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Health Risks From Microbial Growth and Biofilms in Drinking Water Distribution Systems

I. Purpose of the Document

This document is one of a series of papers intended to review what is known about the health risks associated with several distribution system issues, and where relevant, identify areas in which additional research may be warranted. The issues were selected based on the input of distribution system experts. The distribution system issues of concern are: Growth/Biofilms, Cross-Connections, Intrusion, Aging Infrastructure, Decay of Water Quality over Distribution System Residence Time, Contamination During Infrastructure Repair and Replacement, Nitrification, Covered Storage, and Corrosion, Permeation and Leaching.

The goal of this document is to review existing literature, research and information on the potential public health implications associated with the survival and/or growth of pathogens, as well as with the presence of microbial metabolic products in the biofilms of drinking water distribution systems. More specifically, the goal of this document is to review what we know regarding:

- Microbes in or associated with biofilms that may present a public health risk in the distribution system;
- the types of disease each pathogen or metabolic product cause;
- Routes through which pathogens can enter the distribution system;
- factors that influence survival and growth of pathogens within the distribution system;
- the effects of biofilms on some other health-related issues associated with the distribution system;
- suitable measures for controlling biofilm development; and
- possible indicators of the presence of a biofilm problem and the effectiveness of control measures.

II. Executive Summary

Many different microbes have demonstrated the ability to survive in the distribution system, with some possessing the ability to grow and/or produce biofilms. Some of these organisms may be primary pathogens (i.e., those that cause disease in healthy individuals), while others may be opportunistic pathogens (i.e., those that cause disease in individuals with underlying conditions that may facilitate infection). Microbes can enter distribution systems through a wide range of avenues, including treatment processes or through deficiencies of the distribution system infrastructure. Microbial presence in the distribution system can result in colonization of the distribution system infrastructure. Once biofilm development begins, subsequent material, organisms and contamination

introduced to the distribution system can become entrained in the biofilm. The biofilm can protect microbes from disinfection and allow microbes injured by environmental stress and disinfectants to recover and grow. In addition, biofilms may increase pipe corrosion, adversely affect pipe hydraulics and reduce the utility of total coliforms as indicator organisms. Microbial growth in biofilms may result in deterioration of water quality, generation of bad tastes and odors, and proliferation of macroinvertebrates.

Contamination and material in the biofilm may subsequently be released into the flowing water under various circumstances. As a result, biofilms can act as a slow-release mechanism for persistent contamination of the water. The organisms and their products may decrease disinfectant levels (by increasing disinfectant demand), pose a direct public health risk, or create taste and odor problems. Biofilms likely exist in all distribution systems, and are recognized as a normal part of the distribution system.

III. Definitions

Drinking water in the distribution system is not sterile, regardless of the degree to which the water is treated. The water contains microbes that survive the treatment process or enter the distribution system through the pipe network. Many of these microbes can attach to the pipe wall and become part of a biofilm.

Several definitions for biofilms have been published in the literature (LeChevallier, 1999a; Berger et al., 1993; Characklis and Marshall, 1990; Characklis, 1981). There is not one universally-recognized definition for biofilms; however, common among the definitions is that a water distribution system biofilm is a complex mixture of microbes, organic and inorganic material accumulated amidst a microbially produced organic polymer matrix attached to the inner surface of the distribution system. The inner surface of a water pipe may have a continuous biofilm, but usually biofilms are quite patchy (Walch, 1992; van der Wende and Characklis, 1990).

Under certain circumstances regrowth events can occur. The term "regrowth" is not precisely defined in the literature. Some use "regrowth" to refer to any growth that occurs in the distribution system; others restrict the meaning to the recovery and growth of environmentally- or disinfectant-stressed microbes. This document will use the term "growth" as referring to both the recovery and growth of injured microbes, as well as the growth of non-injured microbes.

IV. Microbes that May Present a Public Health Risk in the Distribution System

This section of the paper will discuss the potential public health concern that arises when certain microbes and their products become a component of the distribution system biofilm. While some potential health effects are listed in the tables herein, additional health effects are provided in tables on the EPA Office of Ground Water and Drinking Water website. The organisms and toxins discussed are:

• Bacteria

- Viruses
- Protozoa
- Invertebrates
- Algae and algal toxins
- Fungi
- Microbial toxins

A number of technical reviews of the literature have been published on biofilm organisms in the water distribution system and factors that influence their survival and growth (Geldreich and LeChevallier, 1999; Geldreich, 1996; van der Wende and Characklis, 1990; LeChevallier, 1989a; LeChevallier et al., 1990a; 1990b; 1999b; Costerton and Lappin-Scott, 1989; Marshall, 1992; Mittelman, 1991; USEPA, 1992b; NRC, 1982).

Any microbe (including some pathogens) present in water may attach, or become enmeshed, in the biofilm. Primary pathogens, which cause disease in healthy humans, may survive for a time in the biofilm. However, the survival time for many pathogens in biofilms is uncertain and likely varies depending on the organism For some pathogens, the distribution system is a physical, chemical, and biological environment unsuited for their growth. However, pathogens may accumulate in the biofilm, and the biofilm may extend the survival of primary pathogens by protecting them from disinfectants. These pathogens may be sloughed from the biofilm into the water column due to changes in the flow rate. The persistence of waterborne disease, or of microbial contamination in a distribution system, long after the cause of the distribution system problem has apparently been corrected suggests that there may be an isolated pocket of static or slow-flowing water or biofilm erosion or sloughing is occurring (i.e. the slow-release mechanism).

In contrast to enteric primary pathogens (i.e., those which inhabit the gastrointestinal tract), aquatic microbes are well-adapted to the low nutrient level and cool water temperature of the distribution system, especially in the biofilm. Select aquatic microorganisms may be responsible for the majority of infections and illnesses in humans and other animals. Some aquatic microbes may cause disease in humans under certain circumstances, especially in individuals with a weakened immune system or other major underlying conditions that facilitates infection. These microbes are referred to as opportunistic, or secondary, pathogens. Opportunistic pathogens include *Pseudomonas aeruginosa*, *Legionella pneumophila*, and the *Mycobacterium avium* complex (MAC).

Most waterborne pathogens – both primary and opportunistic – also have routes of transmission other than water. Many of these microbes, especially the primary bacterial pathogens, are important agents of foodborne outbreaks (Schaechter et al., 1998). Direct person-to-person spread is common, especially for the viral and protozoan agents. The importance of water relative to other sources of transmission depends upon the organism and other factors, and is often uncertain.

A. Bacteria

The primary intestinal bacterial waterborne pathogens include *Shigella*, *Salmonella*, *Yersinia*

enterocolitica, *Campylobacter jejuni*, and *Escherichia coli* O157 (Table 1). The potential for them to attach to biofilms exists, and limited growth in some circumstances cannot be ruled out. One primary pathogen that may be waterborne, *Helicobacter pylori*, was found to survive at least 192 hours on stainless steel coupons (inserts used to monitor biofilm buildup) in a chemostat (Mackay et al., 1998). Park, et al., (2001) have also noted the presence of *H. pylori* in biofilms of drinking water mains. In another study, two non-pathogenic *E. coli* strains injected into a pilot distribution system with a biofilm (20°C) grew slightly in the biofilm before eventually dying out (Fass et al., 1996). The building plumbing system may act either as a direct conduit for pathogens sloughed off from distribution system biofilms or as an amplifier of these pathogens. A waterborne disease outbreak caused by *E. coli* O157 persisted for weeks after the suspected source – contaminated water meters and main breaks – were replaced or repaired (Swerdlow et al., 1992). Although a biofilm was not implicated, the potential exists for biofilms to prolong the survival of some microbes. In another study, *Salmonella typhimurium* was able to grow for a short time at 24ºC in non-sterile tap water (Armon et al., 1997).

Table 1: Primary Bacterial Pathogens Capable of Causing Waterborne Disease

Organism	Major Disease[3]	Primary Source	WBDO[1]	CCL[2]
Salmonella typhi	typhoid fever	human feces	X	
Salmonella paratyphi	paratyphoid fever	human feces	X	
Salmonella typhimurium	gastroenteritis	human/animal feces	X	
Other *Salmonella* sp.	gastroenteritis (salmonellosis)	human/animal feces	X	
Shigella	bacillary dysentery	human feces	X	
Vibrio cholerae	cholera	human feces, coastal	X	
Enterovirulent *E. coli*	gastroenteritis	human feces	X	
Yersinia enterocolitica	gastroenteritis	human/animal feces	X	
Campylobacter jejuni	gastroenteritis	human/animal feces	X	
Legionella pneumophila	Legionnaires Disease, Pontiac fever	warm water	X	
Helicobacter pylori	peptic ulcers	saliva, human feces?		X

[1] Documented waterborne disease outbreak in U.S.
[2] Pathogen is on EPA's Contaminant Candidate List (CCL) of March 1998
[3] Disease symptoms described in Benenson (1995)

Much more information is available on the presence of opportunistic bacterial pathogens. Table 2 lists some of the aquatic and soil bacteria that have been associated with both distribution system biofilms and disease. However, strain variation exists within each of the listed bacteria.

Purely environmental strains and clinical strains of the same genus or species may be present in a distribution system. The clinically significant strains are opportunistic pathogens. Infective dose studies on healthy individuals and animals, using the oral or intranasal route, demonstrate that very high doses (10^6-10^{10} cells) are needed for infection or disease, at least for healthy individuals (Rusin et al., 1997). In one study (Olson, 1982), *Acinetobacter* was detected on the surface layer of a mortar-lined pipe at levels up to $10^9/cm^2$. While these studies focused on healthy individuals and animals, little infective dose data are available for more susceptible populations. The clinically significant strains of bacteria listed in Table 2 may cause disease ranging from mild to severe, including pneumonia and septicemia (invasion of the blood). Outcomes are sometimes fatal (Toder, 1998; Inderlied et al., 1993; Jarvis et al., 1985; Pier, 1998; Hardalo and Edberg, 1997; Thomas et al., 1977).

Since opportunistic pathogens affect sensitive individuals, such as some hospitalized individuals, the percent of hospital-acquired (nosocomial) infections caused by these organisms may provide some insight on the effects on sensitive individuals in general. The percent of nosocomial infections caused by various opportunistic bacterial pathogens common in biofilms is given in Table 3. The CDC data (Table 3) indicate that these bacteria cause about 25% of the nosocomial infections (CDC, 1996). There are several sources through which sensitive individuals can come into contact with these bacteria, with some cases being linked to drinking water. Given that a large number of sensitive individuals exist (Table 4), even a very small percentage contribution from drinking water may represent a sizeable number of people.

Table 2: Opportunistic Bacterial Pathogens Detected in the Distribution System and/or Biofilms

Opportunistic pathogens	Health Effects	Information on:			WBDO[1]	CCL[2]
		Disease	Presence in DS	Biofilm Presence		
Acinetobacter calcoaceticus	pneumonia, meningitis, infections of urinary tract, septicemia	Davis et al., 1973, Horan et al., 1988	Geldreich, 1990	LeChevallier et al., 1987; Geldreich, 1990		
Aeromonas hydrophila	sepsis, gastrointestinal illness, respiratory tract infections	Davis et al., 1973	Geldreich, 1990	Reasoner, 1991, van der Kooij and Hijnen, 1988		X
Citrobacter spp.[3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Enterobacter spp.[3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Flavobacterium spp.	septicemia, meningitis	Davis et al., 1973	Geldreich, 1990	Geldreich, 1990		
Klebsiella pneumoniae [3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Moraxella spp.	pneumonia, conjunctivitis, septicemia, otitis, urethritis, meningitis, bronchitis, sinusitis	Benenson, 1995, Davis et al., 1973, Walker, 1998	LeChevallier, 1987	LeChevallier, 1987		
M. avium complex	chronic diarrhea, chronic lung disease	Schaechter et al. 1998	Geldreich, 1990	Norton et al., 2000	X	X
Pseudomonas cepacia	foot infections	Tally, 1998	Geldreich, 1990	LeChevallier et al., 1987		
Pseudomonas aeruginosa	infections when severe burns, cancer patients, lungs when cystic fibrosis, pneumonia, meningitis, others	Toder, 1998	Geldreich, 1990	Geldreich, 1990		
Serratia marcescens [3]	septicemia, pneumonia	Schaechter et al. 1998	Geldreich, 1990			

[1] Documented waterborne disease outbreak in U.S.

