What's New In U.S. UV Action?

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As many of the new U.S. EPA drinking water regulations move towards full enactment, the role of UV treatment technologies in helping public water supplies comply with these regulations becomes more important. The draft preamble to the Groundwater Rule published recently by U.S. EPA has formalized the ability of communities to use UV to comply with this regulation. In light of recent research showing possible promise of several UV technologies to inactivate Cryptosporidium at cost effective dosages, there is much water industry activity in the area of using UV to comply with pending and future EPA rules such as the Interim Enhanced Surface Water Treatment Rule, the LT1ESWTR, the LT2ESWTR and the Disinfectants/-Disinfection By-Product Rule. Most notably:

1. A UV Workshop to evaluate where we are with the application of UV technology to drinking water treatment and identify key data gaps was held by U.S. EPA on April 28 and 29, 1999 in Washington, D.C.

2. AWWARF has solicited three RFPs for research relating to applications of UV or UV-Advanced Oxidation Processes for Drinking Water Treatment (see AWWARF's web site (www.awwarf.org).

3. On March 4, 1999 the University of New Hampshire's Environmental Research Group launched a new research initiative and formed the UV Team. The UV Team is a multidisciplinary (environmental engineering, environmental microbiology, polymer chemistry and chemical engineering) research team dedicated to research on UV Irradiation. UV Team research has a focus on three primary areas: disinfection (in air, drinking water, wastewater); advanced polymer development for UV equipment (fouling resistant sleeve materials, new sensor materials, etc.); and use of UV in advanced oxidation processes to treat organics (in air, drinking water, wastewater). The UV Team was formed to build upon the existing eight years of experience, including twelve projects, that UNH-ERG has completed in the area of UV research. The UV Team initiative has a broad funding base with four on-going projects currently supported by: federal grants (NOAA, U.S. EPA and U.S. DOD), foundation grants (AWWARF and EPRI) and private sector companies. Part of this research initiative includes the development and distribution of UV Mail to aid in rapid technology transfer. For more information contact Jim Malley (jim.malley@unh.edu).

..... *Ultraviolet Light – A Solution to the Cryptosporidium Threat ?* .....

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A Brief History of Waterborne Cryptosporidiosis

Since the first outbreak of waterborne cryptosporidiosis was reported in Braun Station, Texas (D'Antonio et al, 1984), dozens more have been reported world-wide in the U.K. (Lisle and Rose, 1995), USA (Kramer et al, 1996), Sweden (DeJong et al, 1997), Canada (Ong et al, 1997), and Japan (Kuroki et al, 1996). The largest outbreak to date occurred in Milwaukee in 1993, with an estimated 400,000 people affected and over 100 deaths (MacKenzie et al, 1994). The following year, an outbreak of cryptosporidiosis in Las Vegas, Nevada led to 20 deaths in immunocompromised individuals (Roefer et al, 1996).

Most of the cryptosporidiosis outbreaks (D'Antonio et al, 1985; Lisle and Rose, 1995; and Kramer et al, 1996) which have been investigated thoroughly can be related to a deficiency in treatment, improper operations, equipment failure, or inadequate source protection of groundwater supplies. Solo-Gabrielle and Neumeister (1996) reviewed the U.S. outbreaks from an operations viewpoint, and reported that half of the cryptosporidiosis outbreaks were associated with groundwater sources. However, the majority of affected individuals have been served by surface water plants using coagulant addition, filtration and chlorine disinfection. While treatment deficiencies and sub-optimal operational practices were found in some situations, all plants were in compliance with federal and state regulations.

Solo-Gabrielle and Neumeister have questioned how much protection is provided by conventional sand filtration processes, and suggested that filtration systems should be operated at optimum levels exceeding regulatory requirements in an effort to maximize public health protection. The water industry has responded to the threat posed by *Cryptosporidium* with programs such as the voluntary Partnership for Safe Water that assists plants in optimizing their treatment processes to consistently achieve water quality goals beyond those required by law. However, water utility managers are still haunted by the possibility of a cryptosporidiosis outbreak in their system even though every effort has been made to prevent it from occurring. This is due in part to our incomplete understanding of the sources and occurrence of the parasite and its fate and transport in the environment, coupled with the inadequacy of existing controls even when standards are met.

