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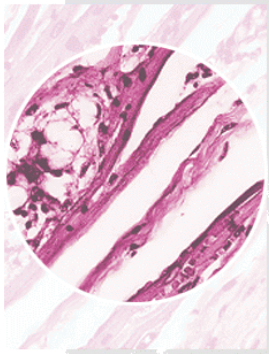
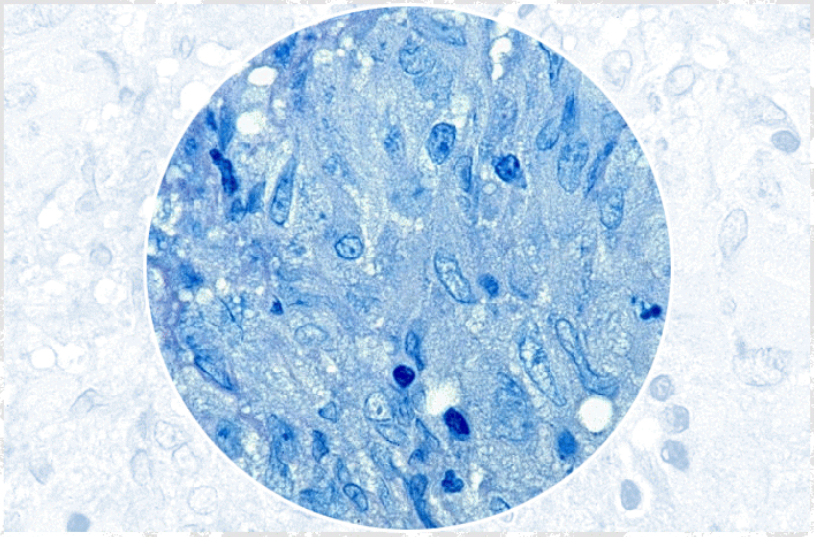
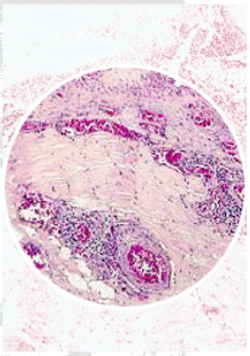
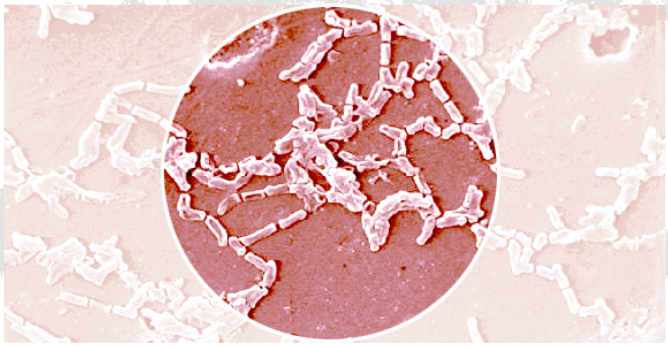
Mycobacteria: Health Advisory









I. INTRODUCTION

The Health Advisory Program, sponsored by the Office of Water (OW), provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with the contamination of drinking water. Most of the Health Advisories (HAs) prepared by the Office of Water are for chemical substances. This Health Advisory however, addresses contamination of drinking water by a microbial pathogen, examines pathogen control, and addresses risk factors for exposure and infection.

Health Advisories serve as informal technical guidance to assist Federal, State and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. The HAs are subject to change as new information becomes available.

This Health Advisory is based on information presented in the Office of Water's Criteria Document (CD) for *Mycobacteria*. Individuals desiring further information should consult the CD. This document will be available from the U.S. Environmental Protection Agency, OW Resource Center, Room M6099; Mail Code: PC-4100, 401 M Street, S.W., Washington, D.C. 20460; the telephone number is (202) 260-7786. The document can also be obtained by calling the Safe Drinking Water Hotline at 1-800-426-4791.

II. GENERAL INFORMATION

History

- Mycobacteria were one of the first types of bacteria recognized to cause disease (tuberculosis and leprosy).

- The name *Mycobacterium*, which means fungus-bacterium, was introduced in 1896. The name does not imply that *Mycobacterium* are fungi; rather it describes the way that the tubercle bacillus grows on the surface of liquid media as mold-like pellicles (Gangadharam & Jenkins, 1998).

- In the 1940s and 1950s, it became apparent that there were many other species of mycobacteria (in addition to those which cause tuberculosis and leprosy) which could contribute to disease, based on their isolation from clinical patients.

Taxonomy

- Mycobacteria belong to the Order Actinomycetales, Family *Mycobacteriaceae* and Genus *Mycobacterium*.

- One of the early techniques used to classify mycobacteria was the system of Adansonian taxonomy (Grange, 1996b). This system was based on the use of cultural and biochemical properties to group related strains. Although this system is rarely used today, this approach facilitated the use of discriminative identification tests for use in diagnostic mycobacteriology.

- Dr. Ernest Runyon performed the initial work and grouping classifications on the taxonomy of *Mycobacterium* in the mid-1950s. In Runyon's classification, mycobacteria, excluding those in the *M. tuberculosis* complex and noncultivable taxa (e.g., *M. leprae*), were divided into four groups based on growth rates and pigmentational properties.

- In 1980, 41 species of mycobacteria were included in the Approved Lists of Bacterial Names (Grange, 1996b). Today, there are 71 recognized or proposed species of mycobacteria (Shinnick and Good, 1994). Over 20 of these species are known to cause disease in humans.

- Mycobacteria not identified as tuberculosis or leprosy complex, have been addressed by a variety of nomenclature including; 'atypical mycobacteria', 'mycobacteria other than tubercle' bacilli (MOTT), 'environmental mycobacteria' or 'non-tuberculous mycobacteria' (NTM) (Wolinsky, 1979). This document uses the NTM nomenclature.

Morphologic and Taxonomic Characteristics

- Aerobic, asporogenous rods, which tend to be acid-fast at some stage of their growth cycle.

- Have been referred to as the 'ducks of the microbial world' due to their thick, waxy, outer coating which enables them to thrive in aquatic environments.

- Have cell walls with very low permeability, contributing to their resistance to therapeutic agents. The mycobacterial cell wall is highly complex and has a lipid content that approximates 60% of the structure (Brennan and Nikaido, 1995). The cell wall characteristics allow the mycobacterial species to survive in different environments (e.g., in biofilms in water habitats or particulate matter in soils and water) and resist disinfection procedures.

Lifecycle

- Most studies have revealed that mycobacteria exist as non-sporing acid-fast rods and replicate by binary fission (Grange, 1996b). However, some investigators have hypothesized that a more complex life cycle (possibly involving cell-wall-free forms or microspores) exists. Several authors have produced evidence for unusual forms and life cycles of mycobacteria.