[2] Pathogen is on EPA's Contaminant Candidate List (CCL) of March 1998

[3] Some species are coliforms

Table 3: Nosocomial Infections[1], 1/90-3/96

Organism	Percent (%)
E. coli	12
Enterococci	10
Pseudomonas aeruginosa	**9**
***Enterobacter* spp.**	**6**
Klebsiella pneumoniae	**5**
***Acinetobacter* spp.**	**1**
Serratia marcescens	**1**
***Citrobacter* spp.**	**1**
Other *Klebsiella* spp.	**1**
Other Enterobacteriaceae	4
Staphylococci	24
Yeast and fungi	7
All others	19

[1] from CDC's Nat'l Nosocomial Infections Surveillance System (5/96)
Note 1: About 5% of all patients develop an infection while in the hospital
Note 2: In **bold** are the opportunistic pathogens that occur naturally in water

Table 4: Immunocompromised Sub-Populations in U.S.

Immunocompromised Sub-population	Number
AIDS patients	274,000[1]
Cancer patients on immuno-suppressive therapy	4.3 million (est.)
People with organ transplants	195,561[2]
Diabetics	8 million[3]
Hospitalized burn patients	75,000 /year[4]
Cystic fibrosis	30,000

[1] through 12/98 (CDC HIV/AIDS Surveillance Report vol 10, no. 2)
[2] 1988-1998 (Scientific Registry, United Network for Organ Sharing, 1999)
[3] CDC, National Center for Health Statistics, data for 1995
[4] J. Burn Care & Rehab., May/June 1992.

Populations susceptible to opportunistic bacterial pathogens that are common in biofilms include infants, young children, pregnant women, the very elderly, and those who have a severely

weakened immune system or other major impairment of the host defense. Table 4 identifies the immunocompromised populations in the U.S., and their numbers, that are susceptible to these pathogens. Cystic fibrosis is included although, strictly speaking, the immune response is normal; however, antibodies cannot reach the bacteria embedded in the thick sticky mucus in these patients (Pier, 1998). Table 4 includes only those groups that are more susceptible to opportunistic pathogens due to being immunocompromised (with the exception of cystic fibrosis), and not those that are more susceptible and not classified as immunocompromised, such as individuals with prolonged antacid or antibiotic use or heavy smokers.

The opportunistic bacterial pathogens common in biofilms that are of most concern include *L. pneumophila*, MAC, and *P. aeruginosa*, although there are others. Depending on the organism, waterborne disease and injury may result from exposure through ingestion, inhalation of aerosols, and by dermal exposure (e.g., through wounds). The link between nosocomial or community infections caused by these organisms and drinking water is summarized below.

1. Legionella pneumophila

At least 39 species of *Legionella* have been identified, and a substantial proportion can cause a type of pneumonia called Legionnaires disease. *L. pneumophila* accounts for 90% of the cases of Legionnaires disease reported to CDC (Breiman, 1993). According to CDC (MMWR Summary of Notifiable Diseases, 12/31/99), 1355 cases of legionellosis (includes Legionnaires Disease and a milder non-pneumonic illness known as Pontiac Fever) were reported to CDC in 1998; however, CDC believes a majority of these illnesses are not reported, and estimates that 8,000 to 18,000 legionellosis cases occur in the U.S. annually (www.cdc.gov/nciod/dbmd/diseaseinfo). The underestimate is probably due to the fact that Legionella testing is not routinely performed, possibly because the organism is so difficult to culture and identify (Stout and Yu, 1997).

L. pneumophila is a naturally occurring and widely distributed organism. In one study, it was isolated from all samples taken in a survey of 67 rivers and lakes in the United States. Higher recoveries occurred in warmer waters (Fliermans et al., 1981). *L. pneumophila* has been found in the biofilms of water mains, although they may not proliferate therein to any extent (States et al., 1990; Armon et al., 1997). Various plumbing materials support the growth of *Legionella*, including latex, ethylene-propylene, polypropylene, polyethylene, polyvinylchloride (PVC) and steel (Rogers et al., 1994). *Legionella* proliferation is facilitated within *Acanthamoeba* and other aquatic amoeba (States et al., 1990; Kwaik et al., 1998), and virulence may be enhanced by their interaction within the amoeba, or may even be a necessary condition for virulence (Cirillo et al., 1999). Small numbers of *Legionella* can occur in the finished waters of systems, including those employing conventional treatment. These organisms can colonize hot water plumbing systems, and aerosols from fixtures, such as showerheads, may cause disease via inhalation (U.S. Environmental Protection Agency, 1989; Tobin et al., 1981). Aerosols from cooling towers, hot tubs, and whirlpools containing *Legionella* have also been implicated as a route of infection (Moore et al., 1993). It is likely that drinking water is an important, if not the primary source, of *Legionella* that seed hot water plumbing systems and cause outbreaks (Schaechter et al., 1998). Especially vulnerable to outbreaks caused by *Legionella* are hospitals and other large institutions that have an extensive hot water plumbing system and cater to susceptible subpopulations.

Several reports have been published on the presence of *Legionella* in the biofilm of dental-unit water systems (Walker et al., 2000; Atlas et al., 1995).

Another indirect effect is primarily associated with the concern about *Legionella* proliferation in the hot water plumbing systems of hospitals and other health care institutions. This concern has prompted some hospitals to increase their hot water temperatures to levels that inhibit *Legionella* growth (about 60°C). The higher temperatures have led to an increase in accidental patient scalding.

2. Mycobacterium avium complex (MAC)

MAC is a small group of bacteria in the genus *Mycobacterium*, primarily consisting of *M. avium* and *M. intracellulare*. MAC is common in the environment and colonizes water systems and plumbing systems (du Moulin and Stottmeier, 1986; du Moulin et al., 1988; Schulze-Robbecke et al., 1992; Norton and LeChevallier, 2000). *Mycobacterium* species are common in pipe biofilms (Kubalek et al., 1995; Schulze-Robbecke et al., 1992). Schulze-Robbecke et al., (1992) detected mycobacteria in 90% of 50 biofilm samples, usually at levels between 10^3-10^4 colonies/cm^2. MAC is relatively resistant to chlorine disinfection (Pelletier et al., 1988). MAC causes chronic lung disease in the immunocompromised population, especially in those receiving cancer chemotherapy, but chronic diarrhea is the major symptom caused by these organisms in AIDS patients (Inderlied et al., 1993; Singh and Yu, 1994). About 40-60% of late-stage AIDS patients suffer from MAC-caused chronic diarrhea. Infrequently, MAC causes disease in otherwise healthy individuals, especially older women (Prince et al., 1989; Inderlied et al., 1993). Clinically important strains have been found in distribution systems (Squier et al., 2000; Aronson et al., 1999), and drinking water has been epidemiologically linked to MAC infections in hospital patients (du Moulin and Stottmeier, 1986).

3. Pseudomonas aeruginosa

P. aeruginosa is widespread in environmental waters, especially in those waters associated with human activity. The organism is often found in finished waters and in pipe biofilms. Although *P. aeruginosa* has not been conclusively implicated in a reported waterborne disease outbreak, it has a significant role in nosocomial illness, including outbreaks. It is a pathogen of concern for people with severe burns and wounds, diabetes, and is the primary cause of injury and death in people with cystic fibrosis (Toder, 1998). Some strains cause pneumonia in general intensive care units (ICUs) and pediatric ICUs. Clinically significant strains have been found in the hospital plumbing system, suggesting that drinking water may contribute to nosocomial infections. A list of nosocomial outbreaks associated with *P. aeruginosa* in contaminated drinking water appears in Highsmith et al. (1986). However, the linking between the water distribution system (as opposed to the hospital plumbing system) and the presence of clinically important strains of *P. aeruginosa* in the nosocomial setting is still open to question (Samadpour, 2001).

B. Viruses

Viruses need a specific host (e.g., humans) to proliferate, therefore, they may accumulate, but not grow, in the biofilm. One pilot-scale distribution system study demonstrated that more poliovirus

1 were recovered from the biofilm than from the water column (Quignon et al., 1997). In the presence of chlorine there were ten-fold more viruses in the biofilm than in the water flow; without chlorine there were two-fold more (Quignon et al., 1997). It is likely that biofilms may harbor other viruses. Vanden Bossche and Krietemeyer (1995) detected coxsackievirus B in biofilms in water mains exiting a treatment plant. Table 5 presents viruses associated with waterborne disease that could potentially become entrained in the biofilm matrix for later release. The biofilm may protect the viruses against disinfectants (Quignon et al., 1997) and thus allow them to survive for a time.

Table 5: Viral Fecal Pathogens Capable of Causing Waterborne Disease

Organism	Major Disease[3]	Primary Source	WBDO[1]	CCL[2]
Poliovirus	poliomyelitis	human feces	X	
Coxsackievirus	upper respiratory disease	human feces		X
Echovirus	upper respiratory disease	human feces		X
Rotavirus	gastroenteritis	human feces		
Norwalk and other Caliciviruses	gastroenteritis	human feces	X	X
Hepatitis A virus	infectious hepatitis	human feces	X	
Hepatitis E virus	hepatitis	human feces		
Astrovirus	gastroenteritis	human feces		
Enteric adenoviruses	gastroenteritis	human feces		X

[1] Documented waterborne disease outbreak in U.S.
[2] Pathogen is on EPA's Contaminant Candidate List (CCL) of March 1998
[3] Disease symptoms described in Benenson (1995)

C. Protozoa

A few studies have also examined the presence of protozoa (i.e, unicellular animals), in the distribution system or in pipe biofilms. A diverse flora of free-living aquatic microbes exist in the pipe biofilm and pipe sediment, and protozoa are a natural part of that community. Ciliates, thecamoebae, amoebae, and flagellates have been detected in the biofilm of pilot distribution systems (Sibille et al., 1998; Block et al., 1993; Pedersen, 1990). Sibille et al. (1998) found an average protozoal count of 10^3 cells/cm^2 in the biofilm. Amoeba were observed in a hospital plumbing system (Michel et al., 1995). Because many protozoa feed on bacteria, it is likely that the protozoan population in the biofilm correlates with bacterial density.

Table 6 presents some primary protozoal pathogens. *Cryptosporidium*, *Giardia*, *Toxoplasma*, *Cyclospora* and other primary human pathogenic protozoa are present in natural water in a non-reproductive protective stage (e.g., cyst, oocyst). Available data suggest that these organisms may

attach to, and accumulate within, the pipe biofilm and persist. For example, in a laboratory experiment, Piriou et al., (2000) placed *Cryptosporidium* oocysts in a recirculated biofilm reactor (PVC pipes) containing glass beads with biofilm, and found that a sizeable fraction of the oocysts became associated with the biofilm. Rogers et al., (1996) also found that *Cryptosporidium* oocysts attached rapidly to biofilms on glass tiles in a chemostat. Although protozoan cysts/oocysts may attach to, and accumulate within, the pipe sediment and biofilm, these organisms do not likely proliferate in such environments. They need a suitable warm-blooded host for this purpose. Reports of ongoing recoveries of oocysts from the distribution system water after switching to an alternate source provided evidence of biofilm involvement following a cryptosporidiosis outbreak (Howe et al., 2002).

