

SWIMMING POOL DISINFECTION TECHNIQUES & PITFALLS

*D.M. Bonnick, BSc, MSc, CChem, MRSC, (Member) **

**Technology Manager, Wallace & Tiernan, Priory Works, Tonbridge, Kent,
TN11 0QL, UK.*

*This paper was presented at CIWEM's 'Swimming Pools & Spa Baths - Effective
Control of Water Quality and Public Health' Conference at the Chancellors
Hotel & Conference Centre, Manchester,
on 30th November 2005.*

Introduction

Swimming pool water is the potential recipient of a wide range of pathogenic micro-organisms. These may be delivered by a variety of routes including direct excretion by bathers, transport on the body, or growth within the filter bed. Without positive steps to inhibit the viability of these organisms the swimming pool would become a major health hazard. In practice the use of disinfection and filtration is practically ubiquitous and swimming pool related infections are relatively infrequent.

In order to maintain effective control of disease causing organisms in the swimming pool it is necessary to appreciate the sources of potential contamination, the types of pathogenic organism, the routes of infection and the mechanisms of disinfection. It is also important to appreciate that the steps taken to reduce the potential for disease transmission may have the potential to adversely affect the swimming environment. This may have consequences both for bather comfort and health.

Effective swimming pool disinfection will take account of the need to inactivate any potential pathogens with the need to provide a pleasant swimming experience without adverse long term health effects.

The Challenge

A typical bather may bring pathogenic organisms into the swimming pool via sweat, urine, mucus, saliva, hair, skin scales, faecal matter and general "dirt". Other potential sources of contamination include faulty plumbing and, for outdoor pools, bird droppings, dust and insects. Pathogenic organisms may be categorised into viruses, bacteria, fungi or protozoa. Once present in the water there is the potential for transfer of viable organisms to other bathers who may ingest them. It is therefore essential to provide a chemical environment in the pool which ensures the most rapid inactivation of the full range of pathogens. It is also desirable to reduce the introduction of pollution into the pool as much as possible by encouraging the use of showering and toilets before bathing.

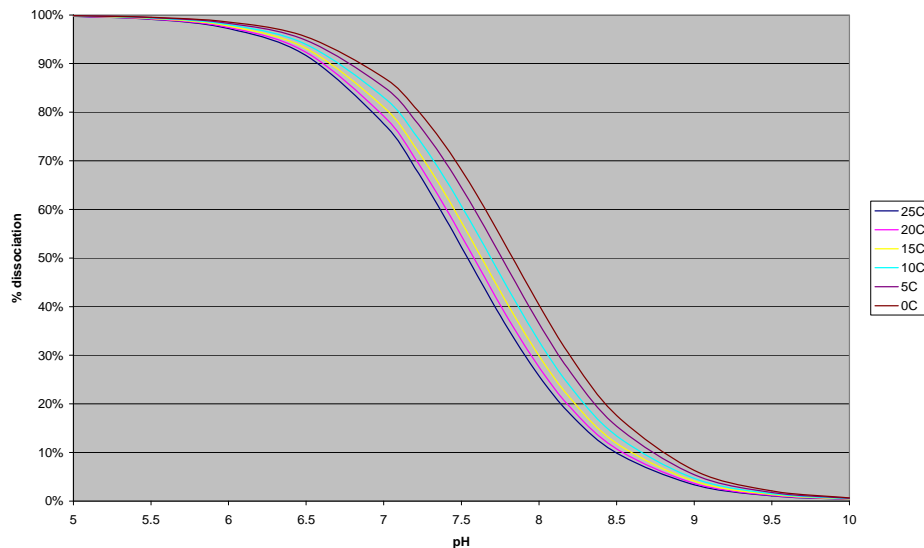
Chlorination

The most common means of providing protection against cross-bather infection is the use of chlorine residual.