- Unusual life cycles may explain the phenomenon of mycobacterial dormancy and persistence (Grange, 1996b). Wayne (1994) reviewed the mechanism of dormancy seen in *M. tuberculosis*. There is ample evidence that these organisms are capable of adapting to prolonged periods of dormancy in tissues, and that this dormancy is responsible for the latency of disease. Wayne (1994) indicates that there may be two or more stages involved in the process leading from active replication to dormancy. One of these steps involves a shift from rapid to slow replication, the second involves complete shutdown of replication, but does not result in death of the cell.

Host Range

- In addition to human, tuberculosis infection also occurs in a wide range of domesticated and wild animals. *M. bovis,* which causes tuberculosis in animals, has one of the widest host ranges of all pathogens. Hosts include the African buffalo, baboon, badger, bison, opossum, cat, elk, Fallow deer, goat, horse, Leche antelope, maral, pig and wild boar, Rock hyrax, and seal (Morris et al., 1994; Grange, 1996b).

- Leprosy is a disease that is primarily transmitted from person to person. Humans were thought to serve as the only reservoir of *M. leprae* until 1974, when scientists discovered nine-banded armadillos in Louisiana with leprosy in an advanced stage. It is now

thought that the nine-banded armadillo is the primary animal reservoir for *M. leprae* and may serve as a vector for transmission of leprosy to humans either by direct or indirect contact (Jenkins, 1991). *M. leprae* has also been found to infect chimpanzees and sooty mangabey monkeys in Africa (Grange, 1996a).

- Several of the NTM species have been isolated from animals, birds and fish. These include: *M. avium* complex, *M. marinum*, *M. ulcerans*, *M. paratuberculosis, M. simiae, M. fortuitum* and *M. smegmatis*. Most of the NTM agents that cause human and animal diseases have demonstrated very little person-to-person contagiousness (Wayne & Sramek, 1992).

III. OCCURRENCE

No evidence was found that water serves as a source of infection for tuberculosis or leprosy. Because the bacterial species that cause these diseases have not been recovered from water sources, the remaining sections of this health advisory will focus only on NTM species.

NTM are ubiquitous in nature and consist of a large number of species which vary in pathogenicity. These infections are more likely transmitted from environmental sources by ingestion, inhalation and inoculation of *Mycobacterium* bacilli. These environmental sources may include aerosols, water, soil, dust, food products and contaminated medical equipment (Gangadharam & Jenkins, 1998).

Because routine environmental monitoring for NTM is not a common practice, the occurrence of these bacteria is often only indicated by outbreaks or sporadic cases of mycobacterial infection. Therefore, this section considers the worldwide occurrence or incidence of mycobacterial infections as well as the occurrence of NTM in water, soil, and air. Environmental factors influencing NTM survival also are discussed.

Occurrence in Water

Environmental mycobacterial species have been repeatedly isolated from natural and municipal waters. They occur in surface water, notably ponds, streams, and estuaries. Piped water supplies are readily colonized by mycobacteria which can thereby lead to more frequent exposure of humans. Mycobacterial characteristics such as surface hydrophobicity and charge, as well as certain physiochemical factors like salinity, temperature, humidity and wind currents can influence the distribution of mycobacteria in water systems (Falkinham, 1996).

Waste Water

- In 1980, a survey conducted in Korea found that both slow- and rapid-growing environmental mycobacteria (EM) were isolated from 67% of the sewage samples

collected. Slow growers constituted 49% of sewage isolates. The isolates from sewage samples included: *M. gordonae*, *M. scrofulaceum*, *M. flavescens*, *M. phlei*, *M. terrae* (, *M. fortuitum*, *M. chelonei* and *M. smegmatis* (Won Jin et al., 1984).

- In Czechoslovakia, environmental mycobacteria have been isolated from waste water in the North-Movarian Region (Kaustova, 1981). Between 1985 and 1991, the reference laboratory in the Czech Republic reported that 8.2% (102) of 1244 waste water samples tested were positive for mycobacterial species (Slosarek et al., 1994).

Surface Waters

- *Mycobacterium-avium-intracellulare-scrofulaceum* (MAIS) organisms were recovered from so-called acid-brown swamps and lake water samples collected in Georgia, Virginia, and West Virginia. The high rate of recovery of MAIS organisms in these regions was attributed to the combination of higher temperatures, low oxygenated waters, low pH soils, higher zinc, and fulvic and humic acids (Kirschner et al., 1992).

- In a study in Finland, MAIS complex organisms were detected in 40% of surface water samples collected from streams. Concentrations of these organisms were found to range from 50 to 1,400 CFU/L (mean 370 CFU/L). Additionally, *M. malmoense* was detected in two stream waters at concentrations of 320 and 750 CFU/L. In all surface water samples (not limited to streams), mycobacteria were detected at a mean of 1,500 CFU/L (Katila et al., 1995).

- In Valencia, Spain, 15 strains of *M. gordonae* and 10 strains of MAC were identified in a variety of surface water samples (Sabater and Zaragoza, 1993).

Swimming Pools/Hot Tubs

- Mycobacterial species including, *M. marinum, M. chelonei, M. scrofulaceum* and *M. gordonae* were isolated from swimming pool samples collected in Araraquara, Sao Paulo, Brazil (Falcao et al., 1993). The number of isolates ranged from 1-3 per site.

- A survey was made of water quality of swimming pools in semi-public areas (hotels, recreational parks and camping grounds) and whirlpools (in sauna institutes and fitness clubs) that were not yet subject to water quality standards under Dutch legislation (Havelaar et al., 1985). Mycobacteria were detected in all water samples. Water temperatures in pools and whirlpools ranged from 18-25^{B}C and 35-40^{B}C, respectively. The mycobacterial numbers in whirlpools on the average were about ten times higher than those in swimming pools, with *M. gordonae* being the predominant species recovered. *M. marinum* was not detected in any of the samples and *M. kansasii* was recovered three times from whirlpools. Only *M. avium* and *M. fortuitum-M. chelonei*

complex were found in both types of pool samples, but the highest densities were seen in whirlpools.

- Species in the *M. avium* complex have been recovered from hot tubs (Embil et al., 1997; Kahana et al., 1997). Aerosols generated by the hot tub action are linked to case reports of disease in humans.

Ground Water

- An investigation was conducted to determine whether ground water could be a natural source of NTM, particularly the MAIS group, in order to explain the geographic bias for infected people in the southeastern U.S. The samples that were tested originated in three geographic regions characterized by different human reactivity to PPD-B. These regions included the Georgia coastal plain, the Virginia coastal plain, and the Valley and Ridge Region of Montgomery Co. Virginia. Relatively low numbers of mycobacterial isolates and very low numbers of MAIS isolates were recovered in the selected regions and were not found to correlate with the distribution of PPD-B reactivity or the incidence of MAIS infection. The data strongly suggest that clean ground waters are not sources of human infection by MAIS or other mycobacteria (Martin et al., 1987).