Table 6: Protozoa Capable of Causing Waterborne Disease

Organism	Major Disease[3]	Primary Source	WBDO[1]	CCL[2]
Giardia lamblia	giardiasis (gastroenteritis)	human & animal feces	X	
Cryptosporidium parvum	cryptosporidiosis (gastroenteritis)	human & animal feces	X	
Entamoeba histolytica	amoebic dysentery	human feces	X	
Cyclospora cayatanensis	gastroenteritis	human feces	X	
Microspora	gastroenteritis[4]	human feces		X
Acanthamoeba	eye infection	soil and water		X
Toxoplasma gondii	similar to infectious mononucleosis	cats		
Naegleria fowleri	primary amoebic meningoencephalitis	soil and water		

[1] Documented waterborne disease outbreak in U.S.
[2] Pathogen is on EPA's Contaminant Candidate List (CCL) of March 1998
[3] Disease symptoms described in Benenson (1995), except as noted
[4] Weber et al., 1994

Several free-living protozoa have been implicated in waterborne disease, especially some *Acanthamoeba* species and *Naegleria fowleri*. *Acanthamoeba* is common in soil and water, including drinking water and home plumbing systems (Sawyer, 1989; Gonzalez de la Cuesta et al., 1987; Seal et al., 1992). Some *Acanthamoeba* species are pathogenic, and can cause corneal inflammation, especially in wearers of soft or disposable contact lenses (Seal et al., 1992). They have also been reported to cause chronic encephalitis and skin lesions in the immunocompromised (Kilvington, 1990; Torno et al., 2000).

Because *Acanthamoeba* have been found in aquatic sediments, they may also be present in the sediment of the water distribution system. These sediments provide a surface for biofilm development. An additional public health concern related to *Acanthamoeba* is the ability of *Legionella* and *M. avium*, after ingestion by *Acanthamoeba* and other aquatic protozoa, to multiply and become more virulent within these protozoa (Steinert et al., 1998; Cirillo et al., 1997). In addition, the

protozoa may protect these ingested pathogens from disinfectants (King et al., 1988). Other bacterial pathogens may be protected by *Acanthamoeba* against disinfection, including *E. coli* O157 (Barker et al., 1999), *P. aeruginosa* (Burghardt and Bergmann, 1995), and possibly *Vibrio cholerae* (Thom et al., 1992).

N. fowleri is a free-living amoeba in soil, water, and decaying vegetation. Though it is common in many warm surface waters, it rarely causes disease. The disease it does cause, primary amoebic meningoencephalitis, is invariably fatal, with death occurring within 72 hours after symptoms appear (CDC, 1992). All disease incidents to date have been associated with swimming in warm fresh waters; however, transmission via drinking water cannot be ruled out. The route of infection is via inhalation, not ingestion. Although *N. fowleri* has not been reported in the distribution system, other *Naegleria* species have been found, including some in the biofilm (Rivera et al., 1979; Block et al., 1993).

D. Invertebrates

Invertebrates include all animals lacking a spinal column. Loosely defined, macroinvertebrates include the larger invertebrates such as insects and worms. A number of macroinvertebrates may colonize the distribution system, including tiny nematodes (worms), mites, insect larvae, rotifers, and tiny crustaceans (Levy, 1990; Geldreich, 1996). These organisms can enter the distribution system through the treatment plant, broken mains, backsiphonage, taps and hydrants (van Lieverloo et al., 1997). However, only some taxa can survive in mains, with these organisms being dependent on the presence of bacteria as a food source (van Lieverloo et al., 1997). Water mites, cladocerans, copepods (and their larvae), oligochaetes and asellids have been observed in water flushed from distribution system lines (van Lieverloo et al., 1997). These have not been implicated in waterborne disease outbreaks, however. Various aquatic bacteria have also been found within the gut of nematodes collected from natural water, and laboratory feeding studies have demonstrated that nematodes can ingest bacterial pathogens and protect them from water disinfectants, and enhancing their survival in biofilms and through treatment processes (Levy et al., 1986). The most recognized problem is that macroinvertebrates occasionally occur in the tap water, or discolor the water, causing frequent consumer complaints (Levy, 1990).

E. Algae and algal toxins

A few algal species, primarily cyanobacteria, or blue-green algae, produce algal blooms in fresh waters, which can result in elevated toxin levels. The toxins, which include hepatotoxins and neurotoxins, may be sufficiently potent to kill an animal within minutes (USEPA, 1992a; Yoo et al., 1995). Numerous reports, summarized by Yoo et al. (1995), show that cyanobacteria blooms can kill large animals such as cattle, sheep, horses, pigs, and dogs within a few minutes or hours after ingesting lake water containing an algal bloom. An outbreak of waterborne gastrointestinal illness in the U.S. was associated with an algal bloom in an uncovered finished water reservoir (Lippy and Erb, 1976). In addition to acute effects, cyanobacterial toxins have been shown to be mutagenic, i.e., cause mutations to DNA (Falconer and Humpage, 1996), and epidemiological data suggests a link between an algal hepatotoxin and liver cancer (Ueno et al., 1996).

A search of the published literature found information from two studies about the presence of

algae or their toxins in pipe biofilm. These studies, using scanning electron microscopy on a pipe surface, found diatom and other algal fragments or "microfossils" embedded in the biofilm (Nagy and Olson, 1986; Allen et al., 1979; 1980). Other authors have found that some algae can grow heterotrophically in the dark (Berger et al., 1979), and cyanobacteria have been found at low levels in ground water (Sinclair, 1990). In addition, algal toxins are relatively stable in the dark (Jones and Sivonen, 1997), and may last at least one week in water (Drikas et al., 2001).

F. Fungi

Fungi are categorized as molds if they have branching, threadlike filaments, and yeasts if they are single-celled organisms that reproduce by budding. Fungi are ubiquitous in the environment. A diverse group of fungi has been found in water distribution systems (Rosenzweig et al., 1986; Niemi et al., 1982; Zacheus and Martikainen, 1995; Frankova and Horecka, 1995; Nagy and Olson, 1982; Geldreich and LeChevallier, 1999). Several studies report that filamentous fungi and yeast are common on water pipe surfaces, even in the presence of free chlorine residuals (Nagy and Olson, 1986; Doggett, 2000). Elevated open storage tanks and fire hydrants may be a significant source of fungi (Rosenzweig and Pipes, 1988; Rosenzweig and Pipes, 1989). Low flow rates are frequently maintained in these structures, which can enhance biofilm development (Geldreich, 1990).

Table 7: Pathogenic Fungi Found in Water Distribution Systems and Biofilms

Name	Some diseases/symptoms	References for Disease	Presence in Biofilms
Aspergillus fumigatus	pulmonary disease, allergies	Benenson, 1995	Rosenzweig et al., 1983; 1986
Aspergillus flavus	pulmonary disease, allergies	Benenson, 1995	Doggett, 2000; Rosenzweig et al., 1986
Aspergillus niger	ear infection	Benenson, 1995	Rosenzweig et al., 1983; 1986
Cryptococcus neoformans	meningitis, lung infections	Benenson, 1995	genus: Doggett, 2000; Rosenzweig and Pipes, 1988,1989
Candida albicans	vaginal, urinary, and esophageal infections, thrush	Benenson, 1995	genus: Rosenzweig and Pipes, 1988,1989
Mucor	thrombosis, infarction, nasal or paranasal sinus infections, GI disorders	Benenson, 1995	Rosenzweig & Pipes, 1988, 1989; Doggett, 2000; Nagy & Olson, 1986
Petriellidium boydii (*Pseudallescheria boydii*)	extracutaneous infections, brain abscess, systemic central nervous system infection	Fisher et al., 1982	Roesch and Leong, 1983
Sporothrix schenkii	skin infection (dermatomycoses)	Benenson, 1995	genus: Doggett, 2000
Stachybotrys chartarum	infant pulmonary hemosiderosis	Jarvis, 2002	Doggett, 2000
Trichophyton	scalp infections	Benenson, 1995	Frankova and Horecka, 1995

A few studies have identified the fungi present in the distribution system (Doggett, 2000). A few fungi are known to cause disease, primarily in immunocompromised individuals. Those fungi are listed in Table 7. Of the listed fungi, those species that have been observed in the pipe biofilm are *Aspergillus flavus*, *Stachybotrys chartarum*, and *Pseudallescheria boydii*. In addition, the fungal genera *Mucor*, *Sporothri*x, and *Cryptococcus* have been detected in the biofilm. These genera include pathogenic species, but apparently speciation was not performed.

Although fungi have been found in drinking water distribution systems and biofilms, fungi have not been conclusively implicated in waterborne disease. Those pathogenic fungi that have been detected in the distribution system are opportunistic and infrequently cause illness, whatever the route of infection. However, *A. flavus* and several other *Aspergillus* species detected in distribution systems produce potent toxins (mycotoxin), including aflatoxins. Also, a pathogenic yeast, *Candida albicans* (candidiasis), can establish itself in the gastrointestinal tract (Schaechter et al., 1998) and thus could potentially be spread by the fecal-oral route. It has been found in seawater and in sand at marine beaches. *C. albicans* and other important fungal pathogens are also associated with soil. Thus, the potential for waterborne disease caused by fungi in the biofilm exists, but the significance is unknown.

G. Microbial toxins

A number of human enteric pathogens produce a variety of toxins to facilitate their entry and proliferation within their human hosts. In contrast to the enteric pathogens, many microbes adapted to the aqueous environment release toxins as a survival mechanism or during cell autolysis.

Virtually all gram-negative bacteria, which represent the vast majority of bacteria in water, release a complex lipopolysaccharide known as endotoxin upon their death and autolysis. Endotoxin is capable in sufficient quantities of causing a non-specific response in humans such as fever. Using the *Limulus* lysate procedure, which is sensitive to picogram levels of endotoxin, various investigators have found this substance in the drinking water of most cities investigated (Diluzio and Friedmann, 1973; Jorgensen et al., 1976). Jakubowski and Ericksen (1980) discussed the health significance of the levels found in water, and determined that additional data were needed to provide a clear answer.

V. Effects on Other Health Related Issues Associated with the Distribution System

Some health issues associated with biofilms are indirect. Biofilms may compromise the use of total coliforms as drinking water indicators or, by corroding pipes, weaken pipe integrity. Although aesthetic problems may not directly represent a public health risk, the appearance of aesthetic problems may signal pipe deterioration, heavy biofilm, or other flaw that may represent, or lead to, a health concern. The indirect health issues include:

- Microbially-induced corrosion
- Loss of indicator organism utility
- Taste, color and odor problems
- Others

A. Microbially-induced corrosion

Pipe material invariably erodes with time. The rate of this process is affected by the composition of the pipe, the corrosivity of the water within the pipe and the soil and water external to the pipe, the microbial activity in the pipe biofilm, and other factors (Geldreich, 1996). Over time, corrosion may become serious enough to restrict water passage, produce taste and odor problems, cause pipe breaks, and accelerate biofilm development (Geldreich, 1996).

