ABSTRACT

Minimizing the risk from Legionnaires Disease - a potentially fatal form of pneumonia - is becoming increasingly controlled by legislation as high profile outbreaks continue to occur throughout the world. Chlorine dioxide is recognized in the UK guidelines and in other studies as being an effective biocide against Legionella. The reasons for this are attributed to the importance that biofilms play in the growth of Legionella bacteria and the superior effectiveness of chlorine dioxide in the control of these biofilms..

1. INTRODUCTION

Legionnaires Disease has had a high profile in the US for many years following major outbreaks in many countries. The recent outbreak in Holland has further increased the awareness of this problem in the rest of the world. This paper looks at the background to the bacteria that cause this disease, how it is transmitted, and the main risk areas. The treatment of water systems to reduce the risk of Legionnaires Disease usually requires non oxidizing biocides or disinfectants such as chlorine or bromine, however the properties of chlorine dioxide make it an effective alternative. This is backed up by case histories where chlorine dioxide has been used to control the risk in various water systems and discussion as to why ClO2 is particularly effective for this application.

2. LEGIONNAIRES DISEASE

Legionnaires disease is a potentially fatal form of pneumonia. Initial symptoms include fever, headaches, muscle pains, a dry cough and breathing difficulties. Some patients also develop diarrhea, vomiting and may become delirious. infection is caused by breathing in droplets of water infected with the bacteria that cause Legionnaires' disease. about 1 in 100 people who are exposed to an infection source actually contract the disease, of these it is fatal in around 12% of cases depending on the susceptibility of the population. For example in hospitals or retirement homes some people will be more at risk due to age or illness.
The disease was first recognized following an outbreak in the US amongst a convention of the American Legion in 1976. 221 people attending the convention or working at the hotel caught Legionnaires Disease and 34 died. The source of the infection was traced to the air conditioning system and eventually the bacteria responsible was isolated and named ‘Legionella pneumophila'.
Although this was the first time it was identified it is not a new bacterium and has since been found to have been present in cases previously thought to be pneumonia as far back as 1943. In the UK a major outbreak occurred in 1985 at Stafford Hospital where 101 people contracted the disease and 28 died. The current UK legislation was issued

mainly as a result of the outcry following this and other high profile incidents such as the outbreak from a cooling tower on top of the BBC Offices in central London. 231 cases of Legionnaires Disease and over 20 deaths were reported following an outbreak in Holland at a large flower show near Amsterdam.

The total number of people who catch Legionnaires Disease is not known, in the UK around 150 - 200 cases are reported annually but an estimate of actual cases is given as 1,100 [1]. Some estimates for the US alone suggest that between 10,000 and 15,000 people catch Legionnaires Disease each year.

The same bacteria have also been found to be responsible for outbreaks of a non-fatal flu like illness called 'Pontiac Fever'. There are a number of theories as to why these outbreaks are different such as virulence of the strain or where it attacks the lung.

3. THE BACTERIA THAT CAUSES LEGIONNAIRES' DISEASE

Legionella pneumophila - the bacteria that is associated with over 90% of cases of Legionnaires Disease - is a rod shaped bacteria of the Legionella ceae family. Further sub-divided into different Sero groups (usually 1 to 16) and Sero group 1 is most commonly found to be the cause of the disease in the UK. There are at least another 37 different species of Legionella now recognized and others such as L. micdadei and L. feelei have also been associated with a small number of cases.

Legionella bacteria are found naturally in rivers, lakes and soil although usually in low numbers. Conditions that favor growth are water temperatures between 20°C and 45°C and a source of nutrient - particularly other biological matter or sediment such as rust or scale deposits.

Bringing the favorable conditions together for growth of Legionella bacteria and the possibility of a water spray being produced of the correct size to be inhaled (thought to be between 3 and 5 microns) the systems that are most likely to present a risk are:

- Water systems incorporating a cooling tower
- Water systems incorporating an evaporative condenser
- Hot and cold water services in the workplace such as taps and showers - particularly those fed from storage tanks rather than directly from the mains.
- Air conditioning or humidifying systems
- Other plant and systems containing water which is likely to exceed 20°C and which may release a spray when being used or maintained. Examples include spray ponds, spa baths, ornamental fountains and paint spray booths.

4. GUIDELINES ON THE CONTROL OF LEGIONNAIRES DISEASE AND THE USE OF CHLORINE DIOXIDE

The main legislation in the US governing the control of Legionnaires Disease are the Health and Safety at Work Act 1974 and the Control of Substances Hazardous to Health Regulation 1994. Under section 16 of these regulations the Health and Safety Commission has issued a 'Code of Practice' with the purpose of providing practical guidance as regards the risks from legionellosis [2] which came into force in January 1992. This Code does not address any technical aspects of controlling the risk but for this refers to the Health and Safety rules referred to as HS9G) 70 [3] .

