Abstract

Human infection from a free-living ameba is oftentimes the result of some type of exposure to water. Although this is not always true for all species of ameba, amebic infection due to *Naegleria fowleri* can invariably be traced to a specific interaction with water. Most often it results from swimming in waters contaminated with the organism. *Naegleria fowleri* is a small free-living ameboflagellate found mainly in warm water habitats worldwide. The organism has been found to be the etiologic agent of a disease in humans known as primary amebic meningoencephalitis (PAM). This is a rare disease that attacks the central nervous system and most often results in rapid death. Although this infection does not occur on as wide a scale as some other more well known diseases, what sets it apart is its very high mortality rate. Approximately ninety-six percent of persons infected intranasally with this microbe will die within two weeks after the first symptoms appear. This study used the International Life Sciences Institute (ILSI) / US Environmental Protection Agency (EPA) quantitative risk assessment (QRA) analytical approach to estimate the risks of swimming-associated PAM from *Naegleria fowleri*.

Using data from mice experimentally infected by swimming in contaminated water, the risk of acquiring this disease was best predicted using a linear two population model. The greatest risk to humans was found to be 1.48 E-06 when the highest environmentally measured concentration of one pathogenic free-living ameba per five milliliters was used. The data from QRA were compared to epidemiological data for swimming-associated PAM. Estimated risks of PAM by QRA were generally consistent with epidemiologically reported risks of PAM.
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I. Introduction

Infections caused by a variety of microbial pathogens and acquired as a result of exposure to contaminated water are not uncommon and occur worldwide. Even though public health measures have been established to address the risks from organisms responsible for these infectious diseases, the continued occurrence of certain waterborne diseases implies that the control measures currently in place are not adequate. This is why some illnesses caused by pathogens thought to be nearly eradicated or controlled are now reemerging and causing more harm to the public’s health.

Furthermore, there are some microorganisms known to cause waterborne disease that have not been addressed at all by current regulations. One such example is the free-living ameba Naegleria fowleri. This organism is known to be the causative agent of a disease known as primary amebic meningoencephalitis (PAM). It attacks the central nervous system by way of the nasal route in a rapid and fulminant manner and has an extremely high mortality rate of >95%. The majority of the confirmed cases have been associated with swimming or some other recreational activity in warm waters. It is now known that this organism thrives in thermal waters, which helps explain why PAM is most often contracted during hot, summer months. Despite the documented cases of PAM and the recognition that the causative organism is ubiquitous in natural surface waters used for primary contact recreation, a quantitative risk assessment of this public health problem has not been reported.
There are two important reasons why it is difficult to perform a quantitative risk assessment of PAM caused by *Naegleria fowleri*. First, it is very difficult to isolate and quantify this free-living ameba from the natural environment. There have been cases of PAM suspected of being associated with a particular body of water, yet examination of water samples yielded no *Naegleria* species. Second, most PAM patients die and therefore it is not possible to experimentally infect humans with known amounts of *Naegleria fowleri* and develop dose-response data. Because the infective trophozoites multiply once inside the host, it is not possible to surmise just how many amebas originally entered the nasal passages. These two aspects must be addressed to assess the risk from this pathogen and deal with the uncertainties of the available data.
II. Statement of Objectives

The overall purpose of this report is to identify and attempt to assess the risk of acquiring the disease primary amebic meningoencephalitis from recreational water contact. The specific objectives are as follows:

- Obtain information regarding the presence and occurrence of *Naegleria fowleri*.
- Acquire animal dose-response data on *Naegleria fowleri* in order to estimate the number of amebas necessary to cause illness.
- Extrapolate from animal to human to get an approximation as to the dose needed for human infection to occur.
- Use information obtained from previous steps to create a risk characterization for *Naegleria fowleri*.

Once all objectives have been met, there will be a risk assessment formulated for *Naegleria fowleri* that will give insight as to whether there is any real danger of acquiring PAM from normal, recreational water contact.

- Compare calculated risks to actual risks – Actual rates calculated as a rate based on annual numbers of reported cases and episodes of recreational water exposure.
III. Literature Review

A. General Overview of Amebas

Amebas are considered the most primitive of all animals and are classified in the Kingdom Protista (Word 1996). They are further categorized as protozoans because they are unicellular, eucaryotic organisms. They also lack internal organs but possess thin cell membranes, a semirigid layer of ectoplasm, a granular, jellylike endoplasm, and an oval nucleus. In particular, they are characterized by their method of locomotion, which consists of extending cytoplasm outward to form pseudopodia in order to achieve movement (Word 1996). Amebas also use these "false feet" for feeding. Smaller organisms, usually bacteria, serve as the primary substrate for these organisms (Salvador Rodriguez-Zaragoza 1994). Formation of pseudopodia is induced by chemical stimuli given off from soon-to-be engulfed cells (Word 1996). The hundreds of different organisms comprising the ameboïd group range in size from about 0.25 to 2.5mm. Most are inhabitants of fresh water and are commonly found living on aquatic plants and in moist ground. Most amebas are harmless, microscopic creatures that spend their lives wandering about in soil, mud and water, occasionally catching food and reproducing by binary fission (Schmidt, GD & Roberts, LS 1989). However, there are a few species of amebas that are parasites of other organisms (Schmidt, et al 1989), and, the ones of utmost public health importance are the species that have been found to infect and cause
illness and even death in humans.

A.1. Natural History of Free-Living Amebas

Free-living amebas are given their name because of their ability to live in the environment where all of their food is available naturally (Visvesvara, GS 1993). In addition, they possess the capability to survive and persist in the environment without the need for hosts to aid in their development. They are ubiquitous in nature and live in a wide range of diverse environments (Rodriguez-Zaragoza 1994), including fresh and frozen water; vegetables; heating, ventilating and air conditioning units; cooling towers of electric and nuclear power plants; sewage; medicinal pools; dental treatment units; dialysis units; bacterial, fungal, and mammalian cell cultures; contact lens solutions; corneal scrapings; skin lesions; and the central nervous system of humans (Visvesvara 1993). These organisms live at interfaces such as water-soil, water-animal, water-plant, water-air, etc., where they feed on bacteria, fungi, yeasts, algae, and protozoa including other amebas (Rodriguez-Zaragoza 1994). Free-living amebas are so cosmopolitan as a group that they were virtually overlooked by medical scientists for a long time (Duma, R.J., Helwig, W.B., Martinez, A.J. 1978).
A.1.a. Background And Discovery of Free-Living Amebas

In 1930 Sir Aldo Castellani found and isolated a free-living, apparently harmless ameba growing in a yeast culture. However, it was not until 1957 that amebas were observed to render cytopathic effects on contaminated cell cultures. One year later, Clyde Culbertson, of Eli Lilly Laboratory, was the first to produce disease caused by these amebas in laboratory animals. Prior to 1958 they were thought of as harmless creatures and more of a biologic curiosity rather than any possible threat to health (Duma, et.al 1978). The discovery that a free-living ameba was the etiologic agent of disease in mammals came about quite haphazardly (Martinez 1993). When the vaccine for polio was being tested for safety on tissue culture plates, one group of plates were noticed to contain plaques, which often suggest the presence of live virus particles. These plates were washed and mice were inoculated with the plate washings. Shortly thereafter, the infected mice died from meningoencephalitis, an inflammation of the membranes of the brain and spinal cord and the brain itself. Because the plaques were thought to have been produced by a virus, a nonattenuated or virulent form of poliovirus was originally suspected as the culprit. However upon further evaluation, abnormal cells were found in the brains of the inoculated mice, and reexamination of the plates uncovered the presence of a free-living ameba. Soon after, a common, small, free-living ameba noted to occur at times as an airborne contaminant of cell cultures was identified. Of the hundreds of ameba species that naturally exist, only those belonging to two genera, *Naegleria* and
Acanthamoeba, and the newly discovered leptomyxid ameba have been found to cause human disease (Visvesvara 1993).