Pipe corrosion may be caused by non-biological factors such as rapidly flowing water (causes erosion corrosion and impingement attack) or chemical oxidation processes (Schock, 1999). However, microbes in the biofilm can also play an important role in pipe corrosion. Ultimately, corrosion can lead to pipe leaks, creating a pathway for pathogen intrusion into the drinking water. Biofilms on the inner surface of the pipe represent a complex and dynamic ecosystem that collectively influences the pipe corrosion process. The microbes most closely identified with pipe corrosion are the iron and sulfur bacteria. Iron bacteria such as *Gallionella* oxidize soluble reduced iron (Fe^{2+}) in the pipe and water to the insoluble oxidized form (Fe^{3+}), which precipitates (AWWA, 1995). Microbes involved in the oxidation of iron and steel surfaces can deposit oxides of iron and manganese in raised hard outgrowths from the pipe known as tubercles (Walch, 1992). Sulfur-oxidizing microbes such as *Thiobacillus* generate sulfate and hydrogen ions, which lowers the pH, often resulting in a highly acidic environment that pit and gouge metal. More important, sulfur-reducing microbes can generate hydrogen sulfide gas, which has a rotten egg odor, and which can accelerate corrosion (AWWA, 1995). Nitrifiers may decrease the pH by oxidizing ammonium to nitrate and other nitrogen compounds, and thus corrode copper and other pipe material (Schock, 1999). Other bacteria produce polymers that may complex with pipe material or change the redox potential of the pipe surface, accelerating corrosion (Schock, 1999).

Higher corrosion rates are associated with both high-flow and low-flow areas; warmer, poorly buffered water; presence of high levels of iron, sulfur, and chlorides; and a well-developed biofilm. Factors affecting corrosion are reviewed in detail by Schock (1999). Microbially-induced corrosion may penetrate 5/8-inch steel within six months (Costerton and Lappin-Scott, 1989).

B. Loss of indicator organism utility

An extensive, well-developed pipe biofilm may compromise the effectiveness of total coliforms as an indicator of drinking water quality in two major ways. Firstly, a high level of heterotrophic bacteria in the pipe biofilm and sediments may interfere with the analysis of total coliforms. This may occur when high levels of heterotrophic bacteria detach from the biofilm and enter the water flow. As a result, water samples collected for the analysis of total coliforms may contain a large number of heterotrophic bacteria that, by competitive inhibition for nutrients and production of various toxins, may prevent the growth, and thereby detection, of coliforms with at least some normally used analytical media. This phenomenon has been examined by Geldreich et al. (1978) and Seidler et al. (1981), among others, and reviewed in EPA's Drinking Water Criteria Document for Heterotrophic Bacteria (USEPA, 1984). The problem is partially obviated by using the presence or absence of coliforms in a sample, rather than a density measurement.

Secondly, the conditions that facilitate microbial growth on pipes can result in the growth of coliforms as part of the biofilm. Instances of coliform proliferation in pipe biofilms are well-documented in the literature, as reviewed by LeChevallier (1990) and Geldreich (1996). Biofilm coliforms may detach into the flowing water and result in coliform-positive samples. For some systems this phenomenon reduces the usefulness of the coliform test for detecting problems in water treatment or distribution system integrity. However, a coliform-positive test resulting from coliform growth in the biofilm may represent a distribution system deficiency because the conditions that permit its proliferation in the biofilm may also permit the growth of many other microbes as well, including opportunistic pathogens. In addition, an extensive coliform biofilm could reflect a high degree of pipe corrosion and deterioration, as well as water system operational problems.

C. Taste, color and odor problems

Aesthetic concerns such as discoloration of the water and taste and odor problems may result from a number of processes, some of which are microbially-mediated. Microbes most often linked to aesthetic problems in drinking water are the actinomycetes, iron and sulfur bacteria, and algae, especially the blue-green algae (Cohn et al., 1999; AWWA, 1995; Burlingame and Anselme, 1995). Many algal species and some actinomycetes produce geosmin and 2-methylisoborneol, both of which produce earthy-musty odors. Some pseudomonads can also produce foul-smelling sulfur compounds. The bacteria in the genus *Hyphomicrobium*, when sloughed off a biofilm, can cause episodes of black water (van der Wende and Characklis, 1990).

In many cases, the microbially-produced metabolites that produce objectionable aesthetic effects enter the distribution system and accumulate in static water areas or stratified storage tanks. This is especially true for the algal metabolites. However, the microbes indicated above, with the possible exception of the algae, are common in the pipe biofilm (Geldreich, 1996).

The percentage of complaints to water suppliers associated with aesthetic concerns is often high and may change with the season. According to a national survey, 60% of responding utilities reported taste and odor to be their most common water quality problems, with red water ranking second with 47.7% (O'Conner and Banerji, 1984).

D. Others

Biofilms react with chemical disinfectants, thereby reducing the level available for inactivating pathogens in the water (Berger et al., 2000). An extensive biofilm may reduce the disinfectant levels to a point that may increase the public health concerns. If a system counters this problem by raising the initial disinfectant dose, the level of disinfectant byproducts generated by this process may become notable.

VI. Routes Through Which Pathogens Can Enter the Distribution System

Pathogens can enter the distribution system via a variety of pathways and become entrained in the biofilm for later release. The pathways discussed in this section are:

- Treatment breakthrough
- Leaking pipes, valves, joints, and seals
- Cross-connections and backflow
- Finished water storage vessels
- Improper treatment of materials, equipment, or personnel before entry
- Inadequate distribution system security

A steady inflow of bacteria, fungi, protozoa, algae, nematodes, and other microorganisms enter the distribution system (Sibille et al., 1998). In general, pathogens may enter the distribution system either through the source water, or at any point within the distribution system (Ratnayake and Jayatilake, 1999). Treated water has ample recontamination possibilities based on the construction characteristics, operation, and maintenance of the water distribution system (Berger et al., 1993). Additional pathways include contamination through uncovered storage facilities, penetrations in covered storage facilities, water main installation and repair sites, cross-connections or during transitory contamination events, i.e. low pressure events leading to intrusion or backflow (Kirmeyer et al., 2001). The severity of the potential health consequences that may result from contamination in the distribution system depends, in part, on the route of entry. An expert panel recently ranked various routes of pathogen entry into distribution systems by the potential health consequences, which took into account the severity of disease, probability of waterborne disease outbreak, volume contaminated and frequency of intrusion (Kirmeyer et al., 2001). These are presented in Table 8. While potential sources of contaminant entry are known, the route of entry of microbes present in distribution systems is still poorly understood (Gauthier et al., 1999).

Table 8: Some Pathways Through Which Pathogens Can Enter the Distribution System

Risk Level	Pathway
High	Treatment breakthrough, intrusion, cross-connections, main repair/break.
Medium	Uncovered water storage facilities.
Low	New main installation, covered water storage facilities, growth and resuspension, purposeful contamination.

Generated based on information from expert panel ranking in Kirmeyer et al., 2001.

A. Entry through the source water (e.g., treatment breakthrough)

It has been shown that the majority of organisms that colonize the pipe materials in distribution systems can be found in the system's source water (Camper, 1996). Some organisms will break through the treatment barriers (Schaule and Fleming, 1997), particularly following rainfall events (USEPA, 1992b). The likelihood of filtration breakthrough depends on several factors, including the condition of the filter media. Filter breakthrough may also lead to coliform episodes (Characklis, 1988).

The principal cause of growth is the failure of primary disinfection and loss of disinfectant residual (Trussell, 1999). *Klebsiella pneumoniae* (a coliform, a few strains of which are opportunistic

pathogens) is protected from disinfectants by several means, including their attachment to carbon fines used to control odor and taste (Morin et al., 1996). Ineffective treatment may also allow fungi and planktonic diatoms to enter the distribution system (Doggett, 2000). Breakthrough due to inadequate treatment may have been responsible for elevated coliform counts in a distribution system in Springfield, Illinois (Hudson et al., 1983).

B. Entry through broken or leaking pipes, valves, joints and seals

Broken or leaking pipes, or leaking valves, joints or seals provide a pathway for potential entry of microbes which can then become entrained in the biofilm. Even in systems using good sanitary practices, main breaks may result in contaminant entry (LeChevallier, 1999b). Aging infrastructure contributes to main leaks and breaks, and may be a significant cause of drinking water contamination. It is estimated that 42-69% of drinking water pipes in the United States are greater than 20 years old, the percentage being dependent on the size of the system (Haas, 1999). Some systems are still using distribution system piping that is up to 140 years old (Haas, 1999). Based on data from a recent survey by AWWA on the distribution systems of 20 cities, 75% of transmission and distribution piping were 20 years of age or more (AWWA, 1999). The main break frequency per system varies by size, ranging from 1.33 breaks/year for systems serving fewer than 500 people to 488 breaks/year for systems serving more than 500,000 people (Haas, 1999). A recent survey of 300 public water systems showed 58 systems that responded to the survey had an average of 2,146 main breaks annually (37 breaks per system) that resulted in reduced distribution system pressures (ABPA, 2000).

Temperature effects can also cause thermal contraction and expansion can lead to main breaks, and therefore, microbial contaminant entry. In northern states a seasonal variation in main breaks is observed (O'Day et al., 1986). Breaks in the pipe was a contributing factor in the Cabool, Missouri outbreak of 1989-1990. This outbreak, which occurred during unusually cold weather, was caused by contamination that entered the distribution system through two major pipe breaks and 45 service meter failures (Swerdlow et al., 1992).

Intrusion may result from water pressure fluctuations in pipes. Transient negative pressure can draw leaked water back into the pipe at any point where water is leaking out of the system (LeChevallier et al., 1999). Even in well-operated systems leakage may represent 10-20 percent of the water produced (LeChevallier, 1999b). Once these leaks or breaks occur, any microbial contamination in the vicinity of the break or leak can potentially enter the distribution system given the pressure changes that occur during breaks or leaks. One major fecal source are nearby sewer lines, which are notorious for leaking (LeChevallier et al., 1999). This may have been a contributing factor in an outbreak of *E. coli* O157:H7, in Cabool, Missouri (Swerdlow, et al., 1992), which caused 243 cases of illness and four deaths. An August, 2000 cryptosporidiosis outbreak in Northern Ireland resulting from ingress of sewage from a septic tank into the drinking water distribution system caused at least 117 cases of illness (Glaberman et al., 2002). Kirmeyer, et al., (2001) found that 42.8% of 32 water samples immediately next to water mains from six states were fecal coliform positive, while 12.5% were positive for culturable enteric viruses using a cell culture assay. Main breaks can also introduce high concentrations of injured coliform bacteria (undetectable by standard coliform techniques) into the distribution system (LeChevallier, 1999b). In addition to contamination in the vicinity of mains

and main breaks, pressure transients may also be common (LeChevallier et al., 1999). Some causes of pressure transients are pump startup and shutdown, flushing operations, opening and closing fire hydrants, sudden change in demand, feed tank draining, power failure, main breaks, altitude valve closure, malfunctioning air-release, vacuum and pressure valves, air-valve slam, surge tank draining, and resonance (LeChevallier, 1999b). In addition to these pressure transient causes, observed causes of pressure reductions include fire flow, elevation changes, service line breaks and main installation (ABPA, 2000). Additional information related to intrusion is being addressed in a separate paper entitled "The Potential for Health Risks from Intrusion of Contaminants into the Distribution System from Pressure Transients".

C. Entry through cross-connections and backflow

Cross-connections have a significant potential to introduce microbial contamination to the distribution system when the cross-connections are not protected by properly operating backflow preventers, and when a pressure change is experienced by the distribution system, particularly when the pressure drops to subatmospheric. Microbes introduced to the distribution system as a result of cross-connections and backflow can become part of the biofilm matrix, and may be released at a later time. Entry of contamination through cross-connections is a major contributor to waterborne disease outbreaks (Geldreich, 1996). Of 57 waterborne disease outbreaks related to backflow events identified in CDC outbreak data from 1971-1994, 20 were associated with microbial contamination (USEPA, 1999). It has been estimated that, at most, 10% of cross-connection incident reports nationwide are submitted to the University of Southern California's Foundation for Cross-Connection Control and Hydraulic Research (USEPA, 1995) in part due to systems' concerns about potential liabilities arising from distribution system contamination. It is likely many more go unrecognized given the transient nature of many pressure fluctuations, understaffing of local cross-connection personnel, and the lack of recognition of actual cross-connections due to their transient nature.