The current HS(G)70 has been revised once in 1993 and mentions chlorine dioxide only as a note in paragraph 107 in the section on 'Methods of Water Treatment'. In this section biocides are advocated as an essential part of the water treatment regime and advice is given on the use of chlorine followed by a note that other biocides such as bromine or stabilized chlorine dioxide and non-oxidizing biocides may be effective if used properly. interesting to note that the HSE have recently published their proposals for a further revision of HS(G)70 and in this consultative document 4 chlorine dioxide appears to be more accepted as a method of treatment.

They also recommend continuous dosing for oxidizing biocides, but if applied as a shot dose they suggest an effective concentration be present for at least 4 out of every 24 hours. Large industrial systems can also be dosed intermittently based on water recirculation rate, the time period depending on the operating characteristics of the system.

A further indication to the acceptance of chlorine dioxide as an effective treatment to control the risk of Legionnaires' disease is given in a Supplement to HS(G)70 issued by the HSE in 1998 called 'The Control of Legionellosis in Hot and Cold Water Systems'. There is a section on chlorine dioxide (Paragraphs 22 to 27) where two references are given that show it to be effective in water systems at levels of 0.5 mg/l 5. Although this guidance does also point out the difficulties of maintaining total oxidant levels below the US Drinking Water Inspectorate level of 0.8 mg/l particularly in systems with a low turnover of water. Failure to comply with either the Code of Practice or HS(G)70 guidelines is not in itself an offense in the US, but in the event of any problems, or a site inspection by the DEP / EPA, the onus is on the owner or manager of the business to prove that they have complied with the requirements in some other way.

5. THE EFFECTIVENESS OF CHLORINE DIOXIDE AGAINST LEGIONELLA BACTERIA

A small number of studies have been carried out on the effectiveness of chlorine dioxide against Legionella pneumophila and all have concluded that is an effective biocide for controlling the risk in water systems. At Ashland, Drew Industrial Division our early laboratory tests in 1987 on Legionella bacteria suspended in cooling tower water showed a very effective kill rate at use concentrations: One of the most important studies was carried out by The Building Services Research Association (BSRIA) 5 who compared the effectiveness of chlorine dioxide against heating water to 60°C as originally recommended in HS(G)70 for water Services. BSRIA have set up three commercial size hot and cold water services systems each 1,350 litres in capacity - equivalent to that used in an office building containing around 50 people. These test rigs were infected with a mixture of waterborne micro-organisms including a non-pathogenic strain of Legionella pneumophila, Serogroup 1 and the bacterial population allowed to become established for eight weeks before treatment. The 16 week trial with chlorine dioxide that followed this showed continually good results with no planktonic Legionella being detected and levels of all bacteria considerably reduced in both hot water (30 – 35°C) and cold water (<20°C) systems. The study concluded that for cold water systems 0.1 - 0.2 mg/l ClO2 was effective while hot water systems required up to 0.35 mg /l ClO2.

Another example is taken from a study following three cases of Legionnaires Disease within three months from a newly built hospital hot and cold water system. The main reasons were traced to poor design; storage of cold water above 20C, a storage capacity at least twice the size of that needed - leading to low flow rates, temperature stratification in the calorifiers, and oil-based organic sealing compounds in pipe joints. Hyper chlorination provided only a short-term effect before the bacteria counts returned to unacceptable levels and Legionella organisms were detected. Following a disinfection with chlorine dioxide at 50-80 mg/l for eight hours in the storage tanks and 1 hour at the taps no Legionella was detected and biofilm was dramatically reduced although not totally removed.

Due to its chemistry Chlorine dioxide is an ideal treatment for some types of cooling systems, particularly where the pH is high (greater than 8.5) or there is some system contamination. Ashland, Drew Industrial Division as a water treatment company is currently treating over 100 cooling systems in the US and 10 in the UK with chlorine dioxide to control microbiological levels. Generally for large cooling systems we recommend adding to recirculation rate rather than make-up rate or volume, as around 50% to 90% of the chlorine dioxide can be lost from the cooling tower depending on its efficiency.

A typical treatment regime would involve daily additions of chlorine dioxide to achieve a minimum of 0.5 -1.0 mg / l of free chlorine dioxide for at least 1 hour. This often involves a longer actual dosing period while a 0.5 ppm reserve is being built up. More chlorine dioxide may be required depending on system conditions; our target is to achieve a general bacteria count of 104 bacteria per ml. or less, as this is generally accepted as indicating that the system is under control.