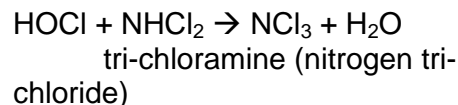
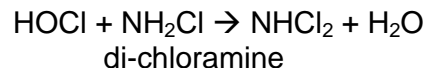
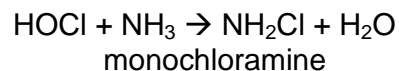
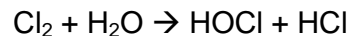
Chlorine may be supplied to the pool water in a variety of forms. Chlorine gas (Cl_2), sodium hypochlorite solution (NaOCl) and calcium hypochlorite are most commonly used but chlorine dioxide is also used and some pools use a combination of chlorine and bromide salts. It is well known that chlorine gas and hypochlorites dissolve in water to produce the compound hypochlorous acid (HOCl). The hypochlorous acid is in equilibrium with its anion,

hypochlorite (OCl^-) and the proportions of each are determined by the pH, temperature and conductivity of the treated water. The ubiquitous DPD test for free chlorine residual measures both hypochlorous acid and hypochlorite ion.

The significance of this for the disinfection of swimming pools and spas is that hypochlorous acid is a very much more effective disinfectant than hypochlorite ion. The familiar graph showing the variation of hypochlorous acid as a proportion of the measured chlorine residual demonstrates the importance of pH control for effective disinfection.



Chlorine reacts with a number of the common contaminants of swimming pools to produce a range of disinfection by-products (DBPs). Some of these are of concern as they pose potential risks to health and may adversely affect the comfort of bathers and spectators. Ammonia is found in pools as a consequence of the presence of urine. Chlorine reacts with ammonia to produce chloramines as follows:



The type of chloramine formed is dependent on the pH and the ratio of chlorine to ammonia. While

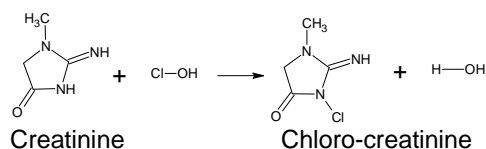
monochloramine is not a particular problem, dichloramine results in a strong chlorine odour and may cause eye irritation. In Germany DIN19643 states that chloramines must not exceed 0.20 mg/l.

Nitrogen trichloride is pungent and will cause extreme discomfort to bathers and spectators. It is released to the atmosphere where it accumulates if ventilation is not sufficient. Levels of 0.1- 0.57 mg. m⁻³ have been detected.¹ An association between nitrogen trichloride levels in the pool atmosphere and increased incidence of asthma has been demonstrated in several studies.^{2 3}

This is of concern for pool-side workers such as swimming instructors and life-guards as they may be exposed to significant levels of nitrogen trichloride over the course of the working day. Correct maintenance of the pool environment with adequate ventilation, dilution, pH control and an adequate level of free chlorine should minimise the formation and accumulation of chloramines. The presence of free chlorine indicates that the so called "break-point" has been reached. In principle this means that chloramines have been oxidised to innocuous compounds. In practice, however this is rarely the case and there will always be a level of chloramines in the pool. However further steps may be taken to hold chloramines at acceptably low levels. These include the dosing of powdered activated carbon onto the filters and ultra-violet treatment.

Other nitrogen containing compounds such as creatinine and amino acids will react with chlorine

to produce N-chloro (chloramine) compounds. These may also cause odour problems. A particular problem arises as many of these compounds are not removed by adding extra chlorine to the pool. This was observed by Lomas⁴ and was attributed to the presence of chloro-creatinine.



Chloro-creatinine is only removed from the pool by dilution, adsorption onto carbon or oxidation by ozone or UV.

