CORONA ENVIRONMENTAL CONSULTING

10 February 2016

To: All Stakeholders Interested in Ballast Water Management
Re: Performance Monitoring of BWMS (46 C.F.R. § 162.060-10(b)(1))

I am a microbiologist and water quality and treatment expert with over 30 years of experience in basic and applied research. I was a founding member and Second International President of the International Ultraviolet Association. I am the recipient of the 2012 Water Research Foundation's Research Innovation Award for contributing to the knowledge of UV disinfection of *Cryptosporidium.* In 2014 I received the American Water Works Association A.P. Black Research Award for excellence in water supply research.

I am writing because I became aware of the U.S. Coast Guard's initial decision not to allow the use of culture methods (most probable number or MPN) to assess the efficacy of ballast water treatment systems to protect U.S. waters from aquatic invasive species 10-50 µm in size potentially transported in ballast water. By not allowing the MPN culture methods, the use of UV for disinfection will not be a practicable or reasonable option for ballast water treatment. The reasoning, based on sound science, is the following.

UV light inactivates microorganisms by targeting the nucleic acids, both DNA or RNA, in the cells. UV irradiation of the cells causes disruptions in the base pairing of nucleotides (mismatches) on the DNA strands, and replication of the nucleic acid cannot occur. This renders the organisms inactivated because they cannot reproduce their DNA. Other types of disinfectants (e.g., chlorine, ozone) are chemical in nature and inactivate cells by disruption of their physical makeup (e.g., creating holes in cell walls, denaturing proteins) or by blocking the energy-yielding or synthetic processes within the cell. Organisms that have been inactivated by UV irradiation have altered DNA but their cell structure remains intact. This is the basis for the inability of fluorescent live/dead stains to accurately assess inactivation of microorganisms by UV. Inactivated oocysts can appear live because the staining measures cell integrity which is unaltered by UV, but they cannot reproduce in a host and cause infection.

This became readily apparent to me in my research on inactivation of the protozoan parasite *Cryptosporidium parvum.* In 1998, I led the team that discovered that UV was highly effective for control of *C. parvum* in water, and at doses much lower than those used traditionally in water treatment for inactivation of bacteria and viruses. *Cryptosporidium* oocysts are resistant to the commonly used drinking water disinfectants and we undertook a project looking at a variety of technologies that might be useful for oocyst inactivation. Traditionally, oocyst viability was measured by the inclusion/exclusion of the fluorescent vital dyes, DAPI and PI or using excystation. Previous research in the literature had concluded that *Cryptosporidium* was not inactivated by UV, even at very high doses, since the cells appeared to be alive (able to exclude vital dyes) after UV treatment.

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In our first research project on UV inactivation of *Cryptosporidium* we incorrectly identified UV as ineffective for *Cryptosporidium* inactivation even when oocysts were exposed to 8000 mJ/cm$^2$ from low pressure lamps because the vital dyes assay indicated that the oocysts were still viable. We did not understand at that time that one could not measure oocyst inactivation with UV using anything but animal infectivity. It was in a second study that we discovered that the mechanism of inactivation dictates the methods that must be used to measure UV efficacy.

Our second study, co-funded by the Water Research Foundation and the United Kingdom Drinking Water Inspectorate, was designed to examine how *in vitro* methods (vital dyes and excystation) used in the UK compared to the *in vivo* animal infectivity assay used in the US. The goal was to find a less expensive alternative to animal studies so that *Cryptosporidium* disinfection research could be done in more laboratories. There were only three labs in the world at that time able to conduct animal studies and research was limited and very expensive. We used three methods to determine whether oocysts were inactivated by UV: 1) animal infectivity, which was the gold standard for assessing whether oocysts were inactivated by a given treatment, and 2) vital dye staining using DAPI/PI, and 3) excystation which mimics chemical conditions in the animal gut to assess oocyst viability. The two *in vitro* methods are much less expensive as they rely on microscopy alone, and were more widely available to researchers.