An M-DBP Federal Advisory Committee concluded that cross-connections and backflow pose a significant health risk (US EPA, 2000). Although some feel the probable occurrence of cross-connections is low in systems with a vigilant cross-connection control program (LeChevallier, 1999b), rarely do all service connections in a system have backflow prevention devices (LeChevallier, 1999b). Drinking water contamination from backflow events may have caused more waterborne disease outbreaks in the US than any other cause (Kirmeyer et al., 2001). Worldwide, the most common sources of contamination result from inadequate pressure and backsiphonage (Geldreich and LeChevallier, 1999). An expert panel convened at a recent workshop regarded cross-connections (Table 8) among the entry pathways of highest risk (Kirmeyer et al., 2001). More details on cross-connections and backflow are being addressed in a separate cross-connection control and backflow prevention paper.

D. Entry through contamination of finished water storage vessels

Both covered and uncovered finished water reservoirs provide opportunities for microbial contamination of the distribution system, and the subsequent inclusion in distribution system biofilms. Contaminated stored water can enter water distribution pipes when the water is drawn from the

vessels for distribution. Contamination introduced through earlier points in the distribution system may be amplified during storage (e.g., biofilm growth). Storage vessels may accumulate sediment, enhancing the ability of microbes to thrive during storage.

Microbial contaminants can enter open storage reservoirs by natural phenomena, animals or humans. Birds and other animals can introduce microbial contaminants through their feces, or through general contact with the finished water. Some open finished water reservoirs may also be subject to surface runoff which may be contaminated. The Interim Enhanced Surface Water Treatment Rule (IESWTR) requires that all newly constructed finished water reservoirs, holding tanks and other facilities constructed for surface water systems or ground water systems under the direct influence of surface water serving 10,000 or more people, be covered (Federal Register, December 16, 1998). The Long Term 1 Enhanced Surface Water Treatment Rule (LT1) extended this requirement to surface water systems or ground water systems under the direct influence of surface water serving fewer than 10,000 people (Federal Register, April 10, 2000).

Inadequately secured covered finished water storage vessels may allow microbial contamination to enter the distribution system. When air is drawn through air vents to replace water leaving the vessel, contamination in the air can enter (USEPA, 1992b). Humans and animals can enter inadequately protected covered finished water vessels and introduce contamination. Underground basins are susceptible to bird, animal and human contamination (USEPA, 1992b), while ground level and elevated finished water storage tanks can also become contaminated by humans and birds. A *S. typhimurium* outbreak in Gideon, Missouri, which caused over 400 cases of illness and seven deaths, was likely caused by bird feces contaminating an elevated storage tank (Clark et al., 1996). More information on contamination of storage vessels is addressed in a separate paper on covered storage.

E. Entry through Improper Treatment of Materials, Equipment or Personnel in Contact with Finished Water

Materials, equipment and personnel introduced to the distribution system also provide pathways for microbial contaminants to enter biofilms. The materials can include filter materials, piping, sealing vials and others (Schaule and Fleming, 1997). Personnel in contact with the water can provide a pathway for contaminant introduction (Schaule and Fleming, 1997) by introducing contaminants during maintenance or repairs of the distribution system or storage vessels. Equipment placed inside water distribution systems, such as tank cleaning equipment or video equipment used to inspect pipelines, can introduce contaminants if not decontaminated prior to use.

F. Entry through inadequate distribution system security

Lack of proper security may result in microbe entry, followed by incorporation of the microbial contaminants into the distribution system biofilm. This may result from intentional security breaches, such as vandalism or terrorism. Also, unintentional contamination can result from unauthorized users tapping into the distribution system and swimmers using storage vessels or reservoirs. Distribution systems can have many miles of pipe, and many storage tanks and interconnections. Because of this, systems can be susceptible to tampering, allowing contamination

(LeChevallier, 1999b).

VII. Factors that Influence Pathogen Survival and Growth in the Distribution System

A variety of physical, chemical, and biological factors affect pipe biofilm development. For a particular system, the interplay among these factors is complex and variable, often making predictions precarious. A number of investigations have shed light on these factors and interactions, and several comprehensive review articles have been published (Geldreich, 1996; Geldreich and LeChevallier, 1999; LeChevallier 1989a;1990a,b). Most of the data on the factors that influence biofilm development are based upon changes in the total viable counts (e.g., heterotrophic plate count) or on changes in the growth of specific coliforms. This document addresses the following factors, below:

- Environmental factors
- Presence of nutrients
- Microbial interactions
- Distribution system materials
- System hydraulics
- Presence of distribution system residual
- Sediment accumulation

A. Environmental Factors

Water temperature affects the microbial growth rate, disinfection efficiency, pipe corrosion rates, and other phenomena associated with biofilm development (LeChevallier, 1989a), as well as opportunity for microbes to enter the distribution system. Where nutrients are adequate, microbes generally grow more rapidly at warmer water temperatures than at colder temperatures (Donlan and Pipes, 1988; LeChevallier et al., 1996). Thus, warmer temperatures likely facilitate the growth of opportunistic pathogens in the biofilm. Predictions are somewhat complicated by the fact that disinfectants are less efficient at lower temperatures in inactivating microbes.

Generally, fecal pathogens can survive longer in colder waters because metabolic processes slow (Atlas and Bartha, 1993). Some may also survive longer in very warm waters with a high organic load. In tropical and subtropical regions, the higher temperatures and organic loading of water is more similar to that of the gut of humans and other warm-blooded animals. In these waters, *E. coli* can survive and even grow (Solo-Gabriele et al., 2000; Jimenez et al., 1989). The density of *S. typhimurium* declined by 90% in 28.8 hours in a temperate source water versus 131 hours in a tropical source water (Hazen and Toranzos, 1990). In contrast, the survival of enteroviruses, *Cryptosporidium,* and *Giardia* decreases with increasing water temperatures (O'Brien and Newman, 1977; Fayer et al., 1998; DeRegnier et al., 1989).

Environmental factors other than temperature that affect biofilm development include finished water turbidity and water pH. Water pH affects the effectiveness of disinfection residuals, and a low pH water is aggressive (Geldreich, 1996). The water turbidity level may interfere with disinfection and turbidity particles can protect pathogens adsorbed to them from disinfection

(Geldreich, 1996).

The release of coliforms from biofilms may be caused by seasonal changes in the water pH. In Wilmette, Illinois, apparently as the result of the near-shore turnover of bottom water, the water pH increased from 7.7 during the summer to 8.2 in December, and then quickly decreased to 7.4 in January (Geldreich, 1996). This resulted in repeated coliform problems. This problem was resolved by adjusting the water to pH 8.3 and slowly adding sufficient lime to develop a more stable coating on the pipe walls (Geldreich, 1996).

B. The Presence of Nutrients

For growth and energy, heterotrophic microbes need a supply of biodegradable organic material (known as assimilable organic carbon, AOC) and sufficient phosphorus, ammonium, and other essential nutrients either from the biofilm or the water (LeChevallier, 1989a; 1990a). These nutrients tend to concentrate at the solid-liquid interface, creating a favorable environment for biofilm growth (LeChevallier, 1989a). The bacterial exopolysaccharide matrix that is part of a mature biofilm can trap and concentrate nutrients (Costerton and Lappin-Scott, 1989). Higher nutrient levels may also facilitate the recovery of disinfectant-stressed microbes (Watters and McFeters, 1990).

Frequently, the AOC level controls the rate and extent of biofilm development. Based on a study of Dutch water systems, many of which do not use chemical disinfectants, maintaining the AOC level below 10 μg C/L was sufficient to limit increases in the density of heterotrophic bacteria, as measured by the HPC (van der Kooij et al., 1999). Many Dutch water systems prefer the use of AOC control over disinfectant residuals to avoid disinfection byproducts, to reduce taste and odor complaints and to reduce costs (van der Kooij et al., 1999). In another study of systems in the U.S., an AOC level less than 50 μg/L was needed to control coliform growth in the distribution system (LeChevallier et al., 1991). In contrast, Gibbs et al. (1993) found no correlation between AOC levels and spatial and seasonal variations in plate counts within a distribution system (conventional treatment with post-chloramination). Rainfall events may increase organic levels in the source water, and thereby increase biofilm growth (LeChevallier et al., 1989a).

Phosphorus and ammonium are sometimes limiting with regard to microbial growth in the distribution system (LeChevallier, 1989a; 1990b). Some forms of phosphorus do not support microbial growth. For example, phosphate-based corrosion inhibitors were found to have an insignificant influence on the growth of some coliform bacteria (Rosenzweig, 1987), while zinc orthophosphate inhibited some coliforms (USEPA, 1992b). Studies investigating the ability of some nitrogenous compounds (nitrate, nitrite, ammonia, organic nitrogen) to stimulate microbial growth have met with mixed results. None of the nitrogen compounds examined by Donlan and Pipes (1988) affected the attached microbial population density, suggesting that nitrogen was not a growth-limiting factor under the experimental conditions. Bacterial survival and growth is frequently supported by ammonia concentrations in ground water supplies according to Rittman and Snoeyink (1984). When systems use chloramines for disinfection, the ammonium added can also be a source of nitrogen, which may support bacterial growth and biofilm formation. The phenomenon is addressed in more detail in a separate paper on nitrification. Other potential growth-limiting nutrients may be highly site-

specific. Iron can stimulate coliform growth (Victoreen, 1980).

The presence or absence of specific nutrients can also select for the microbial population present, including pathogens. However, some pathogens, such as the opportunistic pathogen, *P. aeruginosa*, are especially versatile in the types of organic nutrients they can use. The proportion of multiple antibiotic-resistant bacteria in drinking water was found to increase due to increased copper levels from corrosion (Armstrong, 1981).

C. Microbial Interactions

A microbe faces stiff competition from other microbes for the limited supply of nutrients. Moreover, some aquatic bacteria may produce substances that inhibit other organisms (Waksman, 1941), at the same time they produce extrapolysaccharide and other material that support the growth of these organisms. Protozoan grazing is a major factor in the decrease of enteric bacteria in natural water (Gonzalez et al., 1992; Chao et al., 1988), and possibly in the distribution system as well. However, some opportunistic pathogens thrive in the distribution system environment (Geldreich, 1996).

Some opportunistic pathogens such as *L. pneumophila*, *M. avium*, and primary pathogens such as *V. cholerae*, and *E. coli* O157:H7 survive and even grow within certain common amoeba (Barker and Brown, 1994; Barker et al., 1999; Wadowsky et al., 1991; Cirillo et al., 1997; Thom et al., 1992), and may be protected from disinfection. Some of the biofilm organisms may even supply an essential nutrient to facilitate the growth of an opportunistic pathogen. In one study, *Legionella* only grew near colonies of the bacterium *Flavobacterium breve* on an L-cysteine-deficient medium (Wadowsky and Yee, 1983).