- Environmental mycobacteria were isolated from 27 out of 63 well water samples examined during a nationwide tuberculosis prevalence survey in Korea in 1980. *M. fortuitum, M. terrae*, and *M. gordonae* were the predominant species. (Won Jin et al., 1984).

- Water reservoirs constructed of concrete or steel tanks, located below and above ground in a small town in Texas were reported to contain *M. kansasii* and *M. gordonae* (Steadham, 1980). The investigators postulated that the NTM species could have entered the reservoirs and passed into the water distribution system from deep water wells or through seepage of water and soil which accumulate in both concrete and steel reservoirs.

Drinking Water

- von Reyn et al. (1993) investigated mycobacterial recovery from water supply samples from wells, hot and cold municipal water supplies, showers and stand pipes. M. avium complex organisms were recovered from 1/6 and 2/8 of samples collected in New Hampshire and Boston, respectively.

- Tap water samples from both hot and cold outlets in a French hospital contained NTM. The predominant species included *M. kansasii, M. gordonae,* and *M. fortuitum* (Dailloux, 1991).

- Scarlata et al. (1985) reported detection of NTM in 37% of tap water samples collected in Palermo, Italy.

- Kubalek and Mysak (1995) investigated the prevalence of environmental mycobacteria collected from a drinking water supply system in the North Moravian region of the Czech Republic during 1984 to 1989. Samples were obtained from 16 localities by tap swabs, tap scrapings or collecting running water. The most commonly identified species of NTM were *M. gordonae* (20.4%), *M. flavescens* (13.8%), rapidly growing mycobacteria (5%) and occasionally *M. fortuitum, M. terrae and M. scrofulaceum*.

- von Reyn et al. (1994) investigated mycobacteria in water collected from hospital taps. They found concentrations of *M. avium* ranging from none to 5.2 CFU/mL.

- Falsely reported outbreaks of nosocomial infections by NTM species have been traced to the use of tap water, used on medical devices and equipment during certain surgical and lab procedures, which is contaminated with *M. xenopi* (Sniadack et al., 1993), *M. gordonae* (Stine et al., 1987) and *M. fortuitum* (Jacobsen et al., 1996).

Occurrence in Other Media

Soil

- Mycobacterial species that have been shown to be normal inhabitants of soil include *M. kansasii* (Jones, 1965), MAIS complex (Brooks et al., 1984a; Kirschner et al., 1992) *M. malmoense* (Saito et al., 1994) and rapidly growing species *M. smegmatis* and *M. fortuitum* (Jones, 1965; Wolinsky and Rynearson, 1968).

- Katila et al. (1995) investigated the occurrence of mycobacteria in soils from unpolluted areas of Finland. Mycobacteria were detected in all soil samples with a mean of 3.6×10^5 CFU/g dry weight.

- The presence of *M. avium* of the same serotype in tuberculous lesions in pigs and in sputa of swine workers led Reznikov and Robinson (1970) to postulate that dusts from *M. avium* contaminated soil generated in the swine facility were the source of transmission for both pigs and man.

- Potting soil samples collected from the homes of HIV patients were found to contain serotypes of MAC that were similar to the isolates from the HIV patients of the study group. Although a relationship of exposure to potting soil and acquisition of MAC could not be demonstrated, the data suggest that potting soil may be a potential reservoir of organisms causing MAC infection in San Francisco (Yajko et al., 1995).

Air

- Shelton et al. (1999) recently investigated aerosolized NTM as a possible cause of hypersensitivity pneumonitis in three machine workers. Water-based coolants are typically recycled and may frequently become colonized by microorganisms. In one case study, mycobacteria from the *M. chelonae* complex were identified in concentrations ranging from non-detectable to 6.6 x 10^6 CFU/mL in bulk coolant samples. Air samples collected in the area around the colonized machines revealed concentrations ranging from 56 to 2,256 CFU/m^3. In the second case study, Shelton et al. (1999) found mycobacterial counts of 10^2 to 10^7 CFU/mL in machine fluid samples. Air samples collected near the colonized machines yielded concentrations exceeding the limits of the sampler (greater than 9,424 CFU/m^3). The mycobacteria detected in this case were rapid-growers which were identical to a newly proposed mycobacterial species *M. immunogen.* In the third case study, concentrations of mycobacteria in metal removal fluids were found to range from non-detectable to greater than 10^6 CFU/mL. Again, the mycobacteria were identical to *M. immunogen.*

- Some evidence suggests that some forms of NTM infection may be associated with heavy occupational exposures to dust (Oldham et al., 1975). An apparent association was found between infection with *M. kansasii* and exposure to dusts in the metal grinding trades. The association between dust exposure and mycobacterial infection may have two complementary mechanisms: (1) dust may act as a pulmonary irritant or toxin, resulting in increased susceptibility to mycobacterial infection; and (2) dust serves as a vehicle for mycobacterial exposure.

Surfaces

- In a model system, the accumulation of NTM in biofilms resulted in mycobacterial densities of more than 10^6 colony-forming units per square centimeter (CFU/cm^2) within 10 weeks (Schulze-Robbecke et al., 1991) providing evidence that biofilms may serve as reservoirs for these organisms.

- In a study conducted in Bonn, Germany, 90% of biofilm samples from piped water systems contained mycobacteria at average densities ranging between 10^3 and10^4 CFU/cm^2 with a maximum density of 5.6 x 10^6 CFU/cm^2. Samples collected from organic substances such as plastic and rubber typically had higher concentrations of mycobacteria than did inorganic substances such as copper or glass. The opportunistic pathogens identified in this study included *M. chelonae, M. fortuitum, M. gordonae and M. kansasii* (Schulze-Robbecke et al., 1992).

Food

- Studies of risk factors for MAC infection among AIDS patients in San Francisco did not support the hypothesis that food was a likely source of organisms that cause MAC infection in AIDS patients (Yajko et al., 1995).

Worldwide Distribution of Mycobacterial Infection

- NTM are generally found in the environment as free-living organisms, but many are also known opportunistic human pathogens. NTM diseases are not notifiable or required to be reported; therefore, information on the occurrence of disease is likely to be underestimated. However, human infections due to NTM appear to be increasing at a significant rate across the United States.

- With the emergence of the AIDS pandemic, it is estimated that 25% - 50% of HIV-patients in the United States and Europe are infected with NTM, the primary species of which is *M. avium* (Horsburg, 1991). Presently, the use of highly active anti-retroviral therapy (HAART) has led to a decrease in this estimated risk and rate of infection of NTM in AIDS patients.

- In non-AIDS patients, the occurrence of NTM diseases is also on the rise. This is suggested by the frequency with which MAC has been isolated from clinical samples in regional reference laboratories in the state of Massachusetts from 1972 to 1983 (du Moulin et al., 1985). Reports from Milwaukee, Philadelphia and Portland indicate that the prevalence of pulmonary disease due to MAC in the general population has begun to approach and exceed that of tuberculosis (Iseman, 1998). The Centers for Disease Control and Prevention (CDC) estimates that rates for non-AIDS NTM diseases in the United States are 1.8 per 100,000 per year, of which 1.3 are due to the *M. avium* complex (MAC) (O'Brien, 1989).