A.2 Diseases Caused By Free-Living Amebas Of The Genus Acanthamoeba

Sinusitis, keratitis, and granulomatous amebic encephalitis (GAE) are the human illnesses caused by various species of Acanthamoeba. Currently, Acanthamoeba keratitis is a major concern because of its association with contact lens use. A less common illness associated with this ameba, granulomatous amebic encephalitis, also garners attention because of the grim outlook for persons infected with the organism.

A.2.a Acanthamoeba Keratitis Disease and The Role Of Contact Lenses

Acanthamoeba keratitis is a disease that affects the eye and is preconditioned by corneal trauma. When exposure to Acanthamoeba polyphaga arises, even the slightest damage to the cornea can allow the organism an avenue for entry into the eye. The disease begins with mild inflammation and progresses to include severe pain in the later stages. However, the most important aspect of this infection is its potential to cause permanent vision impairment, and even blindness (Asbell 1993).

Since about 1985, when the use of contact lenses dramatically increased, this infection has become of serious ophthalmologic concern. One study examining the
incidence of this infection found that 80% of reported cases were from lens wearers. Moreover, the association between this infection and contact lens use has been more specifically noticed in soft lens wearers. In addition, seventy-five percent of the investigated cases used daily-wear or extended-wear soft hydrogel lenses. It is known that all modern lenses contain a certain amount of water, which serves as the medium for oxygen influx. The water content of soft hydrogel lenses is 50-75%, but more importantly, the capacity of these lenses to absorb materials is in proportion to their water content. Therefore potential pathogens from lens cases, cleaning solutions, and the person's hands can be transferred to the lenses, and ultimately the person, through direct contact.

A.2.a.1. Sources Of Exposure To Acanthamoeba

It is thought that infection is acquired and perpetuated most often through the cleaning and disinfection processes of contact lens use. Before the link between Acanthamoeba keratitis and contact lens wear was realized, the importance of avoiding tapwater in the cleaning and disinfection processes was not appreciated. Since then, this free-living ameba has been routinely found in tapwater, leading practitioners to advocate the use of preserved solutions when handling lenses in any way. This includes the lens materials themselves, cleaning and disinfection solutions and materials, and lens storage cases. Contamination from any of these sources increases the chance of obtaining this infection (Asbell 1993).
A.2.a.2. Diagnosis Of Acanthamoeba Keratitis

Diagnosing this disease is often a complex undertaking even in the most advanced medical settings. Much of the problem arises from the fact that the infection alternately waxes and wanes, thereby creating difficulty in ascertaining the cause of disease. In addition, confirmation cannot be made unless trophozoites and cysts are present at the time when stained scrapings of the cornea are examined (Tortura, G. J., Funke, B. R., and Case, C. L. 1992). These factors make the diagnosis and treatment complicated and often make Acanthamoeba keratitis infection a precursor to eventual loss of sight.

A.2.b. Granulomatous Amebic Encephalitis

Granulomatous amebic encephalitis (GAE) is the most harmful of the diseases caused by organisms in the genus Acanthamoeba. Several species of Acanthamoeba [as well as the leptomyxid ameba] are known to be responsible for this disease. It is considered the most dangerous of the Acanthamoeba infections because the prognosis is generally poor for patients with this distinct central nervous system infection. The mortality rate associated with this disease is considered high but cannot be conclusively linked to GAE due to the majority of persons infected being already chronically ill and debilitating from other diseases (Martinez 1993).
A.2.b.1. Route Of Exposure And Pathophysiology For GAE

It has been documented that there are four ways through which these organisms have been known to enter the body: lungs, skin, eyes, and directly into the olfactory neuroepithelium. Through hematogenous spread, the amebas reach the target organ, which is the brain, and cell necrosis and hemorrhaging occur in a slow, chronic, and insidious manner. Those same adjectives can also describe the clinical course taken by GAE. The areas most affected by this infection are the posterior fossa structures, diencephalon, thalamus, and brainstem. It usually takes one week to several months for GAE to run its course, after which the afflicted person's outcome is often death (Visvesvara 1993).

A.2.b.2. Population Most Affected With GAE

Research has found that the population most affected with granulomatous amebic encephalitis are persons already debilitated with other diseases or undergoing some type of immunosuppressive therapy. Hence, this infection is considered opportunistic in the majority of cases. It has been noted that half of all cases in the United States have occurred in persons with acquired immune deficiency syndrome (AIDS). Further, almost all patients have no recent history of participation in any water related activities (Martinez 1993).
A 2.b.3 Diagnosis And Confirmation Of Granulomatous Amebic Encephalitis

As is the case with diagnosing most incidents of encephalitis, the most taxing part is determining the specific agent responsible for causing the disease. Almost always, identification is not made and confirmed until microscopic examination has been done on the central nervous system tissue. One diagnostic feature that sets *Acanthamoeba* [and leptomyxid] infections apart from other amebic encephalitis infections is that upon viewing the CNS tissue slides, both trophozoites and cysts will be present in the brain tissue. Another important distinction made during tissue examination is that the trophozoites of *Acanthamoeba* are larger than trophozoites from the genus *Naegleria*. Also, the cysts will have a wrinkled double wall which indicates infection by an organism classified in this genus (Martinez 1993).

A 3. Disease Caused By Free-Living Amebas Of The Genus *Naegleria*

Primary amebic meningoencephalitis (PAM) is the only disease in humans caused by organisms from this genus and *Naegleria fowleri* is the sole species from this genus responsible for the infection. As the term "meningoencephalitis" implies, this disease is characterized by an inflammation of the meninges of the brain and spinal cord as well as the brain itself. It is a rapidly fulminant disease usually beginning with severe headache followed by nausea and vomiting. Next, the affected individual will experience a fever,
after which a coma most often results. Approximately 96% of those infected will die.

A.3.a. Route Of Exposure And Pathophysiology For PAM

There is only one portal of entry for *Naegleria fowleri* and that is by direct invasion into the olfactory neuroepithelium. It is thought that the flagellated trophozoites are forced deep into the nasal passages when the victim dives into the water (Schmidt, et al 1989). Once entrance has been established, penetration of the nasopharyngeal mucosa occurs after which, the trophozoites migrate along the olfactory nerves. Finally the cribiform plate is invaded which allows the amebic trophozoites entrance to the cranium (Bottone 1993). Just as granulomatous amebic encephalitis has the brain as its target organ, it is there that *Naegleria fowleri* produces massive destruction of tissue en route to its rapidly evolving, hemorrhagic primary meningoencephalitis. A distinguishing feature of this disease is the speed with which it does its damage. The incubation period is between two and seven days followed by the manifestation of symptoms. The full clinical course usually takes no more than two weeks with death being the likely result. This time frame may depend on the size of the inocula and the virulence of the amebas (Martinez 1993).
A.3.b. Population Most Affected With PAM

Examination of previous cases has revealed that most persons stricken with this disease are normal, healthy young adults or children, who have had some sort of recent recreational water contact. In addition, statistics show that of the reported cases, 71% have been males while 29% have been females (Visvesvara 1993). There is no known explanation for the disparity among the sexes with regard to the acquisition of this disease. However because of the seasonality of this infection, it can be assumed that persons who participate in water activities regularly during warmer weather increase their chances of obtaining PAM.