All of the systems treated have achieved good microbiological control, unfortunately only three of the cooling systems have been regularly sampled for Legionnaires' disease as currently there is no requirement in the guidelines to do so. The results are summarized below:

Site 1: Borehole water is treated continuously with chlorine dioxide at 0.2 - 0.5 mg / l free residual. This water is supplied to 10 cooling towers of various sizes each fitted with a chlorine dioxide (TwinOxide Dosing system) to achieve 4 hours dosing per day. ClO2 concentrations vary over the towers during this period and although 0.5 mg / l is aimed for, the actual free residual could be as low as 0.05 mg /l and as high as 3 mg/l. During dosing, the bacteria levels are less than 103 bacteria per ml. and outside this time general levels are recorded at 104 or less. Legionella samples have been taken quarterly every year since 1994. Of 260 samples taken over this period only two have been positive at low levels (less than 103 per liter, none were Serogroup 1) with only one of those being in the last four years.
Site 2: A 14 m3 cooling system with recirculating water containing significant amounts of organic contaminant. Microbiological control is through chlorine dioxide at 3 x 1 hour shot dosing per day, each eight hours apart. Maximum ClO2 level is controlled by a redox system to 0.75 mg / l. Microbiological levels are usually 103 bacteria per ml. or less and Legionella samples have been taken twice a year for three years with no positives being recorded.
Site 3: A 79 m3 recirculating cooling system that has regular contamination with a food based contaminant that has a high nutrient content for bacteria is dosed continuously at 0.5 mg / l (TwinOxide) chlorine dioxide, controlled by a redox system. Prior to chlorine dioxide being used this system was very difficult to control and Legionella positives were recorded. Bacteria counts are now less than 104 bacteria per ml and a Legionella test taken since ClO2 has been used was negative.

6. THE ROLE OF BIOFILMS IN THE GROWTH OF LEGIONNAIRES DISEASE

One of the questions we have asked is 'Why use chlorine dioxide over other oxidizing or non-oxidizing biocides for the control of Legionnaires Disease?' and we suspect that the answer lies in its effect on biofilms.
Biofilms build up rapidly on surfaces within a water system. Within a few hours of exposure, a film of organic monosaccharides and fatty acids from microbiological activity elsewhere in the system water will build up, bacteria will attach themselves to the surface by means of extracellular polysaccharides, and grow to form micro-colonies which eventually merge to form a microbial film. This matures as stalked and filamentous bacteria develop and extend further into the bulk water phase. The biofilm entraps inorganic corrosion and scale products, and organic detritus from other microbial growth - providing both nutrients and bulk. Further micro-organisms such as fungi, protozoa, diatoms and even small nematode worms will colonize the biofilm as it becomes established. Biofilms can cause problems such as reduced flow-rate, poor heat transfer, blockages due to sloughing off of the slimes and corrosion due to the

action of sulphate reducing bacteria in the oxygen deficient areas in the lower regions of these films. It is also now known that biofilms play a key role in the growth of Legionella.

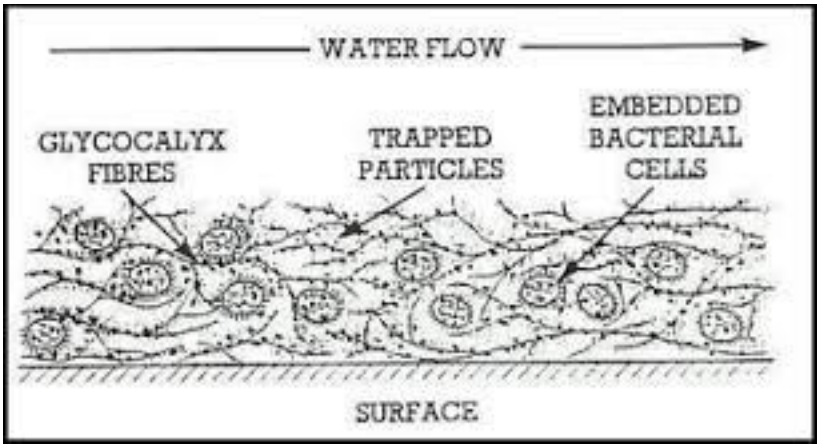

Diagram 1: Typical Biofilm structure

Legionella bacteria are known to be relatively difficult to grow in the laboratory and require special media containing charcoal, iron and amino-acids. It's hard therefore to see how they can multiply to problem levels in cooling water and it is hypothesized that Legionella do not grow within the bulk water but within established biofilms - the slime and sludge that builds up within recirculating water Systems particularly in low flow areas or dead-legs. Research into this has confirmed that this is the case; some estimates suggesting that 99% of the Legionella in cooling Systems will be found associated within biofilms [8].