Regulatory Response

Cryptosporidium control now has become a primary focus of regulatory agencies in the U.S. and U.K., and this pathogen now is regulated as a drinking water contaminant in both countries. In the U.S., *Cryptosporidium* has just been regulated by means of the Interim Enhanced Surface Water Treatment Rule (IESWTR). The rule addresses control of *Cryptosporidium* through establishment of a maximum contaminant level goal (MCLG) of zero and treatment requirement in the same manner that *Giardia* was regulated in 1986 under the Surface Water Treatment Rule (SWTR). Surface water systems that require filtration must achieve at least a 2-log removal of *Cryptosporidium*. Turbidity requirements have been lowered to a level of 0.3 NTU in combined filtered water in 95% of the monthly measurements and cannot exceed 1 NTU at any time. Groundwater systems under the direct influence of surface water (GWUDI) also must comply with the new rules.

Cryptosporidium is added to the watershed protection requirements for systems avoiding filtration. Monitoring of the parasite at a maximum contaminant (MCL) still is not required due to problems in method reliability. Whether these new requirements will protect consumers from cryptosporidiosis remains uncertain. Two outbreaks, Las Vegas, NV (Roefer et al, 1996) and Waterloo, Ontario (Pett et al, 1993) occurred in systems which were meeting these new requirements at the time of the outbreaks.

In the U.K., the Drinking Water Inspectorate (DWI) has introduced a monitoring requirement which has set an MCL and monitoring is expected to begin in late1999. The requirement will be for continuous monitoring of certain surface water supplies. The requirement is unusual in that the monitoring will be daily and continuous (22 hours out of 24). The sampling point will be treated water at the outlet of the plant. The requirement is not health-based, but is a treatment-based standard, with an enforceable limit of 1 oocyst per 10 L. The rationale is that a plant should be removing oocysts to a level of <1/10 L as determined by a new analytical method with

similarities to the U.S. EPA Method 1622: Cryptosporidium in Water by Filtration/IMS/FA (U.S. EPA, 1999). While the monitoring data can and are expected to be used for enforcement, the details of this are still being developed.

The U.K. is the first country to regulate *Cryptosporidium* in drinking water directly by setting a numerical standard and requiring testing to meet that standard. There is a general requirement in the U.K. that drinking water "does not contain any element, organism, or substance .... at a concentration or value which would be detrimental to public health". Put another way, the water provided must be "fit for human consumption". Water that is likely to cause cryptosporidiosis is unfit for human consumption and supplying it would be an offense under Section 70 of the Water Industry Act of 1991 [Water Supply (Water Quality) Regulations, 1989]. The new *Cryptosporidium* regulations would not change this offense nor the penalty. An unlimited fine could be imposed on a water supplier convicted of this offense in a Crown Court.

The U.K. program will require a large effort to support the regulation, both in implementation of the program and continued oversight. The regulation was developed based on what is believed to be achievable analytically, i.e., it is considered possible to detect 1 oocyst in 10 L of water using the new DWI method. However, even if this standard is achievable, there is no guarantee that meeting this standard will prevent an outbreak of waterborne cryptosporidiosis. The regulation is based upon the premise that outbreaks occur when high and intermittent levels of oocysts pass into the distribution system during plant upsets. The DWI feels this regulation will allow them to detect such events and act on them through enforcement, causing plants to tighten operations to avoid prosecution. Whether this rule can predict or prevent outbreaks remains to be seen after implementation. A survey of water suppliers and regulatory agencies worldwide indicated that *Cryptosporidium* is not currently regulated elsewhere at this time, and monitoring for the parasite remains voluntary (Clancy and Hansen, 1999).

Cryptosporidium Control Through Water Treatment

Like *Giardia, Cryptosporidium* oocysts can be controlled by physical removal through filtration pro-cesses, although oocyst removals can be expected to be lower than for *Giardia,* due to their smaller size. *Cryptosporidium* removal has been assessed using a variety of treatment techniques and removals range from 2- to 4-logs in conventional systems (Ongerth and Pecoraro, 1995; Nieminski and Ongerth, 1995; Plummer et al, 1995; Hall et al, 1995). Diatomaceous earth filtration was shown to provide 3.8- to 6-logs of oocyst removal in bench-scale studies (Ongerth and Hutton, 1997). Membrane processes (ultrafiltration and microfiltration) are known to provide high levels (>6-logs) of oocyst removal (Jacangelo et al, 1995).