A.3.c. Diagnosis And Confirmation Of Primary Amebic Meningoencephalitis

Any time a case of meningoencephalitis is diagnosed and bacteria are not found, free-living amebas should immediately be suspected as the causative agent. The best method for ascertaining their presence is by microscopic examination of \textit{fresh} cerebrospinal fluid. This is a mandatory procedure because it may reveal motile amebas which are able to be detected as the trophozoite undergoes changes in shape and configuration (Martinez 1993). Also, viewing central nervous system tissue for the presence of trophozoites is an acceptable method for diagnosis and confirmation of primary amebic meningoencephalitis.
B. Water Regulations

B.1. History of Waterborne Transmission

Of the various uses for water, water for drinking purposes has the most regulations pertaining to it. Much of the reason stems from the fact that waterborne transmission of infectious agents has been recognized since the mid-19th century. Dr. John Snow was the first to recognize water as a method of disease transmission in modern times. Since then, waterborne outbreaks have been repeatedly documented around the world (Juliano 1997).

B.2. History of Water and Amebic Disease

Several different infectious agents can be transmitted by water, with disease outcomes ranging from mild gastroenteritis to death (Juliano 1997). Infection due to free-living amebas is oftentimes the result of some type of exposure to water. Although this is not always true, as is the case with the majority of *Acanthamoeba* diseases, amebic infection due to *Naegleria fowleri* can invariably be traced to a specific interaction with water. In 1965 the first ever case of primary amebic meningoencephalitis was reported from Adelaide, South Australia by Fowler and Carter. The following year Cecyl Butt
reported the first case in the United States in Florida. Even though the ameba held 
responsible in South Australia was thought to have been an *Acanthamoeba* spp., it 
actually turned out that *N. fowleri* was the true causative agent (Martinez 1993). It was 
not until 1973 that the first instance of *Acanthamoeba* infection of the eye was reported 
(Asbell 1993).

**B.3. Epidemiology of Waterborne Amebic Encephalitis**

It has been demonstrated numerous times that water is an established route of 
transmission for various amebic diseases. Although traditionally, it has been bacterial 
pathogens, such as various species of *Salmonella*, that predominate as the culprits of 
waterborne disease. The ubiquity of free-living amebas however, would suggest 
high occurrences of illnesses caused by them. Nevertheless, of all the cases of amebic 
encephalitis recorded at Centers for Disease Control from all over the United States, 59% 
are due to *Naegleria fowleri*, 29% are due to *Acanthamoeba* spp. and 12% are due to the 
leptomyxid ameba (Visvesvara 1993).

**B.4. Current Water Regulations**

The United States Environmental Protection Agency (USEPA) is the 
governmental body responsible for carrying out laws and guidelines legislated by
Congress pertaining to drinking and recreational water in this country. Although water is used in several different ways, regulations are most comprehensive for drinking water. The criteria currently used as a measure for microbiological quality is the presence or absence of specific coliform bacteria. According to the USEPA's National Water Quality Inventory 1994 Report to Congress, indicator bacteria that are found in great numbers in the stomachs and intestines of warm-blooded animals and people are measured in order to qualify a body of water as "safe". Because it is impossible to test waters for every possible disease-causing organism, states primarily measure only indicator bacteria. If the tests are positive for those microbes, it suggests then that the water body may also be contaminated with untreated sewage that more than likely contains other, more dangerous organisms.

B.4.a. Surface Water Treatment Rule

Although Federal regulations to address the suitability of drinking waters continue to evolve, it is the Surface Water Treatment Rule (SWTR) that requires specific reductions of pathogens, particularly Giardia and viruses. As it currently stands, there should be at least a three log or 99.9% reduction in Giardia lamblia and a four log or 99.99% reduction in viruses. Giardia lamblia, a protozoan parasite, was the etiologic agent of disease for more than twenty-four thousand people as of 1985, making it the most widely reported cause of gastrointestinal illness in the U.S. at the time. This is the
most notable reason for it being targeted under this rule.

B.4.b. Shortcomings of Current Regulations

Although it is impossible to check for every known disease-causing microbe, it would be wise to primarily test for organisms with a proven history of producing serious illness and/or death in the human population. This process would undoubtedly spare lives as the result of contact with deadly pathogens. Even though the two agents specified in the Surface Water Treatment Rule are responsible for human illness, there remain organisms known to be present, at times, in water that have yet to be addressed by any current regulations. Until each microbe associated with possibly fatal outcomes is acknowledged in newly created regulations, there will be a chance of acquiring a terminal disease from water.

Cryptosporidium for example, is another protozoan parasite which is smaller and significantly more resistant to disinfection than Giardia. Moreover, C. parvum is responsible for severe gastrointestinal illness and even death in immunocompetent persons as well as those immunocompromised. Since the onset of the AIDS epidemic, this disease has been a major problem in that population. Furthermore, despite intensive trials, no effective drug treatment has been developed to date. For that reason alone, Cryptosporidium should be included in guidelines to ensure safer drinking waters. In addition, Legionella and Vibrio are two genera that also have yet to be specifically
covered by laws pertaining to the quality of water bodies. (Under the SWTR, it is
unwisely assumed that Legionella will be controlled if the Giardia and virus reduction
requirements are met.) However, both are free-living bacteria that contain species
accountable for such ailments in humans as Legionnaire's disease and cholera
respectively. Free-living amebas are another group of microorganisms that have been
omitted by governmental regulations. Naegleria and Acanthamoeba are types of free-
amebas capable of fatal outcomes in infected humans. Although many cases of illness
and/or death have been attributed to species from those genera, neither has ever had any
directive aimed at checking, reducing, or eliminating them from specific bodies of water.
It is evident that all of the aforementioned organisms have real potential of being life-
threatening to those that acquire them. In spite of these facts and the number of deaths
associated with them, there continue to be no efforts to assess or manage the risks from
free-living pathogenic amebas in water.

B.5. Overview of Previous Ambient Water Regulations

Federal interest in clean water programs began with the Water Pollution Control
Act of 1948. Although there were no federally required goals, objectives, limits, or
guidelines detailed in the act, it was the first comprehensive statement sponsored by the
government. The Federal Water Pollution Control (FWPC) Act of 1956, its amendments
were all subsequent statutes established following the initial act. However, it was the passage of the act in 1965 that enabled water quality standards to become a prominent feature of the law. Creation of the Federal Water Pollution Control Administration (FWPCA) evolved from that law and required states to develop their own water quality standards. In 1972 amendments to the FWPC Act established new laws through the EPA. This agency assumed the dominant role in directing and defining water pollution control programs across the country. The Clean Water Act originated in December 1977 after the first major revision was completed on the Federal Water Pollution Control Act Amendments of 1972. Actually only amendments, this new law still endorsed policy directions set forth five years earlier. In 1982, amendments to the Clean Water Act dealt primarily with industrial dischargers and municipal (sewage treatment plant) construction grants.