- The incidence of mycobacterial disease may be influenced by exposure to dusty conditions. Among 154 patients in Great Britain with pulmonary *M. kansasii* disease, 33 had pneumoconiosis and another 31 were coal miners, steel workers or worked in dusty conditions (Jenkins, 1981). In a study of 12 patients with *M. kansasii* pulmonary infections in southern California, seven had pre-existing pulmonary disease and three reported previous exposures to dust (Gorse et al., 1983).

- *M. marinum* has been well established as a human pathogen, based on clusters of cases observed between 1930 and 1970 (Dobos et al., 1999). Infections in humans have been reported in coastal areas of the Middle East (Evan-Paz et al., 1976), in the Far East (Iredell et al., 1992) and in several countries in Europe (Collins et al., 1984) as well as in the United States (Zeligman, 1972).

- In some developing countries, *M. ulcerans* disease is a problem, with hundreds of individuals afflicted with disabling lesions and only limited therapy available (Marston et al., 1995). Although endemic occurrence of the disease has been reported mostly in tropical and subtropical areas of the world (Hayman, 1991), a large outbreak of *M. ulcerans* infection on a temperate southern Australian island was reported by Veitch et al. (1997).

- From the time that *M. haemophilum* was first described as a human pathogen in 1978 up until 1989, only 18 cases of infection had been reported: seven patients were from the United States, and 11 were from Australia, Canada and France (MMWR, 1991). From 1989 to 1991, CDC identified *M. haemophilum* in eight patients from Connecticut, Florida, Georgia, Pennsylvania, Texas and Virginia.

- There is a lack of information on the occurrence of NTM in animals; however, reports raise the possibility that certain animals may represent a natural reservoir for these mycobacteria (Gangadharam and Jenkins, 1998). Strains of disease-causing *M. avium* have been recovered from ducks, geese and swans in an English wildlife reserve. NTM species such as *M. marinum* and *M. scrofulaceum* which are normally associated with water as a habitat, have been found in fish.

Epidemiology and Disease Outbreaks

- NTM diseases are not reportable; therefore, information regarding the occurrence of disease outbreaks is likely to be underestimated. Many infections caused by these organisms are episodic and sporadic (e.g., *M. kansasii, M. marinum,* and *M. ulcerans* infections).

- Waterborne NTM have been associated with a large number of nosocomial and pseudo-outbreaks worldwide. Nosocomial disease outbreaks usually involve sternal wound infections, plastic surgery wound infections or postinjection abscesses. Falsely reported outbreaks are usually related to contaminated hospital equipment and water supplies.

- Outbreaks of mycobacterial disease have been reported after exposures in public swimming areas. **In 1954, an outbreak of 80 cases of *M. marinum* were reported due to exposure in a contaminated swimming pool. An additional outbreak involving at least 290 cases occurred in children who swam in a warm mineral water pool in Glenwood Springs, Colorado (Wolinsky, 1979).**

Factors Affecting Environmental Survival

- The survival of environmental mycobacteria in habitats that are potential reservoirs or sources of infection may be influenced by certain physiochemical factors which include temperature, pH, organic matter salinity and humidity.

- du Moulin et al. (1988) reported that temperatures between 52° and 57°C encouraged the proliferation of *M. avium* in hospital water supplies and recommended raising the temperature of hot-water systems to reduce exposure of patients to organisms of the MAIS complex.

- Other investigators have found that *M. kansasii* colonized cold water systems and mixer taps, whereas *M. xenopi* predominated in hot water systems and mixer taps (McSwiggan and Collins, 1974; Wright et al., 1985).

- An investigation was conducted to measure the heat susceptibility of opportunistic mycobacteria frequently isolated from domestic water supply systems versus the heat susceptibility of *L. pneumophila* (Schulze-Robbecke and Buchholtz, 1992). It was concluded that *M. kansasii* is more susceptible to heat than *L. pneumophila*, whereas *M. fortuitum*, *M. intracellulare* and *M. marinum* are equally susceptible to temperatures between 55º and 60º C. However, *M. avium*, *M. chelonae*, *M. phlei*, *M. scrofulaceum* and *M. xenopi* were found to be more heat resistant than *L. pneumophila*.

- A study designed to evaluate the efficiency of ozone as a disinfectant in secondary wastewater effluent under different environmental conditions, showed that the survival of *M. fortuitum* increased with an increase in pH from 5.7 to 10.1 (Farooq et al., 1977)

- Humic and fulvic acids are known to affect the survival of environmental mycobacteria either directly (Brooks et al., 1984b) or indirectly in combination with other physiochemical variables such as temperature, oxygen content, pH and inorganic substances (Kirschner et al., 1992). The combination of higher temperatures, low oxygenated waters low soil pH, and waters high in zinc, humic and fulvic acids from swamp waters most likely favor the growth and survival of MAIS organisms (Flaig et al., 1975; Schnitzer, 1982).

IV. HEALTH EFFECTS IN HUMANS

Clinical Symptoms

- The clinical symptoms seen following infection with NTM depend greatly on the mycobacterial species and site of the infection. NTM diseases in immunocompetent hosts are relatively rare even though exposure to organisms is common based on their ubiquitous nature in the environment.

- Common clinical syndromes include:
  - < Pulmonary infection

< Lymph infection (lymphadenitis)
< Ear infection
< Skin & soft tissue infection
< Catheter-associated infection
< Disseminated Infection

Pulmonary Infection

- In adults, pulmonary infection is the most commonly recognized form of NTM infections. These infections often present clinically as chronic cough, sputum production and fatigue. Older adults are generally the population in which chronic lung disease due to NTM is observed (ATS, 1997).

- **Two forms of pulmonary disease are recognized: apical cavitary disease that is best recognized with MAC and *M. kansasii* infection in middle aged male smokers, and modular bronchiectasis that is best recognized in MAC and *M. abscessus* infection and is most commonly seen in elderly non-smoking women (ATS, 1997)**.

- Members of the *M. avium* complex or *M. kansasii* are the NTM species most commonly associated with pulmonary infection in the U.S. However, other species known to occasionally cause pulmonary disease include; *M. xenopi*, *M. fortuitum*, *M. abscessus*, *M. szulgai, M. malmoense, M. simiae, M.* ***celatum, M. asiaticum*** **and *M. shimodii* (ATS, 1997)**.

***Lymph Infection (Lymphadenitis*)**

- Lymphadenitis occurs predominantly in young children, between 1 and 5 years old, and typically affects the cervical, submaxillary, submandibular and preauricular lymph nodes (ATS, 1997; Jenkins, 1991). In the absence of HIV infection, this disease rarely affects adults. Historically, the classical cause of cervical lymphadenitis was *M. scrofulaceum* whereas today, the species most commonly involved is the *M. avium* complex. *M. malmoense*, *M. scrofulaceum*, *M. kansasii* and *M. fortuitum* have been implicated to a lesser extent.