Chlorine may also react with organic contaminants to produce a group of compounds known as trihalomethanes (THMs). The trihalomethanes are chloroform (CHCl₃), bromoform (CHBr₃), bromodichloromethane (CHCl₂Br) and dibromochloromethane (CHClBr₂). Tests carried out on eight London pools⁵ gave an arithmetic mean concentration of 121.1 µg/l of chloroform. In Germany the DIN19643 standard stipulates a maximum level for THMs in swimming pools of 20 µg/l. The concentration of chloroform and total trihalomethanes (TTHMs) was correlated to the number of bathers and the Total Organic Carbon concentration. Various health effects have been attributed to the presence of THMs in drinking water supplies. Consideration has also been given to the effects of exposure to THMs in swimming pools. Particular attention has been given to the possible association between THM exposure and bladder cancer⁶ and increased

numbers of birth defects⁷ and low birth weights.⁸ Each different group of pathogens is affected differently by the chlorine residual in the pool. Our knowledge of the way in which chlorine affects micro-organisms has been enhanced in recent years partly as a consequence of discoveries in medical science. White blood cells, also known as leucocytes, are known to respond to potentially pathogenic organisms in the body by engulfing them in the process called phagocytosis. It is currently believed that the leucocyte achieves the destruction of the organism by releasing oxidative toxins including hypochlorous acid.^{9 10} This has led to a number of studies seeking to discover the mechanisms by which these toxins, in particular hypochlorous acid, cause the inactivation of their target.

Viruses

Viruses typically consist of genetic material (nucleic acid) surrounded by protective capsid. The nucleic acid may be either a single strand RNA or a double strand DNA. The capsid consists of protein sub-units. Capsid and nucleic acid are together called the Nucleocapsid. The Nucleocapsid may be contained in a viral envelope. Infection is caused by the viral nucleic acid being released into the host cell and taking over the DNA to produce replicas of the virus.

Viruses which have been shown to have caused disease outbreaks in swimming pools include: Adenovirus, Hepatitis A virus (HAV), Echovirus and Norwalk virus.¹¹

Li et al ¹² have demonstrated that chlorine inactivates the Hepatitis A virus by attacking a section of the nucleic acid known as 5'NTR. They found that a chlorine residual of at least 10 mg/l for 30 minutes was required to completely inactivate the Hepatitis A virus. However, Peterson et al ¹³ have shown that chlorine residuals of 0.5 – 1.5 mg/l for 30 minutes were sufficient to inactivate most, but not all, HAV infectivity while 2.0 – 2.5 mg/l will completely destroy the infectivity. They note that HAV is somewhat more resistant to chlorine than other enteroviruses.

Considerable variations in the susceptibility of different viruses to chlorine have been reported by Engelbrecht et al. ¹⁴ They found that the susceptibility of viruses to chlorine disinfection is a function of virus type, pH and the ionic nature of the surrounding medium. The viruses they investigated were all more rapidly inactivated by chlorine at lower pH values consistent with the greater reactivity of hypochlorous acid relative to hypochlorite ion. They found that pH also affected inactivation independently of the HOCl dissociation and that the presence of other ions notably chloride was very significant.

HAV, Norwalk and Echoviruses are largely spread by the faecal-oral route. This means that, in order for disease transmission to occur in a swimming pool, there has to be a release of faecal matter, or possibly vomitus. At least one recorded HAV outbreak linked to swimming pools was related to accidental contamination by sewage.¹⁵

Adenoviruses may cause pharyngoconjunctival fever, an infection of the pharynx and conjunctiva. Outbreaks of this disease have been associated with swimming pools.^{16 17} Cases of the disease were linked to inadequate levels of chlorination in the swimming pools.

Bacteria

Bacteria consist of a nucleoid containing DNA, ribosomes containing RNA, cytoplasm, a plasma membrane and a cell wall. The energy requirements of bacteria are met via the formation of adenosine 5' –triphosphate (ATP) which is either catalysed by soluble enzymes present in the cell cytoplasm or by enzymes bound in the cell membrane.¹⁸

Bacteria which have been linked to swimming pool and spa related disease include: *Mycobacterium marinum*, *Mycobacterium avium*¹⁹, *Pseudomonas aeruginosa*²⁰, *Escherichia coli*, *Legionella* spp. and *Leptospira interrogans*.

Chlorine in the low concentrations used for disinfection swimming pools and drinking water has been shown to be capable of inhibiting certain enzymes essential for bacterial cell survival. Knox et al²¹ demonstrated that sulfhydryl enzymes were inhibited by chlorine. From studies with *E. coli*, Camper and McFeters²² postulated that the chlorine attacked the sulfhydryl enzymes located in the cell membrane. Barrette et al²³ have shown that hypochlorous acid affects an enzyme in the cell membrane which is responsible for energy transport. The enzyme known as F₁ –ATPase is required for the

synthesis of the ATP. Inactivation of the ATPase will therefore diminish the energy available resulting in the death of the bacterial cell.