The one unifying aspect of these laws was that their only focus was on toxic pollutants released from industries or municipalities. Free-living pathogenic microorganisms known to cause disease were never addressed directly by any of these early laws prescribed by the Federal government (Water Pollution Control Federation 1982). More specifically, organisms that are not of fecal origin and are capable of surviving and proliferating naturally in aquatic environments, such as free-living amebas and Vibrios, were not mentioned in any of these regulations. Only microbes of fecal origin and responsible for gastrointestinal illness have received attention for water quality standards.
B.6. Overview of Previous Regulations on Recreational Waters

Before current regulations were developed to address more recent pathogen concerns, guidelines had been formulated to address microbes responsible for enteric illness. Just as current water regulations fail to address certain classes of pathogenic organisms and certain classes of waterborne disease, previous water regulations also did not address disease-causing organisms even though they were recognized as causes of illnesses then. Although the recognition of organisms that cause waterborne disease may have dramatically changed over the years, the rules established to control their presence unfortunately have not changed as much.

Most regulations on microbes in water target waters used for drinking although there have been some laws established to address waters used for recreational purposes and harvesting edible bivalve molluscan shellfish. The first attempt at designating standards for acceptable recreational water quality came in the form of a report compiled by the National Technical Advisory Committee (NTAC) and presented to the Federal Water Pollution Control Administration in April of 1968. This report only outlined criteria for waters used for primary contact recreation since recommendations for secondary contact recreation had already been drafted. The subcommittee for recreation defined primary contact as "activities in which there is prolonged and intimate contact with the water involving considerable risk of ingesting water in quantities sufficient to
pose a significant health hazard.” Secondary contact refers to activities “in which contact with the water is either incidental or accidental and the probability of ingesting appreciable quantities of water is minimal.” Consequently, fecal coliforms, which are taken as evidence of the presence of microbes causing gastroenteritis in the human population, were utilized in order to measure the quality of recreational waters. Their use was based on microbiological data which showed fecal coliforms to be indicative of human or animal fecal contamination.

A little more than two years after the NTAC gave its suggestion for testing recreational waters for fecal coliforms, the United States Environmental Protection Agency was founded. After its inception on December 2, 1970, its first report on recreational waters was produced as the publication Quality Criteria for Water (QCW) in 1976. In it, the agency advocated fecal coliforms to be used as the bacterial indicators for bathing, or recreational water quality. At that time, 95% of the states and territories of the United States were abiding by this recommendation and following the criteria outlined.

In the 1978 book entitled Water Pollution Microbiology, chapter nine addressed standards for recreational waters. Microorganisms considered as recreational water quality indicators were primarily those indicative of or responsible for gastrointestinal disease. These included such organisms as total coliforms, fecal coliforms, enterococci and Salmonella and Shigella spp. A listing of causative agents of waterborne disease mentioned primary amebic meningoencephalitis, but the responsible free-living ameba
Naegleria fowleri and any other ameba were not included in the tabulated lists of indicators or pathogens. This was mainly due to the fact that diseases caused by these organisms were rare. Moreover, because the source of these pathogens is the aquatic environment itself, it is not possible to index the risk of illness from those microbes based exclusively on indicators of fecal contamination (Cabelli, V. 1978).

The USEPA produced another handbook in 1984 which more specifically detailed its recommendations for recreational waters. In “Health Effects Criteria for Fresh Recreational Waters”, a new recreational water quality research program initiated in 1972 was described and summarized. The purpose of the program was to examine the relationship between swimming-associated gastroenteritis and bacterial indicators of fecal contamination. The results showed a definite correlation among gastrointestinal illness and bacterial densities of either enterococci or E. coli. Fecal coliforms, which were the bacterial indicators currently used at the time, showed no relationship at all. As a result, enterococci and E. coli were found to be the organisms that best measured the suitability of fresh recreational waters.

“Ambient Water Quality Criteria for Bacteria”, released two years later by the USEPA, supported the findings two years earlier with regard to the specific indicators of risk for gastrointestinal illness. The agency did not recommend “a change in the stringency of its bacterial criteria for recreational waters”, but expressed its strong urging to states to begin the transition process toward utilizing the new indicators. This was because the agency believed enterococci and E. coli to be superior to the fecal coliforms
previously used. This publication tabulated bacteriological densities for the accepted indicators. As with all the previous regulations, only the disease outcome of gastrointestinal illness was considered in the examination of safety for recreational waters.

B.7. Regulations on Waters Used for Shellfish Growing and Harvesting

In 1925, public health principles were established "to insure that the shellfish reaching the consumer had been grown, harvested and processed in a sanitary manner." These principles have basically remained unchanged since then, although since then, program procedures have been updated and improved upon at regular intervals (Sanitation of Shellfish Growing Areas 1995). The National Shellfish Sanitation Program, or NSSP as it is sometimes called, uses total or fecal coliforms for the assessment of bacteriological quality for water bodies. Satisfactory compliance is met if the areas in question do not exceed specific coliform MPN values as outlined in the NSSP Manual of Operations.

The suitability of some growing areas for harvesting shellfish is intermittently influenced by the presence of a marine biotoxin known as paralytic shellfish poison (PSP). The presence of this biotoxin can pose severe and potentially fatal illness but the occurrence of the toxin is often unpredictable. During the harvesting season in those areas where shellfish toxins are likely to occur, representative samples of shellfish are
collected and assayed for the presence of marine biotoxins. The toxin concentration, if present, shall not equal or exceed the amount outlined in the NSSP Manual of Operations for the water body to be classified as satisfactorily in compliance (NSSP Manual 1995). There is no evidence that free-living amebas can be transmitted to humans via shellfish. Therefore, the regulations for shellfishing waters do not address these microbes.

C. Organisms Comprising the Genus *Naegleria*

Comprehensive investigation into *Naegleria* has revealed that a total of five species make up this genus. *N. jadini* and *N. lovaniensis* have so far not been associated with causing any pathogenicity to humans. *N. gruberi*, which is also nonpathogenic to humans, is the species morphologically identical to amebas most often isolated from humans (Martinez 1993). *N. australiensis* is actually pathogenic to mice, causing a subacute meningoencephalitis comparable to that caused by various *Acanthamoeba* species. The organism most dangerous and therefore important for humans is *N. fowleri*. This is the only human pathogenic species causing the often fatal primary amebic meningoencephalitis.
C.1. Life Cycle of the Genus *Naegleria*

The life cycle of this particular genus of free-living ameba is quite simple even though the disease that it can cause is not. It is comprised of an active, feeding trophozoite that rapidly multiplies through binary fission and a dormant cyst form. Like many other organisms with life cycles containing an encysted phase, this part of the cycle resists disinfection and dessication and allows the organisms to persist in nature. However, it has been reported that *Naegleria* cysts are very sensitive to free-chlorine (Rodriguez-Zaragoza 1994). The factors controlling whether or not the ameba encysts or excysts are not known. The general conception is that some adverse environmental condition causes the trophozoites to encyst, most probably through a series of complex biochemical processes. Additionally, *Naegleria* has a transient, nonfeeding flagellated form in its life cycle as well (Bottone 1993).