Ear Infection

- *M. abscessus* has recently been implicated in causing sporadic ear (otologic) infections after placement of tympanotomy tubes. This infection is characterized by nonspecific otorrhea and abundant granulation tissue which has lasted over 3 months and is unresponsive to standard antibiotic therapy (Correa and Starke, 1996).

Skin & Soft Tissue Infection

- Multiple species of mycobacteria have been identified as causative agents of skin and soft tissue infections. These include; *M. marinum, M. ulcerans, M. haemophilum, M. fortuitum, M. abscessus, M. chelonae* and species within the *M. avium* complex. These infections can be either community acquired or nosocomial infections.

- *M. marinum* is the species of *Mycobacterium* most commonly associated with skin infections. Most often, infection from this species occurs following an exposure of cut or abraded skin to organisms present in aquariums, pools, natural water supplies and salt water (Feldman, 1974; Wolinsky, 1979; ATS, 1997). The incubation period can range from two weeks to several months (Dobos et al., 1999). The typical outcome of infection is the development of a localized skin lesion on the arms or legs. Occasionally, synovial involvement or development of subcutaneous nodules along lymph channels will occur.

- *M. ulcerans* causes distinctive, often severe, skin lesions. It is thought that the primary mode of infection with this species is through cuts from vegetation (e.g., grass) which allow the organisms to enter the skin. Lesions develop as small, palpable, painless, subcutaneous swellings approximately 4 to 10 weeks after infection. The growing nodule, which is firm and attached to the skin, remains superficial and extends laterally involving fat and fascia around muscle bundles or the muscles themselves. The skin overlying the lesion loses pigmentation, becomes filled with fluid and necrotic and often ulcerates. The ulceration typically has undermined edges and enlarges over many months (Feldman, 1974). *M. ulcerans* has not been found in the United States, but is well recognized in Australia and Africa.

- *M. haemophilum* has been observed to cause joint and skin infections in immunocompromised patients and lymphadenitis and skin lesions in healthy children. The lesions frequently occur on the arms or legs as raised violaceus nodules, which often may become erythematous and ulcerated (Dobos et al., 1999). Recurrence of ulcers may occur in patients who have not undergone a complete excision of the lesion.
- The rapidly growing mycobacterial species *M. abscessus, M. fortuitum* and *M. chelonae* are also a common cause of skin and soft tissue infections following local trauma.

Catheter-Associated Infection

- Although infrequent, catheter-associated mycobacterial infections have most often been associated with long-term central venous catheters and are linked to rapidly growing mycobacterial species. The disease may include exit site infections, tunnel infections or catheter-related bacteremia (Correa and Starke, 1996). Exit site infection is characterized by white to green purulent drainage. Tunnel tract infection is accompanied by erythema and induration of the surrounding tissues.

- The most common NTM species associated with catheter-associated infections are *M. fortuitum, M. chelonae, M. abscessus* and *M. mucogenicum* (Wallace et al., 1993). Rarely, other species may be seen, including *M. avium* complex. Infections may also follow use of other types of catheters, including peritoneal catheters, ventriculostomy tubes and nasolacrimal duct tubes.

Disseminated Infection

- Disseminated NTM infection in HIV patients appears to originate from a primary infection of either the respiratory or gastrointestinal tracts (Correa and Starke, 1996). These infections may involve any organ, but most commonly occur in the lungs, liver, spleen, lymph nodes or bone marrow (Correa and Starke, 1996). Common symptoms include prolonged fevers (often accompanied by night sweats), weight loss and occasional abdominal pain or diarrhea. This disease is most commonly seen in patients with less than 50 CD4 cells (ATS, 1997). The primary *Mycobacterium* species associated with disseminated infections in HIV infected patients is *M. avium.* However, *M. kansasii, M. haemophilum* and *M. genavense* have also been implicated.

- Prior to the HIV epidemic, disseminated infection caused by MAC was rare, occurring primarily in patients with underlying malignancy or immunodeficiency. *M. avium* complex, *M. kansasii, M. chelonae, M. scrofulaceum, M. abscessus* and *M. haemophilum* have all been observed to cause disease in individuals without HIV infection. The typical symptom of disseminated infection with *M. avium* complex is a fever of unknown origin, whereas symptoms caused by the other species consist of multiple subcutaneous nodules or abscesses that drain spontaneously (ATS, 1997).

- In immune-suppressed individuals other than those with HIV, dissemination of disease from a cutaneous infection is the most common form of NTM disease. These infections are usually due to *M. chelonae, M. abscessus, M. haemophilum* and rarely other species such as *M. kansasii.*

Dose Response

- An organism is pathogenic for a specific host if it can infect that host and produce signs and symptoms of disease. Some pathogens, such as *M. tuberculosis*, can successfully infect and cause disease in a completely normal host in whom all defenses are intact. Other organisms have a more limited pathogenicity and cause disease almost exclusively in hosts with one or more defense defects. Such organisms are thus termed "opportunistic". Almost all of the NTM fit into this latter category.

- The dose-response relationship is dependent on the host's immune status, presence of predisposing factors (e.g., broken skin), and the species of NTM. Therefore, a dose of organisms that results in infection in an immunocompromised individual, may have no apparent effect in a healthy host.

- At least one study has shown that inhalation of MAC is more likely to cause infection than oral exposure (Gangadharam et al. 1989; cited in Rusin et al., 1997). In this study, beige mice were exposed once via either the intranasal or oral route to MAC. Via the intranasal route, lung infections were seen within one day and lymph node, liver and spleen infections were seen within 8 weeks. The oral exposure did not result in systemic infection; however, MAC organisms were isolated from spleen and lung tissue samples 6 to 8 weeks post-exposure.

- Bermudez et al. (1992) (cited in Rusin et al., 1997) found that when beige mice were challenged to five oral doses of 10^4 CFU of *M. avium* given on alternate days of exposure, bacteremia was detected in 26.9% and mortality occurred in 11.5% of mice within 4 weeks. By 8 weeks, all of the animals had disseminated disease and as many as 70% had bacteremia caused by MAC strains. After exposure to five doses of 10^8 CFU of MAC, bacteremia occurred in 45% of the dosed animals and mortality was seen in 25%.

Immunity

- The stages of immune response seen in mycobacterial disease are similar to those in other infections and consist of recognition, response and reaction. The first stage consists of 'recognizing' the invading mycobacteria as 'foreign'. In the second stage, the host defense mechanisms are triggered. The last stage represents the interaction between the *Mycobacterium* and the host.

- Cell-mediated immunity is thought to be the host immune response which is mainly responsible for protection against NTM infection. However, some mycobacteria can survive within macrophages.