Unlike *Giardia, Cryptosporidium* oocysts which escape the filtration process are resistant to chlorine-based disinfectants at the concentrations and contact times practical for water treatment (Korich et al, 1990). This makes the physical removal process (coagulation, sedimentation, filtration) the most critical step in conventional water treatment in plants using chlorine for disinfection. Alternative disinfectants do exist.

Ozone is highly effective for *Cryptosporidium* control. Korich et al (1990) demonstrated that oocysts exposed to 1mg/L ozone reduced oocyst viability from 84% to 0% after 5 min at 35°C, and exposure times of 5 or 10 min resulted in 90% to 99.9% reduction in neonatal mouse infectivity, respectively. These data are further supported by Finch et al (1993), who also treated oocysts with ozone and found it to be highly effective for oocyst inactivation. Further work by Finch et al (1997), has shown that a synergistic effect occurs using combinations of certain disinfectants, resulting in a higher log inactivation of oocysts when the chemicals are applied sequentially than when each one is used individually. For example, an initial residual of 2.0 mg/L chlorine for 240 min resulted in a 0.4-log inactivation of *Cryptosporidium*; an ozone dose with an initial residual of 0.75 mg/L for 3.7 min resulted in 1.6-logs inactivation; but treating the oocysts firstly with the ozone, followed by chlorine, resulted in a 2.9-logs inactivation. Other combinations - ozone/monochloramine (1.8-logs), chlorine dioxide/free chlorine (2.9-logs), and even free chlorine/monochloramine (0.6-log) - have proved more useful than each oxidant when applied individually. The discovery of the effectiveness of sequential disinfection provided the first real option for water suppliers to provide a final barrier for *Cryptosporidium* control after filtration.

Let There Be Light

Ultraviolet light (UV) appears to be the newest addition in the war against *Cryptosporidium*, but was almost overlooked in the search for ways to inactivate oocysts. In retrospect, it appears that the early discovery of oocyst resistance to chlorine-based disinfectants left us with the prejudice that oocysts were extremely resistant to "anything and everything". However, when we review the literature, it appeared that the work that was conducted with UV was positive, but was not immediately capitalized upon as data became available. Only in the last six months has the scientific community begun to accept that UV may be a highly effective tool for *Cryptosporidium* control. The history of *Cryptosporidium* inactivation using UV light is brief, as is the review of the published literature that follows.

Lorenzo-Lorenzo et al (1993) used mouse infectivity experiments in a bench top system to assess *Cryptosporidium parvum* oocyst inactivation. There is a lack of clarity in the experimental details and the paper is difficult to interpret. Dr. Andrew Campbell communicated directly with the author to ascertain the details and interpret the findings (Andrew Campbell, personal communication). Lorenzo-Lorenzo used a single inoculum (2.5×10^4 oocysts), and assessed overall infection intensity after treatment by using low pressure (LP) UV. The results estimated a >3-log inactivation at 100-300 mJ•cm^2, although these data cannot be gleaned from the published paper.

Following this work, Campbell et al (1995) examined the Safe Water Solutions, Ltd. *Cryptosporidium* Inactivation Device (CID) designed for oocyst inactivation in clean (<1 NTU) water. These researchers demonstrated 2- to 3-logs inactivation of oocysts using the DAPI/PI (4',6'-diamidino-2-phenylindole and propidium iodide) vital dyes assay and excystation with a dose of LP-UV dose of 8,748 mJ•cm^2. They postulated that their ability to assess the limits of inactivation was restricted by the limits of the standard enumeration procedures that are used with the *in vitro* assays. Additionally, this study also tested the CID in a static rather than flowing mode for which it is designed to be used. These authors recommended a definitive study using mouse infectivity and operating the unit in the flow through mode according to its design.

In 1996, the American Water Works Association Research Foundation (AWWARF) and the Electric Power Research Institute/Community Environmental Center (EPRI/CEC) jointly funded a study to assess innovative electrotechnologies for inactivation of *Cryptosporidium*. This research was undertaken to determine if any commercially available innovative electrotechnologies were capable of inactivating *Cryptosporidium* oocysts in drinking water. Five electrotechnologies were challenge-tested using live oocysts under carefully controlled laboratory or field conditions. The five systems tested included:

- advanced UV light (represented by the CID),
- pulsed UV,
- conventional UV,
- acoustic shock, and
- resonant electric current.