C.2. History Of *Naegleria fowleri* and Primary Amebic Meningoencephalitis (PAM)

Cecyl Butt is credited with inventing the term “primary amebic meningoencephalitis” to refer to the disease caused by *Naegleria fowleri*. As of September 1, 1991, one hundred thirty-four confirmed cases of the disease have occurred around the world, with approximately 47% of those in the United States. Even though it was thought that the first ever case caused by this organism was recorded in 1965,
Symmers discovered upon review of records stored in a London museum that one patient from Essex, England died as early as 1909 while another person from Belfast, Northern Ireland died from PAM in 1937. Virginia had its first case reported by J.H. Callicott Jr. in 1968. In addition, J.G. dos Santos conducted a retrospective study in 1970 of more than four hundred meningoencephalitis cases that occurred in Richmond, Virginia. Five more cases of PAM were found with the earliest occurring in August of 1937 (Martinez 1993).

IV. Materials and Methods

A. Sources of Information and Data

Information sources included literature searches, microbiology and parasitology books, and correspondence with two experts on free-living amebas. Dr. Govinda S. Visvesvara of the Division of Parasitic Diseases, Centers for Disease Control in Atlanta, Georgia and Dr. David T. John of the College of Osteopathic Medicine, Oklahoma State University, Tulsa, Oklahoma provided information and resources in order to complete this technical report. With the use of Microsoft Word’s Encarta, I collected more general data about amebas in terms of their classification and characterization. Letters were sent to all fifty state health departments requesting information regarding case occurrences of primary amebic meningoencephalitis.
B. Risk Assessment Methods

An assessment of pathogen risk involves evaluating the likelihood of adverse human health effects occurring after exposure to a pathogenic microorganism or to a medium in which the pathogen occurs. Pathogen risk assessments must be scientifically valid and relevant in both the regulatory and public health contexts (ILSI Risk Science Institute Pathogen Risk Assessment Working Group 1996). Although many organizations have developed their own guidelines for assessing human health risk, the one thing they all have in common is the utilization of basically the same concepts in the framework. For this report, I chose to primarily follow the generalized framework drafted by the 1996 ILSI Risk Science Institute’s Pathogen Risk Assessment Working Group. I supplemented this with the guideline formulated by the National Research Council as described in Science and Judgment in Risk Assessment (1994). Both of these guidelines can be implemented to assess the risk of human disease following exposure to pathogens.

B.1. Hazard Identification

An analysis of microbes that may cause disease always begins with performing what is called a “hazard identification” which entails identification of the contaminants
suspected to pose human health hazards, quantification of the concentrations at which they are present in the environment, a description of the specific forms of adverse effects caused by the contaminant of interest, and an evaluation of the conditions under which these effects might be expressed in exposed humans (National Research Council 1994). Another term sometimes used for this initial step is “problem formulation”. Both refer to the phase of the evaluation that identifies the goals, breadth, and focus of the risk assessment, as well as the major factors that will need to be addressed for the assessment process (ILSI 1996). This is a critical step in the analysis process since it provides justification for performing the risk assessment. Before too much time and too many resources are given to a particular organism, it must first be established that there truly is a specific human health effect associated with the organism. Following that, it must be determined how strong the association is between the organism and the disease or illness that results. This can be accomplished through epidemiological studies and/or documented reports of the health effect known or thought to be associated with the organism.

**B.2. Characterization of Exposure**

A next step in a pathogen risk assessment is a characterization of the exposure that is associated with the organism of interest. This is a process that evaluates the interaction between the pathogen, the environment, and the human population. It is an
important step because it specifies the population that might be exposed to the agent of concern as well as identifies the routes through which exposure can occur. Moreover, this step attempts to estimate the magnitude, duration, and timing of the doses that people might receive as a result of their exposure. Many times this part of the assessment can include a pathogen characterization. This involves determining the properties of the pathogen that affect its ability to be transmitted to and cause disease in the host. There are numerous factors that affect a pathogen's ability to cause disease. Some of these elements consist of properties which are intrinsic to the disease-causing agent while others relate to extraneous aspects of the pathogen (ILSI 1996). A pathogen occurrence may also be included at this point of the risk assessment. This aspect of analysis involves characterizing the occurrence, distribution, and physical state of the pathogenic microorganism in the environment. Upon completion of this step, an evaluation of all relevant factors pertaining to the occurrence and distribution of the pathogen will enable a more thorough understanding of the exposure to that particular disease-causing organism. Completion of the exposure characterization results in the creation of an exposure profile which will quantify the magnitude and pattern of human exposure for the situations developed during the initial step of hazard identification and serve as input for the risk characterization (ILSI 1996).
B.3. Characterization of Exposure Response

A description of the exposure response is the next phase in the pathogen assessment. This stage can also be referred to as a characterization of human health effects as a result of contact with the microbe. It is an important step because it specifies the population that might be exposed to the agent as well as outlines what happens to the affected individual due to having been exposed to the pathogen. In addition, oftentimes a person can be exposed to a pathogen by more than one pathway. When this happens, it is necessary to identify all the possible health effects associated with each pathway so that a complete understanding of potential outcomes is recognized. The duration of the illness, a consideration of symptomatic versus asymptomatic aspects, and risk of dying are three important areas to be considered. The range of sensitivity or susceptibility to health effects from the microbe by different members of the population may need to be considered. In most cases, the assessment of health effects will rely on epidemiological and clinical information. Because pathogens often have an affinity for a certain host, animal studies will only rarely be used to predict human illness (ILSI 1996).

B.4. Risk Characterization

The final phase of the risk assessment is to formulate a risk characterization. This is composed of results from both the exposure profile and the host-pathogen profile. The
primary goal of this stage of the assessment is to estimate the likelihood of adverse human health effects occurring as a result of a defined exposure scenario to a microbial contaminant or medium. The two parts of this characterization consist of a risk estimation and a risk description. The former describes the types and magnitude of effects anticipated from exposure to the microbe or medium while the latter expresses the confidence in the risk estimates. Ultimately, whether or not the assessment sufficiently addresses the goals outlined in the problem formulation should be answered in the risk characterization as well (ILSI 1996).

C. Analysis Of Dose-Response Data

In order to execute a risk assessment for *Naegleria fowleri*, examination of dose-response data must be performed. Since this organism is one in which most infected persons die, it has been impossible to obtain reliable data on the initial number of amebas in the dose received by PAM patients. Currently, only animal dose response data have been available for analysis. Therefore, any information generated from this portion of the assessment must be converted or adjusted to apply to human beings. To analyze the dose-response data, it was necessary to use an Excel computer program designed by Dr. Douglas Crawford-Brown. Using a journal article entitled “*Naegleria fowleri* Infection Acquired by Mice Through Swimming in Amebae-Contaminated Water” (David T. John and S. L. Nussbaum 1983), the dose of amebas per inoculum for each group of mice was
entered into the program along with the total number of mice per group and the number of mice surviving at each dose. The best usable data was found to be from the five minute swim time of the mice. From this animal infectivity data, three models were fit to the data and a determination was made as to the model that best represented the data. The program analyzed the information using these three models: linear, two population and beta-Poisson. The equations used for each model ensue along with their descriptions:

1) Linear  \[ P(D) = 1 - \exp(-k \times D) \] where \( P(D) \) means probability of effect, \( \exp \) means exponential, \( k \) is a parameter to be set by fitting to the data and it represents the sensitivity of a person, and \( D \) represents dose.