Treatment

Treatment of NTM infection depends on the location and extent of disease involvement, status of the host's immune system, and the mycobacterial species. Since there are a variety of disease manifestations for the NTM species, a variety of treatment options exist. A statement reviewing treatment of NTM disease has been issued by the American Thoracic Society (ATS, 1997).

Pulmonary Infection

- Recent studies have shown that the macrolides clarithromycin and azithromycin have strong activity against MAC (ATS, 1997). According to a recent statement by the American Thoracic Society (ATS, 1997), initial therapy for this MAC-associated pulmonary disease in adult HIV-negative patients should consist of a minimum three drug regimen, consisting of clarithromycin or azithromycin, and rifabutin or rifampin, and ethambutol. On this regimen, patients should show clinical improvement within 3 to 6 months. For patients whose disease has failed to respond to the regimen, an alternative four drug regimen, consisting of isoniazid, rifampin, ethambutol and streptomycin, has been recommended (ATS, 1997).

- In adult patients with pulmonary infection caused by *M. kansasii*, a three drug treatment regimen consisting of isoniazid, rifampin and ethambutol is recommended (ATS, 1997). In cases where no treatment is provided, there is progression of clinical and radiographic disease.

- Although there are multiple other species of NTM that are known to cause pulmonary infection, treatment recommendations are only available for four species; *M. malmoense, M. simiae, M. szulgai* and *M. xenopi*. Treatment durations typically last between 18 to 24 months (ATS, 1997). Most *M. malmoense* isolates are susceptible to ethambutol, and many are also susceptible to rifampin and streptomycin. In most cases, favorable responses have been seen using the alternative four-drug treatment regimen for MAC. For patients requiring treatment of *M. simiae* infection, initial therapy may be implemented using the alternative four-drug treatment regimen for MAC. Based on susceptibility tests, this regimen can be modified as needed (ATS, 1997). *M. szulgai* is typically susceptible to rifampin and higher concentrations of isoniazid, streptomycin and ethambutol (ATS, 1997). Most patients treated with these drugs in combination respond favorably to therapy. In vitro tests have shown that the susceptibility of *M. xenopi* to antituberculosis drugs is variable (ATS, 1997). It is recommended that for initial therapy, patients should be administered a macrolide, rifampin or rifabutin, and ethambutol with or without initial streptomycin. If treatment fails or patients show signs of relapse, surgery may be a consideration.

Skin and Soft Tissue Infection

- Cutaneous lesions caused by NTM may spontaneously regress over a few week period, but the deeper lesions can persist or advance, often requiring treatment (Rosenzweig, 1996). Guidelines for drug therapy of disease caused by rapidly growing mycobacteria have been developed; however, susceptibility testing of isolates is recommended prior to beginning a treatment regimen.

- For serious infection caused by *M. fortuitum* and *M. abscessus,* an initial therapy of amikacin combined with high-dose cefoxitin given intravenously is recommended (ATS, 1997). Surgery may be required in cases of extensive disease, abscess formation or where drug therapy is difficult.

- Several treatment strategies have been identified for cutaneous infection caused by *M. marinum*. These include observation, surgical excision, use of antituberculosis agents and use of single antibiotic agents. The American Thoracic Society (ATS, 1997) has indicated that acceptable treatment regimens for adults include clarithromycin, minocycline or doxycycline, trimethoprim-sulfamethoxazole, or rifampin plus ethambutol daily. Each of these regimens should be administered for a minimum of three months.

- Drugs for treatment of infection by *M. ulcerans* are considered ineffective. Surgical removal is generally considered the treatment of choice for this disease.

Disseminated Infection

- Treatment of disseminated *M. avium* infection requires multi-drug therapy due to problems with drug resistance. Recommendations for treatment, based on currently available data, advise a minimum of three drugs, one of which should be clarithromycin or azithromycin (ATS, 1997). Ethambutol is often used as the second agent and rifabutin as the third. In patients with AIDS, adverse effects resulting from NTM treatment drugs may necessitate frequent changes to the therapeutic regimen.

Sensitive Subpopulations

- In many patients with mycobacterial disease, there are predisposing factors present. These factors include, traumatic breaches of the skin, pre-existing pulmonary disease or damage, lung architectural defects, bronchiectasis and generalized congenital and acquired immunosuppressive disorders (such as HIV) (Grange, 1996a; Dawson, 1990).

- NTM has been recovered frequently in patients with cystic fibrosis, particularly in the southeastern United States.

- Several species of NTM are generally seen only or primarily in patients with suppressed immune status. These species include *M. genavense* and *M. haemophilum*.

Children

- It is difficult to estimate the true incidence of disease caused by NTM in children. These infections are typically underestimated due to a lack of mandatory reporting and the fact that NTM disease is seldom a cause of death in children.

- In general, symptoms seen in children are similar to those presented in adults. The superficial lymph nodes of the head and neck are the most common sites of clinically significant NTM infection in children, with onset occurring more rapidly in younger children. Of the mycobacteria isolated from children under 12 years of age with lymphadenitis, 65% to 80% are MAC, and 10% to 20% are *M. scrofulaceum* (Correa and Starke, 1996). Pulmonary disease is relatively rare in the pediatric age group.

- Symptoms of disseminated NTM infection in pediatric patients include recurrent fever, failure to thrive, neutropenia, night sweats, abdominal pain, anemia and anorexia (Correa and Starke, 1996).

- Mycobacterial lymphadenitis in children most commonly occurs as a lump in the neck of a child who is otherwise healthy (Colville, 1993). The involved nodes are typically high in the neck, just under or near the jaw. There is little or no associated pain or tenderness (Wolinsky, 1979). The nodes typically progress rather rapidly to softening, rupture, sinus formation and prolonged drainage. The most common treatment for lymphadenitis caused by NTM is complete surgical excision. This treatment has a greater than 95% cure rate.

- Therapy for otologic infections consists of surgical debridement, removal of foreign material, and long-term antimicrobial therapy determined by susceptibility testing. This disease is almost invariably due to *M. abscessus*. Clarithromycin is the treatment drug of choice for this infection.

- The primary treatment for skin and soft tissue infection in children is antimicrobial drug therapy, often accompanied by surgical debridement if necessary (ATS, 1997).

V. HEALTH EFFECTS IN ANIMALS

- Several of the NTM species have been known to cause disease in animals. These include: *M. avium* complex, *M. marinum*, *M. ulcerans*, *M. paratuberculosis, M. simiae, M. fortuitum* and *M. smegmatis*.

- Strains of the *M. avium* complex have been recovered from numerous animals including pigs, ducks, geese, swans, and monkeys (Gangadharam and Jenkins, 1998). Most are presumed to be *M. avium*. Most strains of MAC are non-pathogenic in guinea pigs and mice, although some have been shown to be virulent in chickens (Gangadharam and Jenkins, 1998). Progressive disease in laboratory mice has been seen after infection with several strains of *M. avium-intracellulare*.