Only advanced and pulsed UV were shown to inactivate oocysts under the experimental conditions tested. In retrospect, the study design used could have missed the potential of UV to inactivate oocysts entirely had lower doses of UV been applied (Clancy et al, 1998). Depending on one's beliefs, serendipity, blind/dumb luck, or divine intervention occurred next.

In the AWWARF/EPRI study, the objective was to demonstrate whether a given electrotechnology had any potential for oocyst inactivation. The study did not assess levels of inactivation, various doses of energy, or attempt to optimize any technology - it was a quick look-see to screen the various electrotechnologies for further study if warranted. To do this, the research team decided to use the *in vitro* surrogate assays (DAPI/PI, SYTO®-9, SYTO®-59, and maximized *in vitro* excystation) to make initial measurements as these assays are simple to perform and are inexpensive and were thought to correlate to animal infectivity. For those electrotechnologies that showed promise, additional studies would be conducted using animal infectivity, thought by the U.S. EPA and North American researchers to be the 'gold standard' for demonstrating loss of infectivity in disinfection trials. The vendors supplying equipment for assessment were asked to "give it their best shot a/k/a providing a high dose" as this was an initial demonstration and the electrotechnology had to pass this hurdle for future consideration.

Two electrotechnologies appeared to be successful at oocyst inactivation – pulsed UV and advanced UV (low pressure over an extended exposure period). Pulsed UV (Innovatech, Inc.) at 1900 mJ•cm^2 in a 10 gpm system provided >2 logs inactivation and the CID at 8,748 mJ•cm^2 at 400 gpm full scale provided >4-logs oocyst inactivation. Initial studies showed promise with the *in vitro* surrogates and animal studies supported the results. Conventional low pressure UV (180 mJ•cm^2) appeared to have no effect on oocyst viability as measured using the surrogates alone, and based on data accrued with *in vitro* viability assays, Clancy et al (1998) reported that it was ineffective for oocyst inactivation.

At this same time, Clancy et al (1999) were involved in another AWWARF study (in conjunction with the United Kingdom Drinking Water Inspectorate (DWI)), to determine which of the four *in vitro* surrogate assays (DAPI/PI, SYTO®-9, SYTO®-59, and maximized *in vitro* excystation) most closely predicted results of animal infectivity. The objectives were to identify one or more *in vitro* surrogates that correlated well with animal infectivity, allowing disinfection research to continue using an inexpensive, faster, but equally reliable assay. The study was large with a robust statistical design, and involved two U.S. and two U.K. labs so that identical trials could be conducted in both countries. The results of the UV trials using the Innovatech pulsed UV system showed that all four of the surrogate assays significantly underestimated oocyst inactivation when compared to oocyst inactivation as measured by mouse infectivity. It appeared that doses as low as 40 mJ•cm^2 (fresh oocysts) and 14 mJ•cm^2 (aged oocysts) provided over 2-logs inactivation by mouse infectivity while the surrogates showed less than 0.5-log inactivation. Further work on this project has shown that this phenomenon (lack of correlation between the *in vitro* assays and animal infectivity) extends to oocysts exposed to ozone as well.

At this same time, Finch et al, (1997), examining sequential chemical disinfection, also conducted experiments with UV light, and used mouse infectivity to assess inactivation. The unit used was a bench scale low pressure system and they calculated that UV doses of 1280 and 41,400 mJ•cm^2 were applied. The oocysts were stirred in a batch reactor which was a Wheaton glass bottle, with UV applied 11 cm from the glass. Finch et al (1997) reported "no detectable loss of infectivity" but also mentioned that the "data were not comparable with oocysts exposed in thin layers in a Petri dish or membrane filter". They went on to say that their "results are consistent with those reported by others where UV is not a very effective control of cysts or oocysts". These seemingly inconsistent results to those of Clancy et al (1998) can be easily explained. In the Finch experiments, the UV never reached the oocysts as glass is an effective barrier to UV light. Although high UV doses as calculated by lamp output were thought to be delivered, in reality no UV reached the oocysts.