2) Two Population  \[ P(D) = f_1 \times (1 - \exp(-k_1 \times D)) + f_2 \times (1 - \exp(-k_2 \times D)) \] with \( f_1 \) representing the percentage of the population having a low sensitivity, \( \exp \) means exponential, \( D \) stands for dose, \( k_1 \) is a parameter to be set by fitting to the data and it stands for the sensitivity of the \( f_1 \) population, \( f_2 \) represents the percentage of the population having a high sensitivity, and \( k_2 \) is a parameter to be set by fitting to the data and it symbolizes the sensitivity of the \( f_2 \) population.

3) beta-Poisson  \[ P(D) = 1 - (1 + (D/b))^a \] with \( D \) representing dose and \( b \) and \( a \) being constants to be set by fitting to the data.

Parameter values were obtained by fitting each model to the mouse infectivity data.
Once the best-fit parameter values were obtained from the models, they were entered into a program which produced a chart which displayed the results of each model's "goodness of fit" with respect to the data. This goodness of fit was used to determine the maximum likelihood estimates (i.e. the parameter values producing a model that best represented the data). Finally, using only the model that best represented the data, actual concentrations of pathogenic free-living amebas measured from the environment were entered into the program producing calculated risks for each of these concentrations.

D. Extrapolate Data from Animal to Human at Low Doses

To extrapolate the probability of effect from animal to human, it was necessary to multiply each concentration in the mice by a ratio coefficient of 0.6 to simulate interspecies differences in the relationship between environmental concentration and exposure to the nasal epithelium. Such differences would include the fact that mice breathe only through their noses whereas humans can breathe through both their mouths and their noses and the relationship between surface area of the nose and body mass in mice would be smaller than the relationship in humans. This value was determined by dividing the concentration of human surface area of the nose over the human body mass by the concentration of mouse surface area of the nose over the mouse body mass. This can also be expressed in this manner:
E. Compare Calculated Risks of PAM to Reported Risks of PAM from Recreational Water Exposure

Comparison will be made between the risks calculated from the Excel program and actual risks based on case occurrences. Also, using estimated recreational exposure events and responses received from state health departments, the risk of acquiring PAM from normal recreational water activity can be calculated from the available epidemiological data of actual case reports.

V. Analysis Phase

1.0 Hazard Identification for *Naegleria fowleri*

*Only one species of Naegleria, N. fowleri, has been found to be pathogenic to human beings (Bottone 1993), and it causes an often fatal meningoencephalitis in humans. Although precise amounts of the organism in its natural environment have been*
difficult to obtain; attempts at recovery suggest that it occurs in very low concentrations and most often with *N. lovaniensis*, which is a non-pathogenic species. The majority of cases have occurred during summer months to persons in recreational contact with warm waters. Once an individual has been exposed to this ameba by way of the nasal passages, the central nervous system is targeted and attacked, frequently resulting in mortal outcomes.

2.0 Exposure Assessment for *Naegleria fowleri*

Survival and multiplication of *Naegleria fowleri* in the environment is based on the organism being thermophilic and able to tolerate temperatures of 40 to 45 degrees Celsius. There is an active trophozoite, a transient flagellate, and a dormant cyst stage associated with this microbe. Under adverse conditions, the smooth, round, double-walled form of the cyst resists dessication and allows it to persist in nature. At other times, such as a change in ionic concentration of the environment, the trophozoite transforms into the nonfeeding flagellate form (Visvesvara 1993). This will eventually revert back to the trophic stage which feeds on bacteria such as *E. coli*, and rapidly multiplies by binary fission (Bottone 1993). In addition, transportation of this free-living ameba is provided by wind and water currents which facilitate the distribution and cosmopolitan nature of this microbe (Rodriguez-Zaragoza 1994). The encysted stage of this species’ life cycle enables the organism to resist environmental control measures,
such as disinfection, in addition to preventing dessication. The exact factors regulating whether or not the ameba encysts are presently undetermined, but it is hypothesized that adverse environmental conditions trigger the trophic stage to transform into the encysted stage. Naegleria fowleri, like all free-living amebas, are ubiquitous in the environment and prefer natural and artificially heated aquatic and soil environments (Bottone 1993).

Understanding the organism Naegleria fowleri requires a descriptive analysis of the pathogen’s occurrence, which is included under the exposure assessment phase of the risk assessment and addresses several factors.

- **2.1 Seasonality**

  *The population density of small thermotolerant amebas, such as N. fowleri, has its maximum development during the summer months. Correlations between water temperature and the concentrations of this organism have not been established quantitatively, but the seasonal abundance of these types of free-living amebas seems to be related to water temperature changes. In addition, contact of water with hot surfaces, such as heat exchangers, is very important for the occurrence of this species, and it may play a key role in the occurrence of episodes involving Naegleria* (Rodriguez-Zaragoza 1994).

- **2.2 Niche (potential for multiplication or survival in particular medium or material)**

  *Although this organism is present in the air as well as the water, the literature emphasizes the ability of this pathogen to survive based on its existence in water. A*
paper by Cerva (1971) suggests that when there are low concentrations of organic solutes present, the growth of *N. fowleri* is facilitated by these reduced concentrations. Moreover, there is literature to support the notion that the trophozoites benefit greatly when there is a high quantity of organic matter and very low or a complete lack of chlorine in the environment. This explains why it has been reported that poorly chlorinated, warm swimming pools have been shown to be favorable locations for growth of this pathogenic strain. Also, many cases of PAM have been contracted through exposure in these types of swimming areas (Rodriguez-Zaragoza 1994).

- **2.3 Temporal distribution /duration / frequency**

  *Because* *N. fowleri* *thrives in warm waters which are poorly or completely lacking in chlorine*, the temporal distribution associated with this organism would be whenever waters are warmest for a particular body of water.

- **2.4 Amplification, die-off, persistence**

  *N. fowleri* has a cyst form that allows it to persist in nature when adverse conditions arise. However, in the event that the trophozoites have not encysted and remain in the environment, they will be sensitive to dessication, temperatures lower than 4 degrees Celsius, and freezing. Therefore, in areas that have severe winters and/or months when temperatures can fall to below 4 degrees Celsius, the trophozoites will undoubtedly be eliminated (Rodriguez-Zaragoza 1994).

- **2.5 Indicators and/or surrogates for indirect evaluation**

  *Naegleria fowleri* is considered to be a cosmopolitan organism, and has almost
always been isolated together with *N. lovaniensis*, which is non-pathogenic, thermotolerant, and more abundant. This explains why *N. lovaniensis* has been the only isolate in some releases of hot waters, hot springs, and other places where *N. fowleri* was predicted to occur. This does not mean that *N. fowleri* is absent, but rather suggests that the population of *N. fowleri* could be too low to be detected with current methodology (Rodriguez-Zaragoza 1994). This is best supported by the fact that *N. fowleri* has only been measured in the environment at very low concentrations or not been found at all. Moreover, it has been difficult to estimate the number of amebas necessary to produce this disease in humans, primarily due to the inability to quantitate the original dose at post-mortem. *N. lovaniensis* may be an indicator for *N. fowleri*, but it is not known if they occur at a constant ratio that would facilitate estimation of concentrations of the latter based on concentrations of the former.