Other studies [9], [10] have shown that a surprising property of Legionella bacteria is they can survive and grow within protozoa such as amoeba. Photographs have been taken of vacuoles within infected amoeba containing many hundreds of Legionella bacteria and some reports have even suggested that the bacteria can only breed when inside a protozoan [11], [12]. An American report found that 64% of sites with Legionella pneumophila Serogroup 1 also contained the amoeba Hartmanella vermiformis, but this amoeba was only found in 18% of sites that did not harbour Legionella bacteria.

It is stressed that although amoeba can be difficult to kill with biocides, they can generally only exist in any number within established biofilms: therefore removal of the biofilms will ensure effective control of the amoeba.

The importance of biofilms is recognized in the Consultative Document issued by the Health & Safety Executive in the UK who state in paragraph 11 that 'Legionella also require a supply of nutrients to multiply. Sources can include, for example, commonly encountered organisms within the water system itself such as algae, amoeba and other bacteria. The presence of sediment, sludge, scale and other material within the system together with biofilms within a water system are also thought to play an important role in harboring and providing favorable conditions in which the Legionella organisms may grow'.

If biofilms provide conditions, nutrients and protection for Legionella bacteria then monitoring and controlling biofilm formation must become a priority when minimizing the risk from Legionnaires Disease. There are a number of methods available for assessing build up of biofilms in industrial systems. These include direct methods such as 'biofilm monitors' where coupons or studs are placed into a flow of water either directly or on a side-stream to mimic what is happening on the actual system surfaces. The coupons are extracted and the degree of biofouling measured either by removal of the biofilm followed by conventional agar plate determination of the number of bacteria, or directly by fluorescent microscopy where active biofilm can be determined on the coupon. Indirect methods include monitoring the effectiveness of heat exchange surfaces s biofilm is an extremely good insulator.

7. CHLORINE DIOXIDE FOR CONTROL AND REMOVAL OF BIOFILMS

Following the discovery of the role biofilms play in the growth of Legionella bacteria, work has been carried out on the effectiveness of biocides against Legionella contained in biofilms [13], [14]. Not surprisingly it has been found that substantially longer contact times or much higher concentrations of biocide are required when compared to that needed to kill the planktonic bacteria in the water. If this is the case it is reasonable to assume that biocides effective against biofilms will have better results at controlling Legionella in actual system waters. It is interesting to find therefore that there is considerable evidence that TwinOxide chlorine dioxide is extremely effective at removing and controlling biofilms,
examples being:
The BSRIA report mentioned earlier in this paper [5] concluded that around 0,1 mg / l TwinOxide chlorine dioxide produced effective biofilm disinfection in GRP cisterns and hot water pipework running at a reduced temperature of 30 – 35°C. On rubber washers in hot and cold water outlets 0.4 ppm was effective in killing biofilms. At the first of these Symposiums in 1996 a paper was presented 15 that described biofilms and their formation in more detail. They also described work carried out to assess the effect of chlorine dioxide on biofilms [16], [17] and concluded that TwinOxide ClO2 is more effective than HOCl in eliminating biofilm growth. They attributed this effectiveness to inhibition of cell division and biopolymer synthesis in those bacteria which may have become attached to the surfaces. There are many case histories available that highlight how effective TwinOxide chlorine dioxide is at removing biofilms from contaminated systems, examples contained in an Ashland Technical Paper 18
include: A major tomato processing plant where bacterial slimes could not be controlled in the flume system with 8 - 10 mg/l chlorine: TwinOxide ClO2 at slightly over 1.0 mg/l removed old slimes and stopped new slimes from developing. A vegetable oil processing plant with excessive slime in the cooling tower despite chlorine treatment was cleaned up within two days using chlorine dioxide at a tenth of the chlorine addition rate.

One of the earliest examples was given at the 1958 annual meeting of the Institute of Food Technologists in Chicago 19 who were attempting to recycle used wash water. With chlorine they experienced severe slime build up but found that chlorine dioxide was highly effective in curtailing bacteria and slime formation in pea and corn canneries. They also noticed that only minor deposits of slime were detected on equipment or belts that contained or were being sprayed with ClO2 treated water even after many hours of factory operation. It is the experience of Ashland, Drew Industrial Division that in applications such as paper manufacture, food processing and cooling water systems chlorine dioxide is an excellent slimicide and can usually be used alone in situations where both chlorine and a surfactant based bio dispersant were previously required. The reasons for its excellent bio dispersant properties have been mentioned above, [16], [17] another area of investigation is whether the unique chemistry of chlorine dioxide also plays a role. It is known that there is a gradient of reduced oxygen and pH towards the bottom of established biofilms. Generally, the first reduction product of chlorine dioxide is chlorite, it is feasible that in the reduced pH conditions deep within a biofilm this could be converted back to biocidal active chlorine dioxide. This is unproven but may be a reason for Cl02's enhanced biocidal effect against biofilms as alternatives such as chlorine and bromine are more likely to be deactivated as organic halogens.