E. coli has been shown to be very susceptible to chlorine disinfection. It has been widely used as an indicator species to demonstrate effective disinfection. Most strains of *E. coli* are non-pathogenic and commonly found in the gut. However, the serotype *E. coli* 0157 has been shown to cause serious illness. Several outbreaks of disease have been traced to bathing.²⁴ The bathing waters concerned included untreated lakes and an untreated paddling pool.

Pseudomonas aeruginosa is a very common environmental bacterium found in a wide variety of habitats. It can form bio-films and is very resistant to disinfection. *P. aeruginosa* is an opportunistic pathogen which may cause infections in susceptible individuals. It has been shown to be responsible for outbreaks of folliculitis in whirlpools, hot-tubs and, less commonly, swimming pools.²⁵

Legionella spp. Bacteria may cause infection by inhalation of contaminated aerosols. The species most commonly associated with infection is *Legionella pneumophila*. Legionellosis is a form of pneumonia which may result in mortality rates of up to 15% in hospitalised cases.²⁶ Spas are responsible for most known outbreaks of legionellosis in recreational waters. Mycobacteria are particularly resistant to chlorine disinfection. The two main disease causing

species are *M. tuberculosis* and *M. leprae* causing typhoid and leprosy respectively. These are not of significance in swimming pool water. There are, however, a range of environmental mycobacteria which have been implicated in opportunistic infections of people with impaired immunity. They are commonly found in swimming pools but are not a significant cause of disease.²⁷ They can cause problems in some spas and hot tubs. This may be due to the higher bathing load to water ratio and the higher temperature. Mycobacteria are distinguished by their resistant cell wall which is waxy and hydrophobic. This makes them very resistant to disinfection. A study by Taylor et al²⁸ showed that CT values for chlorine of 51 – 204 were required for 99.9% inactivation of *Mycobacterium avium*. Lumb et al²⁹ showed that cases of lung disorders related to *Mycobacterium avium* were related to spas with inadequate disinfection.

Fungi

Fungi may be found in various locations in the swimming pool environment. They are not generally implicated in the spread of serious disease. A survey of fungal contamination in Iranian swimming pools³⁰ indicated that fungal contamination in pools is not significant. People using swimming pools may be more susceptible to opportunistic fungal infections due to the effect of moist conditions on the skin. Changing room floors may well host dermatophytic fungi of the *Trichophyton* genus. These may cause Tinea pedis (athletes' foot) infection. Proprietary disinfectants are available for controlling fungal

contamination of changing room floors.³¹

Protozoa

This is a very diverse group of organisms containing a number of pathogens which create particular challenges for swimming pool disinfection.

The main disease causing organisms are *Cryptosporidium parvum*, *Giardia lamblia*, *Naegleria spp.* and *Acanthamoeba spp.*

Cryptosporidium and *Giardia* are faecally transmitted organisms which can cause acute diarrheal illnesses (Cryptosporidiosis and Giardiasis). The organisms are of particular concern due to the resistance of the infective form of the diseases to chlorination.

Giardia lamblia cysts have been shown³² to require a free chlorine residual of 1.5 mg/l or greater at 25 deg. C for 10 minutes to ensure no detectable organisms were present. At lower temperatures inactivation took considerable longer.

Cryptosporidium parvum oocysts require considerably higher levels of chlorine than *Giardia*. They have been shown to be more susceptible to other disinfectants including chlorine dioxide and in particular to UV³³.

