposium, Sept. 5-6, 1996, Boston, Mass.: Research Foundation of the American Water Works Association.

Craun, G.F., Hauchman, F.S. and Robinson D.E. (Eds.) (2001). Microbial pathogens and disinfection byproducts in drinking water: Health effects and management of risks, Conference Conclusions, (pp.533-545). Washington, D.C.: ILSI Press.
Craun, G.F., Nwachuku, N., Calderon, R.L., and Craun, M.F. (2002). Outbreaks in drinking-water systems, 1991-1998. Journal of Environmental Health, 65, 16-25.
Cross, Brad L and Jack Schulze. *City of Hurst (A Public Water Supply Protection Strategy)*. Texas Water Commission, Austin, TX, 1989.
Curds, C.R. 1992. Protozoa and the water industry. Cambridge University Press, MA. 122 pp.
Curtis, Christopher and Teri Anderson. *A Guidebook for Organizing a Community Collection Event: Household Hazardous Waste*. Pioneer Valley Planning Commission and Western Massachusetts Coalition for Safe Waste Management, West Springfield, MA, 1984.
Curtis, Christopher, Christopher Walsh, and Michael Przybyla. *The Road Salt Management Handbook: Introducing a Reliable Strategy to Safeguard People & Water Resources*. Pioneer Valley Planning Commission, West Springfield, MA, 1986.
Davis, J.V., and Witt, E.C., III, 1998, Microbiological quality of public-water supplies in the Ozark Plateaus Aquifer System: U.S. Geological Survey Fact Sheet 028-98, 2 p.
DiNovo, F., and Jaffe, M., 1984, Local groundwater protection–Midwest Region: Chicago, Ill., American Planning Association., chap. 2-4, p. 5-40.
Dufour, A.P., 1984, Health effects criteria for fresh recreational waters: Cincinnati, Ohio, U.S. Environmental Protection Agency, EPA-600/1-84-004.
Dutka, B.J., Palmateer, G.A., Meissner, S.M., Janzen, E.M., and Sakellaris, M., 1990, The presence of bacterial virus in groundwater and treated drinking water: Environmental Pollution, v. 63.
Edwards, T.K., and Glysson, G.D., 1988, Field methods for measurement of fluvial sediment: U.S. Geological Survey Techniques of Water-Resources Investigations, book 3, chap. C2, 89 p.
Embrey, S.S., 1992, Surface-water-quality assessment of the Yakima River Basin, Washington—Areal distribution of fecal-indicator bacteria, July 1988: U.S. Geological Survey Water-Resources Investigations Report 91- 4073, 33 p.
Fenchel, T. 1974. Intrinsic rate increase: the relationship with body size. Oecologia 14:317-326.
Fenchel, T., T. Perry, and A. Thane. 1977. Anaerobiosis and symbiosis with bacteria in free-living ciliates. Journal of Protozoology 24:154-163.
Flint, K.P., 1987, The long-term survival of *Escherichia coli* in river water: Journal of Applied Bacteriology, v. 63.
Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistology 2:69-212.
Foissner, W. 1988. Taxonomic and nomenclatural revision of Stádecek's list of ciliates (Protozoa: Ciliophora) as indicators of water quality. Hydrobiologia 166:1-64.
Ford, T.E. and Colwell R.R. (1996). A global decline in microbiological safety of water: A call for action, a report prepared for the American Academy of Microbiology.
Foster, Laurence, M.D. 1985. “Waterborne Disease - It’s Our Job to Prevent It”. PIPELINE newsletter, Oregon Health Division, Drinking Water Program, Portland, Oregon 1(4): 1-3.
Foster, Laurence, M.D. 1990. “Waterborne *Disease,” Methods for the Investigation and Prevention of Waterborne Disease Outbreaks.* Ed. Gunther F. Craun. Cincinnati: U.S. Environmental Protection Agency.
Francy, D.S. and Darner, R. A., 1998, Factors affecting *Escherichia coli* concentrations at Lake Erie public bathing beaches: U.S. Geological Survey Water- Resources Investigations Report 98-4241, 42 p.
Francy, D.S., Hart, T.L., and Virosteck, C.M., 1996, Effects of receiving-water quality and wastewater treatment on injury, survival, and regrowth of fecal-indicator bacteria and implications for assessment of recreational water quality: U.S. Geological Survey Water- Resources Investigations Report 96-4199.
Francy, D.S., Helsel, D.L., and Nally, R.A., 2000, Occurrence and distribution of microbiological indicators in groundwater and streamwater: Water Environment Research. v. 72, no. 2., p. 152-161.