D. Distribution System Materials

Some types of pipe and appurtenance materials are especially prone to biofilm development. The materials may include the pipes, valves, joints, fittings or joint-packing material. The Netherlands tests materials for biofilm growth potential, and the utilities use approved construction materials and appendages (van der Kooij et al., 1999). Pipe material may be more influential than the level of organic matter in the system (Volk and LeChevallier, 1999). Some materials provide the microbes a protective niche where growth can occur, while some provide nutrients to support microbial growth. The bacterial levels on disinfected iron pipes generally exceed those on disinfected PVC pipes (Norton and LeChevallier, 2000). Biofilms also develop more rapidly on iron pipes, even with corrosion control (Haas et al., 1983; Camper, 1996). In addition, iron pipes support a more diverse microflora compared to PVC pipes (LeChevallier, 1999b). Iron pipes facilitate the development of tubercles, which are primarily iron oxides (Tuovinen et al., 1980), and these tubercles can adsorb organic material (Geldreich, 1996; Geldreich and LeChevallier, 1999). In this manner, the level of corrosion and tuberculation (i.e., buildup of corrosion pitting products) affect biofilm development. Sloughing of biofilms into the water column can also occur as a result of elevated biofilm levels on iron pipes (Norton and LeChevallier, 2000). Biofilm problems also tend to increase when systems have iron pipe that is predominantly 50 years old or more (Geldreich, 1996). The position (e.g., top versus bottom)

of the biofilm on the internal circumference of cast iron pipes can influence the extent of biofilm growth (Holden et al., 1995). In an additional study, Rogers et. al., 1994, found that elastomeric materials supported more abundant biofilms and higher levels of *L. pneumophila* than did glass or steel.

The relative efficiency of disinfection varies with pipe material. With iron pipes, even low corrosion levels can interfere with chlorine disinfection, but not with monochloramine (LeChevallier et al., 1993). A free chlorine residual of 3 mg/L may not be effective in controlling biofilms on iron pipes (LeChevallier, 1990). In contrast, low chlorine or chloramine levels (1 mg/L) can control the development of biofilms on galvanized, copper, or PVC pipes (Lund and Ormerod, 1995).

Some materials used for pipe gasket seals and other pipe appurtenances that come into contact with water are susceptible to biofilms, and some have been found to support the growth of *Legionella* (Rogers et al., 1994), including rubber gaskets and washers (Colbourne et al., 1984). Materials that support microbial growth include rubber, silicon, PVC, polyethylene and bituminous coatings (Schoenen and Scholer, 1985; Frensch et al., 1987; Schoenen and Wehse, 1988). Besides pipe appurtenances, material added to the infrastructure, such as lining materials which may contain additives, solvents, or monomers, can support microbial growth (Rigal and Danjou, 1999).

When corrosion is severe additional problems may result. Corrosion of pipes can lead to pipeline breaks and leaks in pipelines, valves, joints and seals. Corrosion can occur internal or external to the pipe, with each being influenced by a variety of factors, including the water chemistry, presence of iron and sulfur-oxidizing bacteria for internal corrosion, and the soil corrosivity, water table, and electrical grounding for external corrosion. Corrosion will be discussed in more detail in a separate paper.

E. System Hydraulics

The hydraulic characteristics of the distribution system is one of several factors that may be more influential than the levels of organic matter in regulating the biofilm's biological activity (Volk and LeChevallier, 1999). A variety of hydraulic conditions, such as long residence times due to low flow rates or dead ends, high flow rates, or fluctuating flow rates can influence the survival and growth of microbes in biofilms. A simple relationship between the hydraulic effects and microbial growth in biofilms does not exist.

Among the many factors that can influence flow rates are pipe layout, condition and size, demand, pump operation, and elevation. High water velocities may increase the level of nutrients and disinfectants in contact with the biofilm, and cause greater shearing of biofilms that may contain pathogens from the pipe surface. The reversal of flow caused by backflow can also shear biofilms (USEPA, 1992b), which may result in the release of microbes entrained in the biofilm and lead to their accumulation in low flow areas such as dead ends. Biofilm debris can accumulate in the periphery of distribution systems, leading to sediment accumulation and microbial proliferation (van der Kooij, 2000).

Interrupted or pounding water flows (i.e., water hammer), or reversal of water flows, may

dislodge tubercles and shear biofilms (LeChevallier, 1990) that accumulate in these low flow areas, resulting in release of elevated levels of the contaminants to the water column. Opheim et al., (1988) found, the starting and stopping of flow increased bacterial levels in an experimental pipe ten fold. Low velocities may result in stagnant water and a loss of disinfectant residual, which facilitates microbial growth. Some factors contributing to low flow include mains not arranged in a looped fashion, storage used only for high demand, oversized pipelines and lack of valve exercise. Complex structures in the pipe network may also reduce flows and thereby support biofilm development (Geldreich and LeChevallier, 1999). In addition, the distribution system design may influence the formation of biofilms in finished water storage, as the design and operation of tanks, and the presence of oversized lines, dead ends, and closed valves can lead to growth and a lack of a residual due to long residence times (Crozes and Cushing, 2000). According to one study, the level of colonization in a cast iron coupon biofilm was negatively correlated with water velocity (Donlan and Pipes, 1988). However, this relationship may vary with the system and type of pipe, due to the complex interplay between water velocity, the rate of nutrient and disinfectant transport to the biofilm, the concentration of nutrients and biofilm detachment rate (NRC, 1982).

F. Disinfectant Types and Residuals

Organisms attached to biofilms are more resistant to disinfection than are planktonic cells, i.e. those in the water (Berger et al., 1993; LeChevallier 1990b; Crozes and Cushing, 2000). The rates of disinfectant diffusion, reaction and sorption to the biofilm, and the disinfectant demand are important factors in the control of biofilms using disinfectants. In addition, microbes that have sloughed off the biofilm are often aggregated and surrounded in biofilm glycocalyx, which makes inactivation by disinfectants more difficult (Carlson et al., 1975).

Biofilm accumulation, the extent of biofilm development, and the microbial population can be influenced by the chlorine concentration (Characklis, 1988; Holden et al., 1995). With chlorine, a gradient exists between biofilm level and chlorine residual, with the effect that biofilms are more highly developed downstream, where chlorine concentrations are lower (Characklis, 1988). Excessive biofilm growth can result from the loss of the disinfectant residual (Crozes and Cushing, 2000).

Maintenance of a free chlorine residual does not necessarily control biofilms (Wierenga, 1985; LeChevallier, 1990b). Once a biofilm is established, it may take a high level of chlorine residual (much greater than 0.2 mg/L) to reduce microbial levels significantly (LeChevallier, 1989b). Maintenance of high chlorine levels may be complicated by a need to control disinfection byproducts and pipe corrosion, and to minimize taste and odor problems. Chloramine, being less reactive, tends to penetrate into the biofilm to a greater extent than chlorine (Jacangelo et al., 1987; LeChevallier 1990b;de Beer et al., 1994), although the relative efficiencies of chlorine and chloramines for controlling biofilms are not consistently clear-cut (Stewart et al., 1984). Chloramine is more effective than chlorine for controlling *Legionella* in hospital plumbing systems (Kool et al., 1999). Resistance to chlorine increases with biofilm age and low nutrient levels (LeChevallier et al., 1988). In contrast, resistance to monochloramine is not affected by the nutrient level (LeChevallier et al., 1988).

Treatment with ozone before water enters the distribution system increases the level of easily

degradable organic compounds in the water, and is associated with greater densities and variety of biofilm organisms (Lund and Ormerod, 1995).

G. Sediment Accumulation

Significant microbial activity may occur in accumulated sediment (USEPA, 1992b). Organic and inorganic sediments can also accumulate in low-flow areas of the distribution system, and enhance microbial activity by providing protection and nutrients (USEPA, 1992b). Biofilms that slough can accumulate in the periphery of distribution systems leading to sediment accumulation and the proliferation of some microorganisms (van der Kooij, 2000). Sediments may be an important source of nutrients in open finished water reservoirs, by accumulating slowly biodegrading materials which are then broken down and released into the water column (LeChevallier, 1999b). The opportunities for biofilm development may be more abundant in storage tanks than in distribution system piping. Frequently, water is drawn from storage tanks only when water demand is high, such as during drought, fire flow, and flushing operations. This intermittent use results in prolonged storage times that may lead to increased sediment accumulation and lack of a disinfectant residual in the finished water storage vessel. Biological and aesthetic effects can be observed following the release of accumulated sediments from low flow areas of the distribution system (Geldreich, 1990).

Many studies have identified microbes in accumulated sediments, including both pathogens and non-pathogens. These include bacteria, viruses, protozoa, algae, fungi and invertebrates. Opportunistic pathogens that have been detected, and can multiply in sediments, include *Legionella* and mycobacteria (van der Kooij, 2000). Some primary pathogens can also survive for some time in sediments. Hepatitis A virus survived more than four months in sediments at both 5˚C and 25˚C (Sobsey et al., 1986). Other opportunistic pathogens found in sediments include *Pseudomonas fluorescens* and *Flavobacterium* spp. (Berger et al., 1993). Sediments can also release nutrients into the water which stimulate biofilm growth downstream (LeChevallier, 1999b).

VIII. Suitable Measures for Controlling Biofilm Development

Biofilm control is one of the important objectives for ensuring that water delivered to the consumer is of high quality. Many different methods have been used to control biofilms, and some of the most important are included in Table 9. Biofilm control often requires the use of a variety of tools, rather than a single "best" tool, and the relative effectiveness of a control practice may be site-specific. Most biofilm organisms, including opportunistic bacterial pathogens, are indigenous to the aquatic environment. This is not the case with primary fecal pathogens. This section will discuss the following measures for controlling biofilms:

- Nutrient control
- Control of contamination from materials and equipment
- Control and mitigation of system hydraulic problems
- Cross-connection control and backflow prevention
- Disinfectant residuals
- Corrosion control

- Infrastructure replacement and repair
- Security
- Storage vessel management and alteration

The presence of biofilms and the factors that contribute to biofilm development is often determined through sanitary surveys. A sanitary survey that includes the distribution system can often reveal the existence of a biofilm problem and the factors that promote it (Kirmeyer et al., 2001). This can be the first step in identifying the cause of biofilm problems so systems can implement the appropriate measures to control or correct the biofilm problems. It may be necessary to apply specific testing techniques to determine the extent of growth, the water's potential to support or promote growth, and the most effective means for controlling the growth (Crozes and Cushing, 2000). Distribution system sanitary surveys, along with cross-connection control programs and the maintenance of sanitary conditions during main repair, are three areas that must be improved to prevent pathogen intrusion (Kirmeyer et al., 2001). Utilities should focus their attention on their ability to maintain distribution system barriers at critical points, including repair and construction sites, valves, cross-connections and storage facilities (Kirmeyer et al., 2001). It has been estimated that pipeline repair or damage, along with cross-connections, are associated with 15% of all cases of giardiasis in the United States (Craun, 1986).

Table 9: Methods to Control or Mitigate Biofilms in Distribution Systems

Control or Mitigation Measure	Author(s)
Main flushing, pigging and cleaning	Costello (1984), Berger et al (1993), USEPA (1992b), Trussell (1999), Van der Kooij et al (1999)
Disinfectant residual	Trussell (1999), Geldreich and LeChevallier (1999)
Main repair and replacement	Costello (1984), USEPA (1992b), Kirmeyer et al., (2001)
Minimization of dead ends/flow management	Costello (1984), Geldreich and LeChevallier (1999)
Corrosion control program	Berger et al (1993), Volk et al (2000), Trussell (1999), Geldreich and LeChevallier (1999)
Proper Storage Tank/Reservoir O&M	USEPA (1992b), Geldreich and LeChevallier (1999)
Control and mitigation of system hydraulic problems	USEPA (1992b), Van der Kooij et al (1999)
Nutrient suppression	Volk et al (2000), Trussell (1999), Van der Kooij et al (1999), Geldreich and LeChevallier (1999)
Cross-connection control	Kirmeyer et al (2001), Van der Kooij et al (1999)

A. Nutrient Control

Nutrient control is recognized as one of the most effective methods for controlling microbial growth and biofilm formation. This can be accomplished by controlling the source of carbon or

other nutrients (e.g., phosphorus, nitrogen), depending on the growth-rate limiting nutrient for the specific system. Control of nutrients for the subsequent control of growth in the distribution system is one of the main reasons that systems apply biological treatment (Norton and LeChevallier, 2000). Some methods for nutrient control include biological treatment, coagulation, membrane filtration, and granular activated carbon (GAC). Alternative source waters have also been suggested (Crozes and Cushing, 2000).