The experiments used a collimated beam apparatus to expose *Cryptosporidium* oocysts to varying doses of UV up to 40 mJ/cm$^2$. After exposure, oocysts were 1) fed to neonatal mice to look for infection in the animal gut, indicating that the oocysts were alive and infectious, and 2) stained with vital dyes and examined microscopically to look at inclusion/exclusion of the dyes, and 3) subjected to excystation conditions to assess ability to change from the resting oocyst stage (excyst) and release their sporozoites which cause infection, indicating viability. The results showed that oocysts were highly susceptible to UV irradiation based on the animal studies (no infection with high concentrations of oocysts fed to mice), while the vital dye and excystation results indicated that they were 'alive'.

The disparity in the data is the result of the mechanisms of inactivation, described previously. The oocysts were not alive or viable, because their DNA was damaged and they could not cause infection in an animal host. However, the cell walls were intact since UV damages DNA, not other cellular components. As a result, the oocysts excluded the dye, an indicator of viability, and excysted and released sporozoites, which would be interpreted incorrectly as living. Note that in our earlier work, **even when oocysts were exposed to UV doses as high as 8000 mJ/cm$^2$, there were no changes in the physical characteristics observed microscopically.** Neither the vital dye staining nor wet preparations for hemocytometer counting of the exposed oocysts indicated any loss of cell wall integrity. Because of this we had no idea that the oocysts were irreparably damaged.

Our study was the first to use vital dyes, excystation, and animal infectivity to assess oocyst inactivation and the differences in the three assays became obvious. The infectivity assay was the only reliable one, since oocysts that are UV treated cannot cause an infection and therefore are not viable. This work and additional studies by other investigators demonstrated that *Cryptosporidium* was not only easily inactivated by UV, but much more susceptible than bacteria or most viruses. This led to a major change in drinking water treatment. UV was

included in the Long Term Surface Water Treatment Rule used for disinfection of surface sources used for drinking water. Had we relied on vital dye staining or excystation alone we would have concluded incorrectly, as other research had in the past, that UV was not effective for inactivation of oocysts.

Similarly, if inactivation of microorganisms in ballast water must be measured using live/dead staining, the effectiveness of UV will be incorrectly underestimated simply because of the assay technique. For systems using UV for ballast water disinfection, in order to properly assess its efficacy, a method which directly measures living organisms such as MPN culture, must be used. Live/dead staining cannot predict the efficacy of UV and will underestimate its effectiveness.

In addition to being a very effective means to disinfect water, UV is easy to install and operate. UV produces no known disinfection byproducts. In summary I would ask that the Coast Guard include the use of MPN culture methods so that UV can be used in ballast water disinfection. To exclude this highly effective treatment because the test method used to assess its efficacy cannot do so ignores the science on disinfection measurement and is a step backward in the use of best available technology for protecting environmental health.

I would be happy to discuss this further and I can be reached at jclancy@coronaenv.com or 802-393-0024.

Sincerely,

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Clancy References on UV Disinfection

Hargy, T.M., **J.L. Clancy** and L.P. Landry. 2009. Control of *Cryptosporidium* and *Giardia* in Surface Water by Disinfection. In *Giardia* and *Cryptosporidium* from Molecules to Disease (Ortega-Pierres, G. et al, eds.) CABI, Cambridge, MA.

**Clancy, J.L.** and T.M. Hargy. 2008. Waterborne *Cryptosporidium*: Drinking Water. In *Cryptosporidium* and Cryptosporidiosis, 2nd Edition. (Fayer, R and L. Xiao, eds.) CRC Press, Boca Raton, FL.

Fallon, K.S., T.M. Hargy, E.D. Mackey, H.B. Wright, and **J.L. Clancy**. 2007. Development and characterization of nonpathogenic surrogates for UV reactor validation. J. Am. Water Works. Assoc. 93 (3) 73-82. **Received the 2007 AWWA Water Science & Research Division Best Paper Award.**

Wright, H., E.D. Mackey, D. Gaithuma, C. Fonseca, L. Baumberger, T. Dzurny, **J. Clancy**, T. Hargy, K. Fallon, A. Cabaj, A. Schmalweiser, A. Bierman, and C. Gribbin. 2007. Optimization of UV Disinfection. Denver, Colo.: AwwaRF and AWWA.