- The causative agent of Johne's disease is *M. paratuberculosis*. This disease is a slow, progressive infection of the intestine which can result in diarrhea and wasting of the infected animal. The disease is usually associated with cattle, sheep and goats.

However, other ruminants, both domestic and wild, have been affected (Power et al., 1993).

- It is estimated that approximately 10% of dairy cattle and 33% of dairy herds in the state of Wisconsin are infected with *M. paratuberculosis* (Falkinham, 1996). Since the principal routes of infection are from infected to uninfected cattle and from infected mothers to their young, removal of infected animals from herds or of newborns calves from their mothers has been successful in reducing the incidence of the disease.

- Some investigators have observed that paratuberculosis in cattle may be transmitted *in utero*. In fetuses from cows with clinical signs of paratuberculosis, prevalence of fetal paratuberculosis ranged from 21% to 37%.

- Manning et al. (1998) investigated an epizootic of paratuberculosis in farmed elk. Elk infected with *M. paratuberculosis* have clinical signs (e.g., weight loss and diarrhea) and histologic lesions which are similar to those in cattle.

- *M. marinum* is known to infect fish and serve as an important cause of morbidity, mortality and economic loss. Some reports indicate that the prevalence of *M. marinum* infection in fish may be as high as 15% (Gangadharam and Jenkins, 1998). Clinical signs of *M. marinum* infection in fish include emaciation, poor growth and retarded sexual maturation. Infection rates reportedly range from 10% to 100% when there is an infected fish present in the population. Mycobacterial infections in fish are considered non-treatable. It is recommended that infected stocks be destroyed and that disinfection occur prior to restocking (Reed and Francis-Floyd, 1995).

- Cases of disease from infection with *M. ulcerans* have been reported in koala bears inhabiting Australia (Grange, 1996a).

- In 1965, *M. simiae* was described as a new species of mycobacteria upon its isolation from monkeys. Data suggest that transmission occurs from animal to animal as shown by a case study in which 26% of healthy monkeys caged in groups with infected monkeys developed *M. simiae* infection over a 13 to 90 day contact period (Falkinham, 1996).

- *M. fortuitum* and *M. smegmatis* are known to cause mastitis in sheep and cattle and skin and soft tissue infection in domestic house cats. *M. fortuitum* has also been noted to be pathogenic for mice, producing distinctive abscesses in the kidneys and the syndrome of spinning disease as a result of lesions in the cochlea (Wolinsky, 1979). Additionally, *M. fortuitum* may produce localized abscesses, but not progressive disease, when inoculated subcutaneously or intraperitoneally in rabbits and guinea pigs (Wolinsky, 1979).

VI. RISK ASSESSMENT

Pathogen Characterization and Occurrence

- NTM species have been recovered from waste waters, surface waters and ground waters as well as from aerosols associated with water sources. Mycobacteria can multiply in water that is essentially devoid of nutrients, and they are relatively resistant to disinfection with many water treatment chemicals, including chlorine. Although there are data on the occurrence of NTM species in water sources, the data are often not quantitative.

Exposure

- The Centers for Disease Control and Prevention (CDC) conducted a surveillance study of NTM disease from 1981 to 1983. Based on this study, the annual case rate estimates for non-AIDS related NTM diseases was 1.8 per 100,000 individuals, of which approximately 1.3 were attributable to MAC (O'Brien, 1989). In 1996, the rate of NTM infection in the United States population based on reported data was 7.7 per 100,000 individuals (CDC, 1999a).

- There are three primary pathways of exposure to mycobacteria in water by which humans are known or suspected to become infected: ingestion, inhalation and entry through abraded skin. For water ingestion, USEPA (1993) recommends a value of 2 L/day be used as an estimate of a reasonable maximum exposure in adults. Based on this ingestion rate of 2 L/day and concentrations of mycobacteria ranging from 0.01 to 5.2 CFU/mL in municipal water systems, adults could be expected to ingest from <20 to 10,400 CFU on a daily basis. No information was located on the amount of aerosolized water which is inhaled daily, nor the amount of water which can potentially enter through broken or abraded skin, but the exposure rate is presumably much lower than for ingestion.

Human Health Effects

- In many patients with mycobacterial disease, there are often predisposing factors present, including pre-existing pulmonary disease or damage, and generalized congenital and acquired immunosuppressive disorders (Grange, 1996a).

- The most common clinical syndromes are pulmonary infection, lymphadenitis, otologic infection, skin and soft tissue infection, catheter-associated infection and disseminated infection.

Dose Response

- Little is known regarding the Dose-Response relationship between mycobacterial exposure and infection in humans by either ingestion, inhalation or dermal exposure pathways. Some oral ingestion studies have been conducted in animal models (Rusin et al., 1997), however, these studies are limited in that they did not test responses at a range of doses, but instead tested the health effects after oral ingestion of a single concentration.

Risk Characterization

- NTM are opportunistic pathogens with widespread distribution in the environment but a very low rate of infection in the general population.

- Sufficient information is not available to support a quantitative characterization of the threshold infective dose (i.e., the dose required to produce infection) of NTM species.

- Despite the lack of data on factors necessary to perform a risk assessment, there are direct observations which can be utilized to provide a rough overview of risks in the United States. As mentioned above, NTM infections are not required to be reported, therefore the following rates may be underestimated. The annual case rate estimates from 1981 to 1983 for non-AIDS related NTM diseases are 1.8 per 100,000 individuals. This is equivalent to an annual risk of 1.8×10^{-5} for developing NTM disease as a result of exposure to NTM organisms from all sources (including non-water sources) in the United States.

- The goal of the Surface Water Treatment Rule (SWTR) is to reduce risk to less than one infection per year per 10,000 people (risk = 1×10^{-4}) based on exposure to a microorganism in drinking water. Assuming that case rates have not increased dramatically over the last two decades, the population average risk of developing disease associated with NTM organisms from any exposure source is probably below the goal identified by the SWTR. Risks to individuals may be higher.

- It has been estimated that in the United States, 25% to 50% of individuals with AIDS will develop NTM diseases (Pozniak et al., 1996; Falkinham, 1996). In 1998, the annual rate of AIDS in the United States was 17.1 cases per 100,000 population. However, some states have case rates as high as 189 per 100,000 population, as seen in 1998 in the District of Columbia. If it is expected that 25% to 50% of AIDS patients will develop NTM disease, this would be equivalent to a prevalence of approximately 4.3 to 8.6 per 100,000 across the United States and 47.3 to 95 per 100,000 in the District of Columbia. The recent use of highly active anti-retroviral therapy (HAART) has led to a decrease in risk/rate of NTM infiction in AIDA patients.

VII. METHODOLOGY

Sampling and Recovery

- Because the majority of mycobacterial species grow slowly, some method of decontamination is necessary to kill the other bacteria and fungi present in the water. If this decontamination step does not occur, these other microorganisms will overgrow the culture medium and often cause its breakdown by proteolysis. Acids, alkalis and detergents are often used during the decontamination process since mycobacteria are generally more resistant to these chemicals than are other bacteria (Jenkins, 1991).