Naegleria and *Acanthamoeba* amoebae may cause a serious disease called primary amoebic meningoencephalitis. Cursons et al found that *Naegleria spp.* were killed by 0.79 mg/l total chlorine while *Acanthamoeba spp.* required 1.25 mg/l.³⁴

Organisms in combination

Many micro-organisms have the tendency to form associations with other micro-organisms. Where these associations colonise a surface they are referred to as bio-films. A bio-film may consist of a wide range of species including bacteria, fungi, grazing protozoa and nematodes. Where bio-films form they may make disinfection much more difficult to achieve due to the protective effect of substances excreted by certain of the organisms. Bio-films secreted by *P. aeruginosa* and *Klebsiella pneumoniae* have been shown³⁵ to reduce chlorine residuals to 20 – 30% of the bulk concentration of 2.5mg/l at the film surface and falling to zero at deeper levels. Much higher chlorine residuals were required to completely penetrate and inactivate the bio-film.

Protozoa may act as infective hosts for other types of organism. For example *Legionella pneumophila* has been shown to infect the amoebae, *Hartmannella vermiformis* and *Acanthamoeba polyphaga*.³⁶ The infected amoebae may act as *Legionella* infective agents for humans.³⁷

Bio-films may begin to form in areas where disinfection is inadequate, for example in poorly maintained filters. Once the bio-film is established it may be difficult to remove and may release harmful micro-organisms into the pool. The maintenance of appropriate levels of disinfectant at all times should ensure that bio-films are not established.

Summary

Chlorine in the form of hypochlorous acid can be seen to affect different organisms in different ways. Bacteria like *E.coli* require relatively low doses as they are inactivated by the damaging effect on enzymes in the cell membrane. Mycobacteria have much more resistant cell walls and require much higher doses of chlorine. *Pseudomonas aeruginosa* protects itself with extracellular polysaccharides making it very resistant to chlorine. Viruses vary in their response but chlorine appears to penetrate their protein coat to attack the DNA or RNA within.

Fungi are not of major significance within the pool and can be controlled by fungicides applied to pool surrounds and changing room floors. Protozoa generally may be controlled by hypochlorous acid at typical pool water concentrations but *Cryptosporidium* oocysts are resistant and need to be controlled by effective filtration possibly in conjunction with UV.

Strategies for Effective Disinfection

Effective disinfection of swimming pools and spas must take account of the potential contaminants in the pool, the varying susceptibilities of the different disease causing organisms that may be present and the chemistry of the disinfectant to be used.

A critical component of a pool disinfection system is the provision of adequate filtration. Without the continuous removal of suspended material from the pool it will be impossible to maintain adequate disinfection whichever process is

used. Filters should be sized to the capacity and loading of the pool and should employ appropriate coagulant aids which should be used in accordance with suppliers' instructions. Backwashing of the filters to remove accumulated filtrate should be carried out on a routine basis. It is important that accumulated organic material is removed from the filter during backwashing as it may act as a precursor to THM formation. Post filter dosing of chlorine can assist with reducing THM levels. Properly operated filtration with coagulation can remove much of the pollution from the pool water resulting in lower levels of organisms, lower chlorine demand and less disinfection byproducts.

The disinfectant used should be adequate to inactivate most of the potential pathogens in the pool. The inactivation should take place in a sufficiently short timescale to minimise the risk of cross bather contamination. This means that the disinfectant will have to be halogen based. It is likely that the disinfectant used will be chlorine in some form and where this is the case due consideration must be given to the chemistry of chlorine. The pH of the pool must be maintained at a sufficiently low level to ensure that as much of the free chlorine is in the hypochlorous acid form. At the same time the pH should not be so low as to cause discomfort to the bathers or increase the likelihood of corrosion. These considerations lead to a recommended pH range of 7.2-7.4. The pH level should ideally be controlled by an automated system to maintain a consistent pH in the pool at all times.

If chlorine or a chlorine donor is used then the free chlorine residual should be monitored automatically and the signal used to automatically maintain the residual at a level sufficient to ensure disinfection without causing discomfort to the bathers. In a typical UK pool this is likely to be between 1.0 and 1.2 mg/l free chlorine. Pools in other countries, in particular Germany where pool conditions are strictly controlled by the standard DIN 19643³⁸, may operate at lower free chlorine residuals.