By now the Clancy Environmental Consultants, Inc. (CEC) team – Jen Clancy, Marilyn Marshall (University of Arizona), Zia Bukhari, and Tom Hargy - had conducted dozens of UV inactivation studies since 1995 using mouse infectivity and the *in vitro* surrogate, and were feeling confident that UV was very effective for *Cryptosporidium* oocyst inactivation. The next work focused on medium pressure UV, and work was conducted in conjunction with Calgon Carbon Corporation, using bench-scale collimated beam (CB) units, followed with a demonstration project at >200 gpm using the Calgon Carbon Corporation Sentinel™ demonstration-scale unit. The objectives of this work were to:

- Determine the UV dose required from medium-pressure lamps for 3- to 5-logs inactivation of *Cryptosporidium* oocysts in finished water.
- Establish a dose-response curve for oocyst inactivation by using a CB apparatus at bench scale.
- Conduct demonstration-scale studies and compare oocyst inactivation data from the bench-scale studies to data obtained with the demonstration-scale studies.
- Compare the *in vitro* surrogate assays versus animal infectivity assays.

Oocyst viability was assessed using *in vitro* (DAPI/PI and maximized *in vitro* excystation) and *in vivo* (neonatal mouse infectivity) assays. Using the neonatal mouse infectivity assay, the bench-scale studies showed >4-logs inactivation at UV doses as low as 41 mJ•cm^2: the *in vitro* surrogate assays showed little or no inactivation at this and higher UV doses. The *in vitro* assays, which indicate oocyst viability, grossly overestimated the UV doses required to prevent oocyst infection in susceptible hosts. The demonstration studies, carried out under the National Sanitation Foundation (NSF)/EPA Environmental Technology Verification (ETV) program, provided results that agreed with the bench-scale results and showed that a UV dose as low as 19 mJ•cm^2 provided 3.9 logs inactivation of *Cryptosporidium* oocysts (Bukhari et al., 1999). Recent work by Finch and Belosevic (unpublished data) using CB studies appears to support the Bukhari et al. (1999) data. Additional work on medium pressure UV conducted at CEC using CB bench scale studies has shown that doses in the range of 6 to 9 mJ•cm^2 provide oocyst inactivation at >3.5-logs (unpublished data).

Where Are We Now and Where Are We Headed?

As usually is the case, what we know is much less than what we need to know. Regarding the situation of *Cryptosporidium* oocyst inactivation using UV light, the following statements appear to be true:

- ✓ Oocysts (the Harley Moon isolate) are highly susceptible (3- to 4-logs inactivation) to UV light (MP and pulsed) at relatively low doses (10 to 20 mJ•cm^2) using the neonatal mouse (CD-1) assay.
- ✓ Advanced UV is highly effective at high doses (8,748 mJ•cm^2); no information is available for this system at lower UV doses.
- ✓ Animal infectivity is needed to determine inactivation; the *in vitro* surrogates seriously underestimate inactivation.
- ✓ These generalities apply to filtered drinking water, as all experiments have been conducted using this matrix.
- ✓ UV is not a new technology and there is a high level of experience in disinfection applications for both water and wastewater worldwide.

What we don't know is all the rest. Issues to be resolved on the biology of *Cryptosporidium* include:

- Are there strain differences or differences based on oocyst production, processing, or storage that affect UV susceptibility?
- Are there differences in the mouse model (different mouse strains, infectivity assays, etc.) which need to be assessed?
- Is oocyst reactivation, as seen in bacteria, possible? ? If so, what are the minimal UV doses at which this can be prevented?
- Can UV disinfection of surrogate organisms (MS2 coliphage, *Bacillus subtilis*, etc.) be used to predict effectiveness against *Cryptosporidium*?
- Can alternatives to animal infectivity studies be used to demonstrate disinfection effectiveness, allowing significantly more research to be conducted?

Issues to be resolved on the engineering/operations side include:

- Are oocysts which penetrate conventional filters shielded by persistent coagulant or other particles, thereby reducing the effects UV irradiation?
- How can UV systems best be monitored to ensure consistent and effective operation in varying water quality or over time?
- It appears that UV light may be able to provide control of *Cryptosporidium* in drinking water. However, there is never a panacea in drinking water treatment. If UV proves to be as effective for *Cryptosporidium* control as the early studies indicate, it will be simply an additional tool for water suppliers to use. It is now our challenge to make this happen.

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