Francy, D.S., Jones, A.L., Myers, D.N., Rowe, G.L., Eberle, Michael, and Sarver, K.M., 1998, Quality-assurance/quality-control manual for collection and analysis of water-quality data in the Ohio District, U.S. Geological Survey: U.S. Geological Survey Water-Resources Investigations Report 98-4057, 71 p.
Francy, D.S., Myers, D.N., and Metzker, K.D., 1993, *Escherichia coli* and fecal-coliform bacteria as indicators of recreational water quality: U.S. Geological Survey Water-Resources Investigations Report 93- 4083.

Fujioka, R.S. and Shizumura, L.K., 1985, *Clostridium perfringens*, a reliable indicator of streamwater quality: Journal of the Water Pollution Control Federation, v. 57, no. 10, p. 986-992.
Gannon, J.T., Manilal, V.B., and Alexander, M., 1991, Relationship between cell surface properties and transport of bacteria through soil: Applied and Environmental Microbiology, v. 57, n. 1, p. 190-193.
Geldreich, E.E., 1976, Fecal coliform and fecal streptococcus density relationships in waste discharges and receiving waters: CRC Critical Reviews in Environmental Control, October 1976, p. 349-369.
Gerba, C.P., and Bitton, G., 1984, Microbial pollutants—Their survival and transport pattern in ground
Giese, A.C. 1973. Blepharisma. Stanford University Press, CA. 366 pp.
Gilliom, R.J., Alley, W.M., and Gurtz, M.E., 1995, Design of the National Water-Quality Assessment Program— Occurrence and distribution of water-quality conditions: U.S. Geological Survey Circular 1112, 33 p.
Gordon, Wendy. *A Citizen's Handbook on Groundwater Protection*. Natural Resources Defense Council, New York, NY 1984.
Guerra de Macedo, G. (1991). Pan American Health Organization. Ref. No. HPE/PER/CWS/010/28/1.1.
Guerrant, R.L. (1997). Cryptosporidiosis: An emerging, highly infectious threat. Emerging Infectious Diseases, 3, Synopses. [On-Line.] Available: http://www.cdc.gov/ncidod/ied/vol3no1/guerrant.htm
Handzel, T.R., Green. R.M., Sanchez, C., Chung, H., and Sobsey, M.D., 1993, Improved specificity in detecting F-specific coliphages in environmental samples by suppression of somatic phages: Water Science Technology, v. 27, no. 3-4, p. 123-131.
Harrison, Ellen Z. and Mary Ann Dickinson. *Protecting Connecticut's Groundwater: A Guide to Groundwater Protection for Local Officials*. Connecticut Department of Environmental Protection, Hartford, CT, 1984.
Havelaar, A.H., van Olphen, M., and Drost, Y.C., 1993, F specific bacteriophages are adequate model organisms for enteric viruses in fresh water: Applied and Environmental Microbiology, v. 59, n. 9, p. 2956-2962.
Helsel, D.R. and Hirsch, R.M., 1992, Statistical methods in water resources: New York, Elsevier Science Publishing Company.
Hernandez-Delgado, E.A., Sierra, M.L., and Toranzos, G.A., 1991, Coliphages as alternate indicators of fecal contamination in tropical waters: Environmental Toxicology and Water Quality, v. 6, p. 131-143.
Herwaldt, B.L., Craun, G.F., Stokes, S.L., and Juranek, D.D., 1991, Waterborne-disease outbreaks, 1989-1990: Morbidity and Mortality Weekly Report, Centers for Disease Control, v. 40, no. SS-3, p. 1-13.
Hirsch, R.M., Alley, W.M., and Wilber, W.G., 1988, Concepts for a national-water quality assessment program: U.S. Geological Survey Circular 1021.
household supply, Lower Susquehanna River Basin, Pennsylvania and Maryland: U.S. Geological Survey Water-Resources Investigations Report 96-4212.
Howell, J.M., Coyne, M.S., and Cornelius, P., 1995, Fecal bacteria in agricultural waters of the Bluegrass Region of Kentucky: Journal of Environmental Quality, v. 24, p. 411-419.
Hrezo, Margaret and Pat Nickinson. *Protecting Virginia's Groundwater A Handbook for Local Government Officials*. Virginia Polytechnic Institute and State University, Blacksburg, VA, 1986.
Ijzerman, M.M., and Hagedorn, C., 1992, Improved method for coliphage detection based on □-galactosidase induction: Journal of Virological Methods, v. 40, p. 31-36.