- **2.6 Spatial distribution**

  When in water, this free-living ameba must attach to particulate matter suspended in the water column in order to feed. Since it is already known that these organisms live at interfaces and that interfaces concentrate nutrients, high rates of amebic activity occur in these areas, which in turn enhance microbial survival. The quantity of suspended particulate matter plays a very important role in the amount of activity displayed by the amebas in the water column (Rodriguez-Zaragoza 1994).
• Reported concentrations of *N. fowleri* and other free-living amebas in environmental waters

As shown in Table 1 concentrations of free-living amebas, including *N. fowleri*, have varied widely, with as many as 1/5 ml (200/L) in a cooling lake to as few as 1/560 L in thermally polluted and freshwater lakes.

**Table 1. Concentrations of Pathogenic Free-Living Amebas in Environmental Waters**

<table>
<thead>
<tr>
<th>Location of Water</th>
<th>Type of Water</th>
<th>Organisms Sampled For</th>
<th>Amount Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oklahoma</td>
<td>Freshwater</td>
<td><em>Naegleria</em></td>
<td>1 pathogenic free-living ameba / 3.4 liters</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acanthamoeba</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Leptomyxid</em></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>Thermally Polluted And Freshwater Lakes</td>
<td><em>Pathogenic Naegleria</em></td>
<td>1 pathogenic <em>Naegleria</em> / 560 liters</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Man-made Artificial Cooling Lake</td>
<td><em>Thermophilic Amebas</em></td>
<td>1 pathogen / 5 milliliters</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Thermophilic Naegleria spp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Naegleria fowleri</em></td>
<td></td>
</tr>
</tbody>
</table>

3.0 Exposure Response for *Naegleria fowleri*

The only human health effect resulting from exposure to *N. fowleri* is the disease known as primary amebic meningoencephalitis (PAM). Once the amebas invade the nose, they migrate along the olfactory neuroepithelium toward the brain reproducing in the subarachnoid space and then they encroach on the cerebral cortex where they produce the rapidly and oftentimes fatal meningoencephalitis. The incubation period
may vary from two to seven days after which symptoms begin to occur. The virulence and pathogenicity of this microbe is exemplified by the adherence of the trophozoites to nasal mucosal surfaces, rapid locomotion exhibited through tissue at 37 degrees Celsius which aids in their invasive potential, production of the cytopathic enzymes elastase and protease, and the ability to phagocytize host neutrophils (Bottole 1993). The significant pathologic features in PAM have been confined to the central nervous system from which the disease does not spread to other organs within the body (Martinez 1993). Normally healthy children or young adults are the predominant population affected by this disease. The majority of patients have been in the 15-18 year age group although cases have ranged in age from five years to sixty years (Visvesvaran 1993). It is believed that the infection mechanism is initiated when amebic trophozoites are driven deep into the victim's nasal passages after diving into contaminated water. Once in the nose, they invade the olfactory neuroepithelium and migrate to the brain, multiply in the subarachnoid space, invade the cerebral cortex, and produce a rapidly fatal meningoencephalitis. This is the only route of infection associated with this organism (Martinez 1993). Also, people already infected with this disease cannot be a source of infection for others; therefore, there is no potential for secondary spread connected with this organism.
Figure 1

(a) 
- Brain
- Central nervous system
- Spinal cord
- Peripheral nervous system

(b) 
- Dura mater
- Arachnoid
- Pia mater
- Cerebrum
- Cerebellum
- Pons
- Medulla
- Spinal cord
- Central canal
- Subarachnoid space of spinal cord
- Subarachnoid space of brain
- Spinal meninges
The data used to estimate the dose-response of *N. fowleri* in humans are dose-response data for mice swimming in test water seeded with known concentrations of *N. fowleri* and shown in Table 2 (John, D. T. and Nussbaum, S. L.). Of these data only the dose-response data for a five minute swimming exposure were used in the analysis because it provided the best usable data. The results of the analysis using the Excel program are shown in Table 3, which summarizes the values of the key variables in each of the models (linear, two population, and beta-Poisson). Figure 1 plots the actual experimental dose-response data points as well as the point estimates produced by each model as calculated from the best-fit model parameters. These results indicate that the two population model best fit the data, followed by the beta-Poisson model and finally the linear model.
Table 2

<table>
<thead>
<tr>
<th>Conc (am/ml)</th>
<th># dosed</th>
<th># effects</th>
<th>Conc (am/ml)</th>
<th>P(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>1.00E+04</td>
<td>10</td>
<td>1</td>
<td>10000</td>
<td>0.1</td>
</tr>
<tr>
<td>1.00E+05</td>
<td>10</td>
<td>4</td>
<td>100000</td>
<td>0.4</td>
</tr>
<tr>
<td>1.00E+06</td>
<td>10</td>
<td>7</td>
<td>1000000</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>5.00E-62</td>
</tr>
<tr>
<td>k</td>
<td></td>
</tr>
<tr>
<td>Two Subpopulations</td>
<td>0.4</td>
</tr>
<tr>
<td>f1</td>
<td>3.00E-52</td>
</tr>
<tr>
<td>k1</td>
<td>0.6</td>
</tr>
<tr>
<td>f2</td>
<td>5.00E-72</td>
</tr>
<tr>
<td>k2</td>
<td></td>
</tr>
<tr>
<td>beta-Poisson</td>
<td>7.00E-01</td>
</tr>
<tr>
<td>a</td>
<td>1.20E+05</td>
</tr>
<tr>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2

Microbial Risk Assessment (*Naegleria fowleri*)

![Graph showing microbial risk assessment](image-url)
4.0 Risk Characterization for *Naegleria fowleri*

4.1. Risk Estimation

Based on information obtained using the Excel program and experimental data for mouse intranasal infectivity through swimming, the linear two population model provides the best fit for the data, followed by the beta-Poisson, and lastly the linear or exponential model. The linear two population model may fit the data best because it is the only model to acknowledge varying sensitivities within a given population. When actual reported concentrations of pathogenic free-living amebas were used to predict risk using the two population model, the greatest risk to humans was predicted to be $1.48 \times 10^{-6}$ when the highest measured concentration of one pathogenic free-living ameba per five milliliters (200 / Liter) was used. A more moderate concentration of one pathogenic free-living ameba per 3.4 liters produced a risk to humans of $2.17 \times 10^{-9}$. The lowest measured concentration of one pathogenic free-living ameba per 560 liters resulted in a $1.32 \times 10^{-11}$ risk to humans. However, as it currently stands, the United States Environmental Protection Agency does not consider a risk lower than $1.00 \times 10^{-6}$ to require direct and immediate attention. Although the predicted risk from *Naegleria fowleri* at its highest measured concentration to date is slightly above that, no specific regulations have been developed that target this pathogenic free-living ameba. It is noteworthy that the highest reported concentration of *N. fowleri* was from a cooling lake, which is not a body of water typically used for primary contact recreation.
4.1.a. Sources of Uncertainty in the Risk Characterization

- Concentration of amebas in environmental conditions
- Fraction of these amebas which are as infectious as those used in the mouse studies
- Fraction of population swimming in non-treated waters (i.e. pools)
- Length of swim
- Relative fraction of amebas which reach the target tissue (brain) after entering the nasal passages in mice and humans
- Immune reaction efficacy in mice and humans

Before any regulations can be set into place regarding this organism, all of these sources of uncertainty must be addressed prior to providing a final risk estimate.