The Redox potential of the pool water provides a valuable additional control parameter. DIN19643 states that the Redox potential should be between 750-770mV. Systems are available which use a combination of residual measurement and redox potential to enable the use of chlorine to be minimised.

It is essential to keep the level of contaminants entering the pool to a minimum. This can be achieved by encouraging the use of toilets, showers and disinfected footbaths before entering the pool. People who know they have an infectious disease should be discouraged from using the pool. Policies need to be in place to ensure an effective response to faecal or vomiting incidents in the pool.

The levels of pool contaminants may be reduced by maintaining a policy of adding fresh water to the pool in proportion to the bathing load. The publication "Swimming Pool Water"³⁹ recommends that 30 litres per day of fresh water are added for every bather using the pool.

Other approaches to maintaining the levels of contaminants and by-products at low levels include the use of ozone, ultraviolet, and activated carbon.

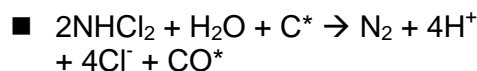
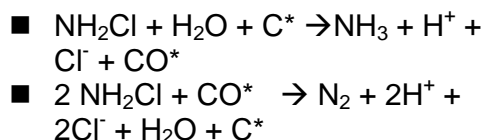
Ozone is used in conjunction with residual chlorination with the aim of improving the environment for bathers and spectators. It is normal to use it in combination with activated carbon filtration. This improves performance and prevents ozone from entering the swimming pool area.

Ultraviolet treatment may be used to reduce the levels of chloramines in the pool. UV at relatively low doses can also inactivate *Cryptosporidium* oocysts.



Wallace & Tiernan Jetpak Powdered Carbon System

Activated carbon powder is prepared into a suspension which is dosed prior to sand filtration. Provided that a uniform grade of sand is used a layer of carbon can then be formed in the filter. This adsorbs and breaks down chloramines in accordance with the following reactions.⁴⁰



THMs are adsorbed and discarded with the waste carbon during backwashing. This system has been widely used in Germany in the last 10 years to ensure compliance with the stringent requirements of DIN 19643 which stipulates a maximum of 200 µg/l chloramines and 20 µg/l THM (as chloroform).

Conclusion

Residual disinfection is essential for the control of the transmission of disease between bathers. Disinfection can result in the formation of undesirable byproducts which may have consequences for the health and comfort of bathers and staff. It is possible to provide safe and pleasant bathing conditions provided that the pool is properly maintained and treatment is properly designed, implemented and maintained.

¹ THICKETT, K.M., MCCOACH, J.S., GERBER, J.M., BURGE, P.S.

Occupational asthma caused by chloramines in indoor swimming-pool air *Eur. Respir. J.*;2002;19:827-832

² MASSIN, N., BOHADANA, A.B., WILD, P., HEREY, M., TOAMAIN, J.P., HUBERT, G. Respiratory symptoms and bronchial responsiveness in lifeguards exposed to nitrogen trichloride in indoor swimming pools. *Occup. Environ. Med.* 1998;55:258-263

³ BERNARD, A., CARBONNELLE, S., DE BURBURE, C., BUCHET, J-P., HERMANS, X., DOYLE, I. Lung hyperpermeability and asthma prevalence in schoolchildren: unexpected associations with the attendance at indoor chlorinated swimming pools. *Occup. Environ. Med.* 2003;60:385-394

⁴ LOMAS, P.D.R. The Combined Chlorine Residual of Swimming bath Water *J.*

Assoc. Publ. Analysts, 1967, 5, 27. p.27-36

⁵ CHU, H., NIEUWENHUIJSEN, M. Distribution and determinants of trihalomethane concentrations in indoor swimming pools. *Occup. Environ. Med.* 2002;59;243-247

⁶ VILLANEUVA, C.M., CANTOR, K.P., GRIMALT, J.O., DOSEMECI, M., MALATS, N., REAL, F.X., SILVERMAN, D., TARDON, A., GARCIA-CLOSAS, R., SERRA, C., CARRATO, A., CASTANO-VINYALS, G., ROTHMAN, N., KOGEVINAS, M. Bladder Cancer and Exposure to Disinfection Byproducts in Water Through Ingestion, Bathing, Showering and Swimming in Pools: Findings from the Spanish Cancer Study. *Epidemiology* Vol.15, No.4, July 2004.