International Association of Water Pollution Research and Control Study Group on Health Related Water Microbiology, 1991, Bacteriophages as model viruses in water quality control: Water Research, v. 25, no. 5, p. 529-545.
International Programme on Chemical Safety (2000). Disinfectants and disinfectant byproducts, Environmental Health Criteria 216.
Jaffe, Martin and Frank Dinovo. *Local Groundwater Protection*. American Planning Association, Chicago, IL, 1987.
Kirmeyer, G.J. (1994). An assessment of the condition of North American water distribution systems and associated research needs. American Water Works Association Research Foundation Project #706.
Koterba, M.T., Wilde, F.D., and Lapham, W.W., 1995, Ground-water data-collection protocols and procedures for the National Water-Quality Assessment Program—Collection and documentation of water-quality samples and related data: U.S. Geological Survey Open-File Report 95-399, 113 p.
Kreier, J.P., and J.R. Baker. 1987. Parasitic protozoa. Allen and Unwin, Boston, MA. 241 pp.

Larson, J.L., Wolf, D.C., and Butterworth, B.E. (1994a). Induced cytolethality and regenerative cell proliferation in the livers and kidneys of male B6C3F1 mice given chloroform by gavage. Fundamentals and Applied Toxicology, 23, 537-543.
Larson, J.L., Wolf, D.C., and Butterworth, B.E. (1994b). Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs. ad libitum in drinking water. Fundamentals and Applied Toxicology, 22, 90-102.
Laybourn, J., and B.J. Finlay. 1976. Respiratory energy losses related to cell weight and temperature in ciliated protozoa. Oecologia 44:165-174.
LeChevallier, M.W., Norton, W.D., and Lee, R.G., 1991, Occurrence of *Giardia* and *Cryptosporidium* species in surface water supplies: Applied and Environmental Microbiology, v. 57, no. 9, p. 2610-2616.
Lee, C.C., and T. Fenchel. 1972. Studies on ciliates associated with sea ice from Antarctica. II. Temperature responses and tolerances in ciliates from Antarctica, temperate and tropical habitats. Archive für Protistenkunde 114:237-244.
Levy, D.A., Bens, M.S., Craun, G.F., Calderon, R.L., and Herwaldt, B.L.,1998, Surveillance for waterborne disease outbreaks—United States, 1995-1996: Morbidity and Mortality Weekly Report—Surveillance Summaries, December 11, 1998, 47(SS-5).
Lindquist, H.D.A. (1999). Emerging pathogens of concern in drinking water. EPA Publication #EPA 600/R-99/070.
Loomis, George and Yael Calhoon. "Natural Resource Facts: Maintaining Your Septic System." University of Rhode Island, Providence, RI, 1988.
Macozzi, Maureen. *Groundwater- Protecting Wisconsin's Buried Treasure*. Wisconsin Department of Natural Resources, Madison, WI, 1989.
Maine Association of Conservation Commissions. *Ground Water... Maine's Hidden Resource*. Hallowell, ME, 1985.
Malard, F., Reygrobellet, J-L., and Soulie, Michel, 1994, Transport and retention of fecal bacteria at sewage polluted fractured rock sites: Journal of Environmental Quality, v. 23, p. 1352-1363.
Massachusetts Audubon Society "Local Authority for Groundwater Protection." Groundwater Information Flyer #4. Lincoln, MA, 1984.
Massachusetts Audubon Society. "Groundwater and Contamination: From the Watershed into the Well." Groundwater Information Flyer # 2. Lincoln, MA, 1984.
Massachusetts Audubon Society. "Mapping Aquifers and Recharge Areas." Groundwater Information Flyer # 3. Lincoln, MA, 1984.
Massachusetts Audubon Society. "Road Salt and Groundwater Protection." Groundwater Information Flyer # 9. Lincoln, MA, 1987.
Mast, A.M., and Turk, J.T., 1999, Environmental Characteristics and Water Quality of Hydrologic Benchmark Network Stations in the Eastern United States, 1963- 95: U.S. Geological Survey Circular 1173-B, 158 p.
McCann, Alyson and Thomas P Husband. "Natural Resources Facts: Household Hazardous Waste." University of Rhode Island, Providence, RI; 1988.
Miller, David W. *Groundwater Contamination: A Special Report*. Geraghty & Miller, Inc., Syosset, NY 1982.
Montagnes, D.J.S., D.H. Lynn, J.C. Roff, and W.D. Taylor. 1988. The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. Marine Biology 99:21-30.
Mullikin, Elizabeth B. *An Ounce of Prevention: A Ground Water Protection Handbook for Local Officials*. Vermont Departments of Water Resources and Environmental Engineering, Health, and Agriculture, Montpelier, VT, 1984.