4.2. Risk Description

*The risk estimate established for this risk assessment is based on certain assumptions. The most important is that mouse infectivity data can be successfully utilized in order to compare with and extrapolate to hypothesized human infectivity. Another is that all the amebas involved in the exposure events have the same virulence properties so that none have an advantage of increased infective capabilities over any others. It is also assumed that all of the contaminated water thrust up the nose has the same amount of contact time in humans as in mice. Moreover, this risk estimate is based*
on an exposure time through swimming of five minutes. It is important to note that if the
length of exposure increases, so too will the risk of acquiring this disease. An
uncertainty associated with this risk estimate is how much water actually enters the nose
during an exposure episode. That amount undoubtedly can depend on such things as the
size of the nose, amount of nose hair present that may filter some of the pathogens, as
well as use of nose clips that can drastically reduce the amount of water entering the
nose originally. In addition, certain host factors may play a role in allowing some
subpopulations of humans to be more susceptible to this disease than others. Hence,
there is moderate confidence in the risk estimates produced for this report because of the
significance of the uncertainties associated with the exposures, human susceptibility, the
infectious organism and the disease it produces. This is also due to the sufficiency of the
data as well as the models used to predict the risk of acquiring the disease.

E. Comparison Between Estimated Annual Exposure Events and Actual Case
Occurrences

Another approach to estimating or characterizing risk is to compare calculated
risks based on dose-response and occurrence data to reported risks based on
epidemiological data and exposure events. There are data for reported cases of PAM and
estimates of recreational activities in freshwater, specifically swimming. Therefore one
can compute the number of reported annual PAM cases per the annual number of
exposure events. Using the 1993 article, "Free-Living Amebas: Infection of the Central Nervous System" by A. Julio Martinez, M.D., as of September 1, 1991 sixty-three cases had been recorded in the United States resulting in roughly two cases of PAM occurring each year since 1965. After analyzing the responses obtained from the state health departments, that data was found to result in the same number of reported cases as referenced in the aforementioned article. (Table 4)
<table>
<thead>
<tr>
<th>State</th>
<th>Reportable Disease</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Arizona</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Arkansas</td>
<td>Yes</td>
<td>7</td>
</tr>
<tr>
<td>California</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>Connecticut</td>
<td>No</td>
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*Note: DNS = Did not state*
When that figure is compared to the estimated number of exposure events thought to have taken place per year, it appears that this disease does occur on a very limited basis. For example, according to the State Division of Parks and Recreation, Recreational Resource Services, and the Army Corps of Engineers, in North Carolina it is calculated that there are nineteen million exposures annually. Yet, there have only been two cases documented of this disease in the state. Consequently, a person who resides in North Carolina and participates in freshwater recreational activities would have a 1.00 E-07 chance of obtaining PAM (Table 5). This risk estimate is even more miniscule than what was predicted by the risk assessment. Because this would be a worse case scenario for a single year in North Carolina, the estimated PAM risk produced for this state is actually an overestimation. For the U.S. in 1994, national statistics report that there were sixty million two hundred seventy-seven thousand exposure events. Again using two as the number of actual cases, there appears to be a 3.32 E-08 risk of obtaining PAM (Table 5). Because the proposed number of exposure events used for North Carolina and the U.S. include all types of water exposures, these risk estimates would be higher if only freshwater exposures were used. Since obtaining the number of freshwater exposures exclusively was not possible, all types of water exposures had to be used. With all of this information in mind, overall there appears to be a low risk of acquiring primary amebic meningoencephalitis from ordinary recreational freshwater contact.
Table 5

Calculated vs. Actual Risks of PAM

<table>
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<th>North Carolina</th>
<th>United States (1994)</th>
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<td>2 Cases</td>
<td>2 Cases</td>
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<tr>
<td>19 Million Exposures</td>
<td>60,277,000 Exposures</td>
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<tr>
<td>Predicted Risk: 1.00 E-07</td>
<td>Predicted Risk: 3.32 E-08</td>
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VI. Conclusion

This paper has established that in the human population there is a low risk of acquiring primary amebic meningoencephalitis from normal recreational exposure to water contaminated with *Naegleria fowleri*. In water contaminated with the pathogen at as high a concentration as one pathogen per five milliliters, a two population model predicted a risk of 1.48 E-06. Since the parameter values used are considered a major source of uncertainty, this estimate would be affected. There may be other data that allow the models to fit better. While there are many assumptions and uncertainties associated with this organism and the actual exposure events associated with it, confirmation that any or all of them may affect whether or not the disease presents itself is either currently unknown or not fully understood. Certain elements intrinsic to the host
are thought to play a role in the susceptibility of some subpopulations of humans. Furthermore, comparisons made between annual exposure events and actual case occurrences show there to be even less of a chance of acquiring PAM through normal recreational water contact than predicted by the QRA. In general, the risks estimated by the QRA and those calculated from epidemiological data are reasonably consistent.

VII. Future Research

The free-living ameba *Naegleria fowleri* is a very difficult organism to isolate from the natural environment. Although it is described as being ubiquitous in nature, to date, very rarely has it been recovered from "suspect" sites. Even though these locations are strongly considered to contain the pathogen, oftentimes no trace of *Naegleria fowleri* is found at all. Therefore, future research needs to focus on improved recovery methods of this deadly microbe. Also, since current data presents an incomplete and complexing view as to all the environmental factors needed that contribute to the growth and proliferation of the organism, more research must be directed toward becoming aware of all factors required for its propagation and distribution in water. There must also be improved awareness of the occurrence and recovered concentrations of *Naegleria fowleri* from the environment. Even though this organism and the disease it is responsible for in humans has been recognized for over thirty years, there is very limited data in these areas present in the literature. Since little attention has been given to this free-living ameba as
a disease-causing organism, the responsibility to prevent infection caused by it will rest mainly with individuals exercising certain personal preventive measures of their own. Since *Naegleria* causes only one disease of human concern and it is most often correlated with water exposure, the best precaution to prevent becoming afflicted with primary amebic meningoencephalitis is to avoid activity in warm, fresh waters and poorly chlorinated swimming pools. This directive should especially be followed during warm summer months since the episodes have all followed a seasonal pattern. If, however, exposure does occur in these types of environments, attention must be paid to prevent water from entering the nasal passages since this is the only route of exposure to this disease. Most importantly though, is to design public health measures that are more proactive regarding this organism. Warnings posted at sites where *Naegleria fowleri* has been recovered and cases of PAM have originated must become routine practices. Even though the risk of obtaining this disease is low, making the public aware of the possibility that a dangerous organism may be lurking in a certain recreational body of water is small price to pay for saving just one person from death caused by this pathogenic free-living ameba.
VIII. References


John, D.T., Personal communications, April and June, 1997.


Visvesvara, G.S., Personal communications, March and April, 1997.