⁷ WHITAKER, H.J., NIEUWENHUIJSEN, M.J., BEST, N.G. The Relationship between Water Concentrations and Individual Uptake of Chloroform: A Simulation Study. *Environmental Health Perspectives* Vol.111, NO.5, May2003

⁸ WRIGHT, J.M., SCHWARTZ, J., DOCKERY, D.W. Effect of trihalomethane exposure on fetal development. *Occup. Environ. Med.* 2003;60;173-180

⁹ HURST, K.J. BARRETTE, W.C. Leucocytic Oxygen Activation and Microbicidal Oxidative Toxins. *Critical Reviews in Biochemistry and Molecular Biology*, Vol. 24 Iss. 4 (1989) p. 271 -328.

¹⁰ KLEBANOFF, S.J. Phagocytic Cells: Products of Oxygen Metabolism. *Inflammation: Basic Principles and Correlates*. Eds. J.I Gallin, I.M. Goldstein, and R. Snyderman. Raven Press, Ltd. New York. 1988.

¹¹ WHO. Guidelines for Safe Recreational-water Environments Final Draft for Consultation Vol. 2: Swimming Pools, Spas and Similar Recreational-water Environments August 2000

¹² LI, J.W., XIN, Z.T., WANG, X.W., ZHENG, J.L., CHAO, F.H. Mechanisms of Inactivation of Hepatitis A Virus by Chlorine. *Applied & Environmental Microbiology*, Oct. 2002, p.4951-4955 Vol.68, No.10

¹³ PETERSON, D.A., HURLEY, T.R., HOFF, J.C., AND WOLFE, L.G. Effect of Chlorine Treatment on Infectivity of Hepatitis A Virus. *Applied and Environmental Microbiology*, Jan. 1983, p. 223 – 227. Vol. 45, No.1

¹⁴ ENGELBRECHT, R.S., WEBER, M.J., SALTER, B.L., AND SCHMIDT, C.J.

Comparative Inactivation of Viruses by Chlorine. *Applied and Environmental Microbiology*, Aug. 1980, p. 249-256. Vol.40 No.2

¹⁵ WHO. Guidelines 2000

¹⁶ WHO Guidelines 2000

¹⁷ HARLEY, H., HARROWER, B., LYON, D., DICK, A. A primary school outbreak of pharyngoconjunctival fever caused by adenovirus type 3. *Commun. Dis. Intell* 2001;25:9-12

¹⁸ HADDOCK, B.A. AND JONES, C.W. Bacterial Respiration. *Bacteriological Reviews*, Mar. 1977 p.47-99 Vol. 41, No. 1.

¹⁹ LUMB, R., STAPLEDON, R., SCROOP, A., BOND, P., CUNLIFFE, D., GOODWIN, A., DOYLE, R., BASTIAN, I. Investigation of Spa Pools Associated with Lung Disorders Caused by *Mycobacterium Avium* Complex in Immunocompetent Adults. *Applied and Environmental Microbiology*, Aug. 2004, p. 4906-4910. Vol. 70, No. 8

²⁰ RATNAM, S., HOGAN, K., MARCH, S.B., BUTLER, R.W. Whirlpool-Associated Folliculitis Caused by *Pseudomonas aeruginosa*: Report of an Outbreak and Review. *Journal of Clinical Microbiology*, Mar.1986, p.655-659.

²¹ KNOX, W.E., STUMPF, P.K., GREEN, D.E., AND AUERBACH, V.H. The Inhibition of Sulfhydryl Enzymes as the Basis of the Bactericidal Action of Chlorine. *J. Bacteriol.* 1948 55 (4) p. 451 – 458.

²² CAMPER, A.K. AND MCFETERS, G.A., Chlorine Injury and the Enumeration of Waterborne Coliform Bacteria. *Applied & Environmental Microbiology*. Mar. 1979, p.633-641. Vol.37, No.3.