While control of nutrient levels is the most direct method of controlling biofilm growth, it is also the most difficult (USEPA, 1992b). Biofilm control through nutrient reduction may not be immediate. Several months were required for biological filtration to have an observable impact on bacterial water quality, according to one study (Volk and LeChevallier, 1999). Careful disinfectant selection is also necessary. Oxidation with chlorine or ozone may increase the amount of biodegradable organic matter in the finished water (Joret and Prevost, 1992; LeChevallier et al., 1992).

Since organic carbon is the primary carbon and energy source for much of the distribution system microbial activity, the rate and extent of biofilm formation can be minimized by control of the organic carbon concentration (Characklis, 1988). To control the influent organic carbon, treatment for control of AOC is applied at the treatment plant. Control of the AOC concentration has proven so effective at controlling bacterial survival and growth that some systems have discontinued applying secondary disinfection (Schellart, 1986; van der Kooij, 1987).

Biological treatment is one technology for systems to control AOC. Biological treatment uses microbial activity at the point of treatment to reduce the AOC concentration in the water entering the distribution system, thereby reducing the rate and extent of microbial growth and biofilm formation. Preozonation is commonly used, followed by biological filtration with GAC (Morin et al., 1996). Preozonation oxidizes organic matter to a more readily degradable form prior to biological treatment. When using biological treatment it is important to encourage the growth of bacteria in the GAC filter. Several instances have been noted in which biofilms accumulated in systems where bacterial growth in the GAC filter was hindered. In addition, changes in the disinfectant type and dose, and point of disinfectant application can impact AOC levels in finished drinking water (LeChevallier, 1999a).

Activated carbon, as both GAC and the powdered form (PAC), can effectively remove AOC from drinking water prior to distribution. GAC and PAC filter out the AOC by sorption. However, research suggests coliforms and opportunistic pathogens can be associated with GAC particles released from GAC filters (Camper et al., 1986; Stewart et al., 1990). The GAC and PAC processes require careful control, as well as careful monitoring of breakthrough of GAC particles containing organisms. The controls may include preozonation (Morin, et al., 1996).

AOC is mainly low molecular weight non-humic substances that are difficult to remove by coagulation (Volk and LeChevallier, 1999). However, coagulation has been effective at removing dissolved organic carbon (DOC) and biodegradable dissolved organic carbon (BDOC) (Volk and LeChevallier, 1999).

Both nanofiltration (NF) and reverse osmosis (RO) have been suggested for the removal of

nutrients (Crozes and Cushing, 2000). In addition to decreasing the concentration of organic matter from drinking water, NF and RO are also effective at removing microbes (Sibille, 1998).

Control of finished water phosphorus concentrations may also help limit microbe survival and growth and biofilm formation where phosphorus is the limiting nutrient. Often, systems with high organic carbon levels have phosphorus as the growth-limiting nutrient (Miettinen et al., 1997). Phosphorus limitation has been shown to be site-specific within the distribution system (Herson et al., 1984). This emphasizes the importance of understanding site-specific characteristics of the system, as treatment for non-growth-limiting nutrients will not control growth. In general, for most systems, phosphorus is not the growth limiting factor (Donlan and Pipes, 1988). Some common technologies that are used for reducing phosphate levels in wastewater treatment are biological treatment, and precipitation using lime or metal salts such as ferric chloride, or aluminum sulfate (alum).

Because organic carbon is usually the growth-limiting substrate, control of nitrogen levels may be ineffective at controlling microbial growth and biofilm formation. According to LeChevallier et al, (1987), AOC concentrations decreased during travel through a distribution system, whereas nitrogen compound concentrations remained adequate to maintain balanced growth (Camper, 1996). Careful control of the correct ratio of chlorine to ammonia when practicing chloramination can minimize the stimulation of microbes (LeChevallier, 1999b). Systems can remove ammonia from drinking water by biological removal associated with GAC (Dahab and Woodbury, 1998; Snoeyink, 1990). In some systems with poor water quality, a process of superchlorination-dechlorination can be used to oxidize ammonia (Haas, 1990). Ion exchange, biological denitrification, reverse osmosis, electrodialysis and distillation can be used for nitrate removal (Dahab and Woodbury, 1998). Ion exchange and RO are listed as best available technologies for both nitrate and nitrite removal in drinking water systems, while electrodialysis is also listed for nitrate removal. However, most bacteria use nitrogen in a reduced form (e.g., ammonia).

B. Control of Contamination from Materials and Equipment

Control of contamination from materials and equipment can reduce the subsequent contamination of the distribution system. When microbial contamination is present on materials or equipment used in distribution system maintenance, the microbes can become a part of the microbial community of the distribution system (i.e. provides a biological seed to the distribution system). By reducing the biological seed entering the distribution system, biofilm problems can be reduced (Trussell, 1999). Poor operations and maintenance procedures facilitate pathogen entry into the distribution system (Berger et al., 1993). Therefore, good distribution system maintenance techniques are viable alternatives for biofilm control (Camper, 1996). Equipment may have been idle for a long time or may not have been adequately cleaned following previous usage, and may, therefore, harbor microbial contaminants. Disinfection of equipment and a high pressure flush of various tools with tap water could reduce the pathogen seed.

When replacing or repairing infrastructure, systems can take steps to prevent microbial contaminant entry when the system returns to service. Thorough disinfection and flushing is important before placing the system back into service. (To reduce biofilm sloughing, the most

effective type of flushing is unidirectional flushing.) Following a main break systems usually isolate the section of the pipe to be repaired before superchlorinating and flushing the section (LeChevallier, 1999b). Sanitary conditions are difficult to maintain, and quality control procedures are to follow, given that water main repairs often involve working in wet environments (Kirmeyer et al., 2001). System maintenance of sanitary conditions during main repair is one of three areas (along with cross-connection control and sanitary surveys) in which systems need to improve for preventing pathogen intrusion (Kirmeyer et al., 2001).

C. Control and Mitigation of System Hydraulic Problems

System hydraulic control is important in controlling microbial contamination in the distribution system. Among the several measures systems can use to control the hydraulic condition of the pipelines and biofilms is to flush and/or pig (use of a water-propelled device) the pipeline at regular intervals. Flushing and pigging can remove the biofilm, sediments (Crozes and Cushing, 2000), and tuberculation, improving the system hydraulics. Where tuberculation is severe flushing may not suffice, and pigging, or even the use of cable-drawn devices may be necessary (AWWA, 1987). Routine systematic flushing is a primary component of proper distribution system maintenance (USEPA, 1992b). Drinking water systems should also thoroughly flush the distribution system following a contamination event (LeChevallier, 1999a). Flushing, along with valve-turning proved effective in helping a system in Washington, D.C. regain compliance following violations of the Total Coliform Rule (TCR) in1996 (Clark et al., 1999). Flushing was one of two interim measures used to control biofilm containing high coliform levels in a system in Connecticut (CDC, 1985).

Distribution system cleaning practices, such as flushing and pigging will not prevent recolonization (Walker and Morales, 1997). Therefore, flushing and pigging are measures that many systems routinely conduct. Furthermore, flushing and/or pigging the entire distribution system at frequencies necessary to maintain low biofilm densities may not be cost effective. Although repeated flushing and/or pigging are effective for localized contamination (USEPA, 1992b).

The elimination of low-flow areas and dead ends can improve system hydraulics (Camper, 1996), thereby reducing microbe survival and biofilm formation. Dead ends (causing excessive residence times) can be eliminated by valve exercising, and eliminating excess storage, while low flow areas can be eliminated by line resizing (Crozes and Cushing, 2000). Some of the practices that can lead to changes in water velocity are routing flow to fire hydrants, and proper pipe network design and pipe size which can eliminate low flow areas (Smith et al., 1989; Geldreich, 1988). Systems should avoid sudden flow increases (USEPA, 1992b) or hydraulic disturbances (Characklis, 1988). These can cause accumulated biofilm to shear or slough, resulting in release to the water column.

Continual positive pressure throughout the distribution system is recommended (Kirmeyer et al., 2000), and is a best available technology (BAT) in the Total Coliform Rule (USEPA, 1992b). Lack of positive pressure may commonly occur, as circumstances producing transient pressure waves may be common to every water system (LeChevallier et al., 1999), and occur frequently in many water distribution systems. One modeling study of three distribution systems, saw low or negative pressure from loss of pumping power, loss of flow, fire flow or main breaks in 13 to 31% of pressure model

nodes (Kirmeyer et al., 2001). Systems should also avoid extreme changes in pressure gradients (Kroon, 1984), and consider surge control devices as part of the design (Kirmeyer et al., 2001). Pressure monitoring in all parts of the distribution system can ensure that positive pressure is maintained by alerting operators to the need to manage pumps and other components of the distribution system. However, negative pressures may last for only a few seconds, and systems may not detect them using conventional pressure monitoring (LeChevallier, 1999b). Record keeping of the events that contribute to pressure changes may aid systems in minimizing such events.

D. Cross-Connection Control and Backflow Prevention

Backflow prevention devices are an important barrier against entry of contaminated water (LeChevallier, 1999b). Installation of backflow prevention assemblies or devices are also frequently accompanied by the regular inspection of the assemblies and devices, as well as regular testing of the assemblies. Kirmeyer et al., (2001) recommends annual inspection and testing. Where backflow is a problem cross-connection control has been identified by an expert panel as one area of improvement necessary to prevent pathogen intrusion (Kirmeyer et al., 2001). A US General Accounting Office report on the review of 200 sanitary surveys and a nationwide questionnaire of States identified inadequate cross-connection control programs as the most common deficiency (USGAO, 1993). The specific methods for controlling contamination due to backflow are being addressed in a separate paper on cross-connection control and backflow prevention.

E. Disinfectant Residuals

Systems provide disinfectant residuals throughout the distribution system (i.e., secondary disinfection) for protection of the finished water from microbial contamination in the distribution system. Among the reasons for secondary disinfection are the inactivation of coliforms and pathogens entering through cross-connections and line breaks, and the suppression of bacterial growth and biofilm in static water areas (Geldreich, 1996; Trussell, 1999). Secondary disinfection also protects against reinoculation of the flowing water by microbes trapped in the biofilm (Haas, 1999), which can occur through sloughing or erosion of the biofilm. Disinfectant residuals can also reduce the amount of viable organisms available to become adsorbed to the biofilm. Although contamination from cross-connections and backflow may be controlled by a disinfectant residual (Snead et al., 1980), some water supply professionals believe a disinfectant residual is not effective when cross-connections result in massive contamination (LeChevallier, 1999b; Snead et al., 1980).

The residual disinfectant concentration is important in determining biofilm bacterial density and composition (Norton and LeChevallier, 2000). Bacterial growth can be controlled with adequate disinfectant residuals (Morin et al., 1996), and bacterial density will remain low (Berger et al., 1993). However, many factors influence the concentration of the disinfectant residual in the distribution system, and therefore the ability of the residual to control microbial growth and biofilm formation. These factors include the AOC level, the type and concentration of disinfectant, water temperature, pipe material, and system hydraulics. Because of these many factors, preventing pathogen survival and growth requires strict attention to the residual disinfectant throughout the system (Trussell, 1999). Disinfectant residual penetration into biofilms can also be inhibited by corrosion products due to the

reactions between the residuals and the corrosion products (LeChevallier et al., 1993; LeChevallier et al., 1996).