8. SUMMARY

Evaluation of the effectiveness of biocides against Legionella bacteria in bulk system water will not provide adequate information on how they will perform in actual systems. It is the interaction with complex microbiological biofilms that is important in practice.
It is likely therefore that TwinOxide chlorine dioxide is proving to be the most effective biocide in cooling water systems, process systems and water services due to its superior activity against the biofilms that provide an environment where Legionella bacteria can thrive.

REFERENCES

[1] Joseph, Carol; PHLS Communicable Disease Surveillance Centre
Paper presented at the WMS Conference 'Managing Building
Services Effectively to Prevent Legionnaires Disease' Sept 1996, London
[2] HSE 'The Prevention or control of legionellosis (including legionnaires' disease)
Approved Code of Practice (L8) HMSO 1991 ISBN 0 11 885659 6
[3] HSE The control of legionellosis, including legionnaires' disease
HS(G)70 HSMO 1991 ISBN 0 11 885660 X
[4] HSE Available on the Internet www.open.gov.uk/hse/condocs/ as CD143
'Proposals for the revision of the Approved Code of Practice "The prevention and control of legionellosis (including legionnaires' disease)" and associated guidance' June 1999
[5] BSRIA 'Chlorine dioxide water treatment for hot and cold water services
Technical Note TN 6/96 BSRIA ISBN 0 8602 2486 4
[6] Chlorine dioxide field study Thomas et al New Gross Hospital, Wolverhampton
ProMinent page 6/7
Chlorine Dioxide Published by Ashland / Drew Ameroid
2nd International Conference on Chlorine Dioxide in Paris
Bello Zon® Chlorine Dioxide Generation Systems
WV10 0QP (unpublished study)
[7] Walker J.T. et al 'Control of Legionella pneumophila in a hospital water system by chlorine dioxide' Journal of Industrial Microbiology (1995)15384-390
[8] McCoy, B: Talk given at the CTI 1997 Education Seminar on Legionella Control and reported in the CTI Journal, Vol 18. No. 2
[9] Rowbotham TJ. Preliminary report on the pathogenicity of Legionella pneumophila for freshwater and soil amoeba. J Clin Pathol 1980 33:1179-1183
[10] Anand CM et al. 'Interaction of L. pneumophila and a free living amoeba'
J Hyg Camb 198391:167-178
[11] Hart C., Monteil H. Interactions between free-living amoeba and Legionella in the environment Wat Sci Tech 1988; 20:235-239
[12] Holden EP. et al. Intracellular growth of Legionella pneumophila within Acanthamoeba castellani Neff. Infection and Immunity 1984; 45:18-24
[13] Green P.N. 1993, Efficacy of biocides on laboratory generated Legionella biofilms. Letters in Applied Microbiology 17, 158-156
[14] Wright J.B, Ruseska I., Costerton J.W., Decreased biocide susceptibility of adherent Legionella pneumophila Journal of Applied Bacteriology 1991 71 531 –538
[15] Belluati M., Bartole L., Bressan G. 'Once-through Cooling Systems Antifouling Treatment by Chlorine Dioxide Proceedings of First European Symposium on Chlorine Dioxide and Disinfection Rome 1996 p 133-151
[16] Bartole L., Bressan G. (1993) Effects of Chlorine Compounds on Periphyton (Primary Slime: A Preliminary Report Ichem, Biodegradation and Biodeteriation 9: 123-127
[17] Bartole L. et al Fouling Control Systems using chemicals inside industrial pipings: a Laboratory Simulation Oebalia 19 (suppl): 375 –382
[18] Ashland Chemical Co. Drew Industrial Division 'Chlorine Dioxide: An Alternative to Chlorine for Disinfection in Water Systems OX-TP-009 Presented at the Engineering Panel of the Campden Food Preservation Research Association Chipping Campden, UK 1995
[19] Welsh and Fotinazzo: Use of Chlorine Dioxide for Cannery Sanitisation and Water Conservation Food Technology March 1959
Source M.J. Turvey, Ashland Chemicals