²³ BARRETTE, W.C., HANNUM, D.M., WHEELER, D.W. AND HURST, J.K. General Mechanism for the Bacterial Toxicity of Hypochlorous Acid: Abolition of ATP Production. *Biochemistry*, Vol. 28, No. 23, 1989 p. 9172 – 9178.

²⁴ WHO. Guidelines 2000

²⁵ RATNAM ET AL 1986

²⁶ WHO Guidelines 2000

²⁷ LEONI, E., LEGNANI, P., MUCCI, M.T. AND PIRANI, R. Prevalence of mycobacteria in a swimming pool environment. *Journal of Applied Microbiology* 1999, 87, 683-688

²⁸ TAYLOR, R.H., FALKINHAM, J.O., NORTON, C.D., LECHEVALIER, M.W., Chlorine, Chloramine, Chlorine Dioxide, and Ozone Susceptibility of *Mycobacterium avium* *Applied and*

Environmental Microbiology, Apr. 2000, p.1702-1705

²⁹ LUMB, R., STAPLEDON, R., SCROOP, A., BOND, P., CUNLIFFE, D., GOODWIN, A., DOYLE, R., BASTIAN, I. Investigation of Spa Pools Associated with Lung Disorders Caused by *Mycobacterium Avium* Complex in Immunocompetent Adults. *Applied and Environmental Microbiology*, Aug. 2004, p. 4906-4910. Vol. 70, No. 8

³⁰ NANBAKSH, H., DIBA, K. HAZARTI, K., Study of Fungal Contamination of Indoor Public Swimming Pools. *Iranian J. Public Health*, Vol. 33, No.1, pp.60-65 2004

³¹ BOBICHON, H., DUFOUR-MORFAUX, F., PITORT, V. In Vitro Susceptibility of Public Indoor Swimming Pool Fungi to Three Disinfectants. *Mycoses*. 1993 Sep-Oct; 36(9-10):305-311

³² JARROLL, E.J., BINGHAM, A.K., & MEYER, E.A. Effect of Chlorine on *Giardia lamblia* Cyst Viability. *Applied & Environmental Microbiology*, Feb. 1981, p.483-487. Vol.41, No.2

³³ MORITA, S., NAMIKOSHI, A., HIRATA, T., OGUMA, K., KATAYAMA, H., OHGAKI, S., MOTOYAMA, N., FUJIWARA, M., Efficacy of UV Irradiation in Inactivating *Cryptosporidium parvum* Oocysts. *Applied & Environmental Microbiology*, Nov. 2002, p.5387-5393 Vol.68, No.11

³⁴ CURSONS, R.T.M., BROWN, T.J., KEYS, E.A. Effect of Disinfectants on Pathogenic Free-Living Amoebae: in Axenic Conditions. *Applied & Environmental Microbiology*, July 1980, p. 62-66 Vol, 40, No.1

³⁵ DE BEER, D., SRINIVASAN, R. & STEWART, P.S. Direct Measurement of Chlorine Penetration into Biofilms during Disinfection. *Applied & Environmental Microbiology*, Dec.1994, p.4339-4344

³⁶ ABU KWAIK, YOUSEF, GAO, LIAN-YONG, STONE, B. J., VENKATARAMAN, CHANDRASEKAR, HARB, O. S. Invasion of Protozoa by *Legionella pneumophila* and Its Role in Bacterial Ecology and Pathogenesis *Appl. Environ. Microbiol.* 1998 64: 3127-3133

³⁷ ROWBOTHAM, T.J., Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *Journal of Clinical Pathology* 1980;33:1179-1183

³⁸ DIN 19643

³⁹ Swimming Pool Water - Treatment and Quality Standards. Pool Water Treatment Advisory Group. 1999.

⁴⁰ SCARAMELLI, A.B. AND DIGIACOMO, F.A. Effect of sorbed organics on the efficiency of ammonia removal by chloramine-carbon surface reactions. *J. Wat. Pollut. Control Fed.*, 1977, 49, No.4, 693-705