Murphy, Jim. "Groundwater and Your Town: What Your Town Can Do Right Now." Connecticut Department of Environmental Protection, Hartford, CT.
Myers, D.N., 1992, Distribution and variability of fecal indicator bacteria in Scioto and Olentangy Rivers in the Columbus, Ohio, area: U.S. Geological Survey Water-Resources Investigations Report 92-4130, 61 p.
Myers, D.N., and Sylvester, M.D., 1997, National field manual for the collection of water-quality data—Biological indicators: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, 38 p.
Myers, D.N., Koltun, G.F., and Francy, D.S., 1998, Effects of hydrologic, biological, and environmental processes on sources and concentrations of fecal bacteria in the Cuyahoga River, with implications for

management of recreational waters in Summit and Cuyahoga Counties, Ohio: U.S. Geological Survey Water-Resources Investigations Report 98-4089, 38 p.
National Academy of Engineering (2000). Greatest engineering achievements of the 20th century. [On-Line]. Available: (http://www.greatachievements.org/ greatachievements/) (accessed 2-10-03).
National Research Council. *Ground Water Quality Protection: State and Local Strategies.* National Academy Press, Washington, D.C., 1986.
Natural Resources Defense Council, 1998, Testing the waters—Volume VIII: New York, 145 p. Novotony, V., Sung, Hung-Ming, Bannerman, R., and Baum, K., 1985, Estimating nonpoint pollution from small urban watersheds: Journal of the Water Pollution Control Federation, v. 57, p. 339-348.
New England Interstate Water Pollution Control Commission. "Groundwater: Out of Sight Not Out of Danger." Boston, MA, 1989.
Niederlehner, B.R., K.W. Pontasch, J.R. Pratt, and J. Cairns. 1990. Field evaluation of predictions of environmental effects from multispecies microcosm toxicity test. Archives of Environmental Contamination and Toxicology 19:62-71.
Noake, Kimberly D. Guide to *Contamination Sources for Wellhead Protection*. Draft. Massachusetts Department of Environmental Quality Engineering, Boston, MA, 1988.
Office of Drinking Water. *A Local Planning Process for Groundwater Protection*. U.S. EPA, Washington, D.C., 1989.
Office of Ground-Water Protection. *Guidelines for Delineation of Wellhead Protection Areas*. U.S. EPA, Washington, D.C., 1987.
Office of Ground-Water Protection. *Survey of State Ground Water Quality Protection Legislation Enacted From 1985 Through 1987*. U.S. EPA, Washington, D.C., 1988.
Office of Ground-Water Protection. *Wellhead Protection Programs. - Tools for Local Governments.* U.S. EPA, Washington, D.C., 1989.
Office of Ground-Water Protection. *Wellhead Protection: A Decision-Makers' Guide.* U.S. EPA, Washington, D.C., 1987
Office of Pesticides and Toxic Substances. *Citizen's Guide to Pesticides.* U.S. EPA, Washington, D.C., 1989.
Office of Underground Storage Tanks. *Musts for USGS. - A Summary of the New Regulations for Underground Storage Tank Systems.* U.S. EPA, Washington, D.C., 1988.
Ohio Environmental Protection Agency. *Ground Water.* Columbus, OH.
Ontario Ministry of the Attorney General, The Honorable Dennis R. O'Connor (2002). Part one: A summary: Report of the Walkerton inquiry: The events of May 2000 and related issues.
Otterstetter, H. and Craun, C. (September, 1997). Disinfection in the Americas: A necessity. Journal of the American Water Works Association, 8-10.
Palmer, M.D., Lock, J.D., and Gowda, T.P.H., 1984, The use of bacteriological indicators for swimming water quality: Water and Pollution Control, v. 122, no. 3, p. 14-15, 17-18, and 74.
Payment, P., and Franco, E., 1993, *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts: Applied and Environmental Microbiology, v. 59, no. 8, p. 2418-2424.
Redlich, Susan. *Summary of Municipal Actions for Groundwater Protection in the New England/New York Region.* New England Interstate Water Pollution Control Commission, Boston, MA, 1988.
Research, v. 13, p. 33-41.
Robertson, J.B., and Edberg, S.C., 1997, Natural protection of spring and well drinking water against surface microbial contamination. 1. Hydrogeological parameters: Critical Reviews in Microbiology, v. 23, no. 2, p. 143-178.
Rose, J.B. (2002). Water quality security. Environmental Science and Technology, 36, 217-256.