Various studies have obtained different results with respect to the ability of chlorine to control biofilms, depending on the chlorine level used and other factors. At higher levels (1 mg/L), free chlorine or chloramine was effective in disinfecting biofilms on galvanized iron, copper or PVC pipes (LeChevallier et al., 1990b). However, some investigators have found that significant biofilms can develop in the presence of low levels of residual free chlorine, and even at high levels, biofilms are not eliminated (Characklis, 1988). According to Nagy and Olson (1986), bacterial densities in biofilms were unaffected by the presence of free chlorine residuals. Camper (1996) noted that free chlorine residuals were ineffective in controlling coliform occurrences when the AOC concentrations are greater than 100 μg/L. Characklis (1988) noted that biofilm sloughing has not been documented in the presence of chlorine. Some research has shown that most of the free chlorine is depleted before penetrating the biofilm (Haas et al., 1991). In contrast to biofilms, chlorine controls the level of heterotrophic bacteria and viruses in water (Characklis, 1988; Quignon et al., 1997).

Following TCR violations in Washington, D.C., in 1996, an increase in the residual chlorine level, along with a close monitoring of the temperature, was one of three factors that helped the system return to compliance (Clark et al., 1999). Increased chlorination was also used to control high total coliform counts in a system in Connecticut (CDC, 1985) and in Springfield, Illinois (Hudson et al., 1983).

Utilities can also use chloramines for controlling microbial growth and biofilm formation in the distribution system. Chloramines may be preferred over free chlorine when the disinfecting objective is biofilm control (Trussell, 1999), as chloramines may be more effective than free residual chlorine at controlling biofilm formation. In support of this position, LeChevallier (1999a) found that in filtered systems, coliforms were present in 0.97 percent of systems using free chlorine, but only 0.51 percent of systems using chloramines. While chloramines can be used to retard biofilm formation, no disinfectants completely eliminate biofilms. Mycobacteria were frequently detected in eight well-characterized systems in the presence of a chloramine residual (LeChevallier, 1999a). Water treatment plant operators can more effectively control the concentration of the disinfectant in distant reaches of the distribution system due to the increased stability of chloramines over free chlorine (LeChevallier, 1999b). The increased effectiveness of chloramines over free chlorine for biofilm control is more pronounced in systems using iron pipes (LeChevallier et al., 1990b). However, chloramine disinfection was not able to control coliform organisms in a system in Springfield, Illinois, following an increase in coliform organisms, whereas chlorine was (Hudson et al., 1983).

Like free chlorine, the chloramine concentration impacts the ability of chloramines to control biofilm formation. Monochloramine concentrations below 0.5 mg/L in distribution system dead ends, result in more coliform occurrences than when higher monochloramine concentrations are present (LeChevallier et al., 1996). The types of chloramines present also influence their effectiveness, with monochloramines being less effective than dichloramines, but more effective than nitrogen trichloride (trichloramines). Monochloramines are more widely used, however, as dichloramines have a lower odor threshold, are more corrosive, and decrease in predominance at pH values of 7-8. One

study found that neither chlorine residuals nor chloramines residuals alone were able to control biofilm development, however when used in combination biofilms were controlled (Momba and Binda, 2002).

F. Corrosion Control

The ability of corrosion control to inhibit biofilm formation is widespread in the literature (Smith et al., 1990; LeChevallier et al., 1990a; Smith et al., 1990). Corrosion control may be better in controlling biofilm activity than reducing organic matter levels (Volk and LeChevallier, 1999), and may be the most important factor for control of biofilm development (Volk et al., 2000). Corrosion control may include the inhibition of biofilm formation or the prevention of biofilm sloughing by coating the biofilm (Berger et al., 1993). Corrosion is among the fundamental factors leading to the release of biofilms into the water (LeChevallier et al., 1998). The effectiveness of zinc orthophosphate or polyphosphate in reducing biofilm densities has been shown in one study (Abernathy and Camper, 1997), while the South Central Connecticut Regional Water Authority saw a long term decrease in coliform occurrences after increasing the zinc metaphosphate mixture concentration (Smith et al., 1989). However, according to Rosenzweig (1987), the growth of some coliform bacteria are not significantly influenced by the presence of phosphate-based corrosion inhibitors.

Several factors influence the effectiveness of corrosion inhibitors for controlling microbes and biofilm formation. One of the factors includes high concentrations of organic material (Volk et al., 2000). Corrosion control can also impact disinfection effectiveness on biofilms, with an increase in free chlorine disinfection effectiveness being observed when using corrosion control on iron pipes (LeChevallier et al., 1990b; Lowther and Moser, 1984; Martin et al., 1982). However, using corrosion inhibitors can be detrimental at excessive concentrations. A legionellosis outbreak in Lanzarote, Canary Islands in 1993 may have been amplified due to excessive polyphosphate concentrations (Crespi and Ferra, 1997).

G. Infrastructure Replacement and Repair

In some cases where growth is severe, replacement of pipe sections may be necessary (USEPA, 1992b). The temporary fixes (e.g., where flushing may break corrosion-weakened pipes) may be more costly in the long run than the more permanent solutions of pipeline rehabilitation or replacement (USEPA, 1992b). Main reconditioning or replacement is a practice recommended for the control of microbial growth (Costello, 1984), and is listed as a Best Available Technology (BAT) under the Total Coliform Rule (TCR) (USEPA, 1992b). When systems have frequent contamination problems resulting from deficiencies in or due to the nature of the infrastructure, the system may opt to initiate a repair or replacement program. This may enable the system to resolve existing problems without catastrophic failures or the associated costs. Although in many cases problems may be more severe in older pipes, this is not always true. In general, pipe replacement should target sections of the system experiencing the greatest number of leaks (Kirmeyer et al., 2001). However, mains are not the only infrastructure whose failure can lead to contamination. Failure of other appurtenances , such as isolation valves, air valves and surge control devices may lead to contamination (Kirmeyer et al., 2001).

Some materials used in drinking water distribution systems may provide limiting nutrients to biofilm organisms and enhance microbial growth. In some instances, replacement of these materials may be a viable solution. These materials may include the pipes, valves, joints, fittings or joint-packing material. Bacteria can colonize piping, pipe joints, valves, elbows, tees, and other fittings due to the changing water movement and stagnant areas of the distribution system (Berger et al., 1993). Studies have also found that iron pipes require higher monochloramine concentrations than other pipe materials, and that monochloramines were more effective than free chlorine in eliminating surface-associated bacteria on iron pipe (LeChevallier et al., 1990b).

H. Adequate Security

The issue of adequate water system security has recently become highly visible, and marked attention has been directed to this theme. This paper will not address intentional sabotage of the distribution system, except to state that adequate security includes fencing, alarms, and regular surveillance. Unintentional breeches should also be minimized.

I. Proper Storage Vessel Management and Alteration

Proper storage vessel management and alteration, when necessary, can prevent contamination of the distribution system. Following TCR violations in 1996 in Washington D.C., one measure that proved effective in bringing the system back into compliance was the cleaning, inspection and disinfection of storage tanks and reservoirs (Clark, et al., 1999). To reduce pathogen presence and biofilm development, systems should have a scheduled program to rehabilitate all water storage facilities (USEPA, 1997). Proper operation and maintenance of storage tanks and reservoirs is listed as a BAT in the TCR (USEPA, 1992b). Storage tanks and standpipes should be pressure flushed or steam cleaned, then disinfected before returned to service (USEPA, 1992b), preferably with a disinfectant solution. This may not only remove microbial contamination from the vessel's inner surface, but also nutrients that may be present. Proper operation of storage vessels can also reduce excessive residence times, which can lead to microbial survival and growth, and biofilm formation. Properly designed inlets and outlets, and the overall system design can improve problems caused by dead ends (Trussell, 1999). Pathogen contamination due to air introduction can be reduced by installing air filters to guard against pollution entering covered water reservoirs (USEPA, 1992b). Covering finished water reservoirs can protect against contamination from airborne sources, surface runoff, accidental spills and animals, such as insects and birds (USEPA, 1992b). EPA's Uncovered Finished Water Reservoirs Guidance Manual describes recommended contamination control measures related to birds and other animals, human activity, algal growth and insects and fish (USEPA, 1999b). An understanding of the storage hydraulics and operation is important in reducing contamination of the finished water.

Proper turnover of the water in finished water storage facilities eliminates what amounts to dead ends and can reduce the extent to which biofilms develop, minimize nutrient availability and prevent the accumulation of sediments. To accomplish this systems can exercise valves to reduce stagnation, and eliminate excess storage (Crozes and Cushing, 2000).

Systems can exercise additional control over biofilm accumulation and microbial growth in finished water storage vessels by preventing sediment accumulation. This can be accomplished through periodic flushing (Crozes and Cushing, 2000) and cleaning. Pigging of pipes to reduce the biofilm seed that leads to sediment accumulation in storage vessels may help.

IX. Indicators that Signal the Presence of Biofilm Problems

Several indicators have been mentioned as a potential means of signaling the presence of a significant biofilm problem. One such indicator is a high level of heterotrophic bacteria (Geldreich, 1996; Carter et al., 2000). Microbial density in a pipe biofilm has been estimated in several ways. Some investigators removed a small section of pipe, or a pipe coupon, and examined it under epifluorescent or scanning electron microscopy. In other studies, biofilm material was scraped from a pipe with a spatula or brush, and cultured.

In addition, where other problems are not evident (e.g., cross-connections, treatment upsets), a persistent coliform problem may indicate extensive growth problems (Crozes and Cushing, 2000). Several species of coliforms that can grow in pipe biofilms, including *Enterobacter cloacae*, *Klebsiella* spp., *Citrobacter freundii* and *Enterobacter agglomerans* (Geldreich, 1996). Another potential indicator is the loss of disinfectant residual (Snead et al, 1980)

X. Additional Research Needs

As with most areas, further opportunities exist for research to result in greater certainty of the health impacts associated with drinking water distribution systems. To better control pathogen survival and growth in the biofilm and other public health problems associated with the biofilm in the distribution system, the following areas of research are important:

- Link between organisms present in distribution system biofilms and human health impacts

- Effectiveness of potential indicators of extensive biofilm growth, including loss of disinfectant residual, high AOC levels, pipe corrosion, and the presence of red or black water

- The relative effectiveness of preventive and corrective measures

- Potential problems created by cleaning deteriorated pipes

- Level of public health protection provided by adding disinfectant residuals to the distribution system

Some specific research opportunities related to drinking water distribution systems are outlined in two reports being prepared for EPA as part of Comprehensive Drinking Water Research Strategy and the Microbial/Disinfection Byproducts (M/DBP) Research Council.

XI. Summary

JUNE 17, 2002

A wide range of primary and opportunistic pathogens have demonstrated the ability to survive, if not grow, in biofilms. These pathogens are of both fecal and non-fecal origin, and have a multitude of pathways through which they can enter the distribution system. Some of the pathogens identified as growing or potentially surviving in biofilms include *Legionella*, *Mycobacterium avium* complex, *Pseudomonas aeruginosa*, poliovirus 1, coxsackievirus B and several species of fungi. Microbes can enter the distribution system through inadequate treatment, cross-connections, leaking pipes and appurtenances, as well as other means. Once becoming established as part of the biofilm, pathogens can be protected from disinfection. Systems may select from a range of measures which can limit the growth of microbes in the biofilm, prevent microbes from entering the distribution system, and/or minimize the number of microbes present. Some of these measures are most effective if performed regularly. Systems can also choose from a range of methods to detect biofilm problems.

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