- Carson et al. (1988) found that sodium hypochlorite (0.2 ug/mL of free chlorine) appeared to be the most effective in reducing gram-negative bacterial populations stemming from background contamination while still allowing good recovery of NTM in test samples.

- Iivanainen et al. (1997) compared two decontamination methods for the isolation of mycobacteria from brook waters. The decontaminates were 0.7 mol/L NaOH followed by 50 g/L oxalic acid and 0.9 mol/L H_2SO_4 combined with 0.5 g/L cycloheximide. The authors reported that in general, the NaOH-oxalic acid method resulted in lower contamination and higher isolation of mycobacteria than the H_2SO_4-cycloheximide method.

- In addition to decontamination methods, recovery of mycobacteria is often dependent on the culture medium. Some media favor rapid-growing mycobacteria and may result in obscured growth of the slower growing species. One of the most common media used in culturing mycobacteria, the Lowenstein-Jensen medium, contains eggs, asparagine, glycerol and some mineral salts.

Detection, Quantification and Identification

- Numerous methods have been developed for the detection of mycobacteria in samples. Although the majority of these methods have been developed for the analysis of clinical specimens (e.g., blood, sputum), they can also be applied to detection in water sources. These methods include:
  - < Polymerase chain reaction (PCR)
  - < Radiometric methods (BACTEC)
  - < GC/MS
  - < Nucleic acid probes
  - < Cultural and biochemical tests

  Overall, the most common and reliable method for the detection and identification of mycobacteria is culture isolation.

- Polymerase chain reaction (PCR) techniques take a small quantity of bacterial DNA and enzymatically produce multiple (as many as a billion) copies of the target DNA segment. These segments can then be separated via gel electrophoresis. This technique can be used to identify, the disease-causing bacteria. One drawback of PCR techniques is that they can result in a positive reading even if the bacteria have been inactivated, due to the presence of residual genetic material in the water source. This may lead to inappropriate assertions that a drinking water source is contaminated, when the detected genetic material may in fact be from non-viable cells (Cormican et al., 1992).

- Radiometric detection of mycobacteria, also known as BACTEC methods, is becoming widely used. The BACTEC TB460 system can distinguish between *M. tuberculosis* and NTM via the use of a selective growth inhibitor called NAP (ATS, 1997). The average time for performing this test is five days. Although the BACTEC system can detect growth of mycobacteria; it is not quantitative. Additionally, this system can not be used for species identification.

- Gas chromatography-mass spectrometry (GC/MS) has been used for the detection and quantificiation of various mycobacterial strains in drinking water.

- Species of mycobacteria can also be identified via High Performance Liquid Chromatography (HPLC) analysis of species-specific mycolic acids (Butler et al., 1991, 1992; CDC, 1999b). Most commonly reported species can be rapidly identified using this method. CDC has developed a document entitled "Mycolic Acid Pattern Standards for HPLC Identification of Mycobacteria" (CDC, 1999b) to serve as a resource in species identification.

- Nucleic acid probes are a tool which can be used for rapid identification of pure cultures of mycobacteria (Gangadharam and Jenkins, 1998). Probes have been developed for the following species: *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. avium* complex, *M. kansasii*, and *M. gordonae*.

- In addition to the above methods, identification of mycobacteria is often performed by evaluating both cultural and biochemical characteristics (Grange, 1996; Gangadharam and Jenkins, 1998). Until more methods are developed for species identification, the conventional methods based on morphology, growth rates and biochemical parameters will continue to be the most utilized.

VIII. ANALYSIS AND TREATMENT

Drinking Water Treatment Methods

- In general, drinking water treatment methods can be separated into two modes of action: removal and disinfection. Removal methods typically are physical rather than chemical techniques. Disinfection is defined as the destruction or inactivation of pathogenic microorganisms including bacteria, amoebic cysts, algae, spores and viruses (Montgomery, 1985).

- The quality of the raw or source water and the measures that are taken to improve water quality prior to disinfection will have a significant effect on the efficacy of any treatment method.

- Water quality factors that influence disinfection efficiency include particulates or aggregates (suspended solids or turbidity), dissolved organic matter, inorganic constituents, pH and temperature (Sobsey, 1989).

- Physicochemical treatment methods are generally used prior to disinfection to 'clarify' the source or raw waters for improvement of water quality. These treatment methods are also used to improve the efficiency of disinfection (called disinfection demand) by reducing the inorganic and organic loads present in source water prior to drinking water treatment. These methods include:
    - < Sedimentation
    - < Flocculation and coagulation
    - < Filtration
    - < Adsorption

Disinfection/Inactivation

- Disinfection treatment methods consist of a number of different processes that are used to destroy or inactivate pathogenic microorganisms. These include treatment with: chlorine (free chlorine), chloramines, chlorine dioxide, bromine, iodine, ozone and ultraviolet radiation.

- Many species of mycobacteria have proven resistant to chlorine treatment. This is of concern, since most waters that are intended for human use are treated with chlorine. In fact, it is estimated that 98% of municipal water suppliers in the United States use chlorine for water treatment.
- The susceptibility of mycobacterial strains to chloramines has not been fully evaluated (Pelletier et al., 1988).

- Preliminary studies have shown that *M. fortuitum* is more resistant to ozonation than is *E. coli*. Farooq et al. (1977) concluded that ozone residual is the controlling factor in the inactivation of *M. fortuitum*.

- No information was located regarding the disinfection efficacy of bromine, iodine or hydrogen peroxide against NTM species.

- Inactivation by UV radiation is believed to act through direct absorption of UV energy by the microorganism, causing molecular rearrangement and disruption of unsaturated chemical bonds. Studies have shown that UV radiation can be effective for disinfecting water contaminated with pathogenic mycobacteria (Kubin et al., 1982).

IX. RESEARCH NEEDS

NTM are an important cause of community- and hospital-acquired illness, and they can be associated with morbidity and mortality when an infection is not rapidly diagnosed and treated. In addition, NTM are widely distributed in the environment, including treated water supplies. Additional information is needed to institute optimal prevention and control measures and to minimize the morbidity and mortality associated with these organisms. Specific information gaps include the following:

- More information is required on the latency and dormancy periods of NTM diseases. This information will facilitate the identification of environmental sources which lead to disease outbreaks and may help identify species-specific transmission factors.

- More comprehensive data on the concentration of NTM species in water sources is needed, especially as it relates to potable water supplies in order to accurately estimate exposures to these organisms.

- Further information is needed regarding the nature of the dose-response relationship for NTM species, particularly for exposures from potable water. More specifically, research is needed to establish the minimal infectious dose for high-risk and general populations. This information will help support development of acceptable levels of these organisms in water supplies.

- Identification of the most effective (and most cost-effective) biocidal treatments for NTM species in water sources.

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