Rose, J.B., Atlas, R.M., Gerba, C.P., Gilchrist, M.J.R., Le Chevallier, M.W., Sobsey, M.D., and Yates, M.V., 1999, Microbial pollutants in our Nation's
Rose, J.B., Gerba, C.P., and Jakubowski, W., 1991, Survey of potable water supplies for *Cryptosporidium* and *Giardia*: Environmental Science and Technology, v. 25, no. 8, p. 1393-1400.
Southern Arizona Water Resources Association. "Water Warnings: Our Drinking Water.... It Takes Everyone to Keep It Clean." Tucson, AZ.
Sponenberg, Torsten D. and Jacob H. Kahn. *A Groundwater Primer for Virginians.* Virginia Polytechnic Institute and State University, Blacksburg, VA, 1984.

Taylor, W., and R. Sanders. 1991. Protozoa. Pages 37-93 in J.H. Thorp and A.P. Covich, eds. Ecology and classification of North American freshwater invertebrates. Academic Press, New York.
Texas Water Commission. "On Dangerous Ground: The Problem of Abandoned Wells in Texas." Austin, TX, 1989.
Texas Water Commission. *The Underground Subject: An Introduction to Ground Water Issues in Texas.* Austin, TX, 1989.
U.S. Centers for Disease Control and Prevention (1997). Summary of notifiable diseases. U.S. Centers for Disease Control and Prevention (April 12, 1996). Morbidity and Mortality Weekly Report, CDC Surveillance summaries: Surveillance for waterborne disease outbreaks, United States, 1993-1994.
U.S. Centers for Disease Control and Prevention (December 11, 1998). Morbidity and Mortality Weekly Report, CDC Surveillance summaries: Surveillance for waterborne disease outbreaks, United States, 1995-1996.
U.S. Centers for Disease Control and Prevention (May 26, 2000). Morbidity and Mortality Weekly Report, CDC Surveillance summaries: Surveillance for waterborne disease outbreaks, United States, 1997-1998.
U.S. Centers for Disease Control and Prevention (November 19, 1993). Morbidity and Mortality Weekly Report, CDC Surveillance summaries: Surveillance for waterborne disease outbreaks-United States, 1991-1992.
U.S. Centers for Disease Control and Prevention, (2002). National Center for Infectious Diseases, Infectious Disease Information, Diseases related to water. [On-Line]. Available: http://www.cdc.gov/ncidod/diseases/water/drinking.htm
U.S. Centers for Disease Control and Prevention, (November 22, 2002). Morbidity and Mortality Weekly Report, CDC Surveillance summaries: Surveillance for waterborne disease outbreaks, United States, 1999-2000.
U.S. Environmental Protection Agency (1991). Letter from Wilcher, L.S. to Guerra de Macedo, G.
U.S. Environmental Protection Agency (1998a). National Primary Drinking Water Regulations: Disinfectants and Disinfection Byproducts; Final Rule. Federal Register Vol 63, No. 157. Wednesday, Dec.16, 1998.
U.S. Environmental Protection Agency (1998b). Regulatory Impact Analysis of Final Disinfectant/ Disinfection byproducts Regulations. Washington, D.C. EPA Number 815-B-98-002-PB 99-111304
U.S. Environmental Protection Agency (2001a). Toxicological review of chloroform in support of summary information on the Integrated Risk Information System (IRIS). EPA Number 635/R-01/001.
U.S. Environmental Protection Agency (2001b). Controlling Disinfection byproducts and Microbial Contaminants in Drinking Water. EPA Number 600/R-01/110.
U.S. Environmental Protection Agency (2002). Public drinking water systems: Facts and figures. [On-Line]. Available: http://www.epa.gov/safewater/pws/factoids.html (accessed 11-22-02).
U.S. Environmental Protection Agency. *Seminar Publication: Protection of Public Water Supplies from Ground-Water Contaminants.* Center for Environmental Research Information, Cincinnati, OH, 1985.
Waller, Roger M. *Ground Water and the Rural Homeowner.* U.S. Geological Survey, Reston, VA, 1988.
water, *in* Groundwater pollution microbiology: New York, John Wiley and Sons, p. 65-88.
water—Environmental and public health issues: Washington,D.C., American Society for Microbiology,
World Health Organization (2002a). Water and Sanitation: Facts and Figures. [On-Line]. Available: http://www.who.int/water_sanitation_health/General/ factsandfigures.htm
World Health Organization (2002b). Water and Sanitation: Guidelines for drinking water quality. [On-Line]. Available: http://www.who.int/ water_sanitation_health/GDWQ/Microbiology/ Microbioladd/microadd5.htm



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