McCuin, R.M. and **J.L. Clancy**. 2006. Occurrence of *Cryptosporidium* oocysts in US wastewaters. J. Water and Health 04: 437-452.

McCuin, R.M. and **J.L. Clancy**. 2005. Methods for the recovery, isolation and detection of *Cryptosporidium* oocysts in wastewaters. J. Microbiol. Methods 63 (1) 73-88.

McCuin, R.M. and **J.L. Clancy**. 2005. *Cryptosporidium* occurrence, removal and inactivation methods for wastewaters. WERF Final Report. WERF/IWA Publishing, London.

**Clancy, J.L.,** M.M. Marshall, T.M. Hargy, and D.G. Korich. 2004. Susceptibility of five strains of *Cryptosporidium parvum* oocysts to ultraviolet light disinfection. J. Am. Water Works Assoc. 96 (3) 84-93.

LeChevallier, M.W., G.D. DiGiovanni, **J.L. Clancy**, Z. Bukhari, S. Bukhari, J.S. Rosen, J. Sobrinho, and M.M. Frey. 2003. Comparison of Method 1623 and cell culture-PCR for detection of *Cryptosporidium* in source waters. Appl. Environ. Microbiol. 69 (2) 971-979.

**Clancy, J.L**., T.M. Hargy, M.M. Marshall, D.G. Korich and W.L. Nicholson. 2002. Susceptibility of multiple strains of *Cryptosporidium parvum* oocysts to UV light. Denver, Colo.: AwwaRF and AWWA.

Widmer, G., C.A. Bowman, G. Batzer, B. Stein, C. Pearson, M.A. Buckholt, S. Tzipori, T. Clancy, Z. Bukhari, D. Miller, **J.L. Clancy**, H.D. Ward. 2002. Molecular mechanisms of chemical inactivation of *Cryptosporidium* oocysts and *Giardia* cysts. Denver, Colo.: AwwaRF and AWWA.

**Clancy, J.L.,** Z. Bukhari, T.M. Hargy, J.R. Bolton, B. Dussert, and M.M. Marshall. 2000. Using UV to inactivate *Cryptosporidium*. J. Am. Water Works Assoc. 92 (9) 97-104.

Bukhari, Z., M.M. Marshall, D.G. Korich, J.S. Rosen, C.R. Fricker, H.V. Smith, and **J.L. Clancy**. 2000. *Cryptosporidium parvum* viability and infectivity: inter-laboratory comparisons to determine correlation between assays. Appl. Environ. Microbiol. 66 (7):2972-2980.

Korich, D.G., M.M. Marshall, H.V. Smith, J. O'Grady, Z. Bukhari, C.R. Fricker, J.S. Rosen, and **J.L. Clancy**. 2000. Inter-laboratory comparison of the CD-1 neonatal mouse dose response model for *Cryptosporidium parvum* oocysts. J. Eukaryotic Microbiol. 47(3):294-298.

**Clancy, J.L**., Z. Bukhari, R.M. McCuin, T.P. Clancy, M.M. Marshall, D.G. Korich, C.R. Fricker, N. Sykes, H.V. Smith, J. O'Grady, J.S. Rosen, J. Sobrinho, and F.W. Schaefer, III. 2000. *Cryptosporidium* Viability and Infectivity Methods. Denver, Colo.: AwwaRF and AWWA.

Bukhari, Z., T.M. Hargy, J.R. Bolton, B. Dussert, **J.L. Clancy**. 1999. Medium-pressure UV light for oocyst inactivation. J. Am. Water Works Assoc. 91(3):86-94. **Received the International Ultraviolet Association Classic UV Paper Award, 2011.**

Bukhari, Z., W.G. Glen, and **J.L. Clancy.** 1999. Effects of pH on a fluorogenic vital dyes assay (4', 6'-diamidino-2-phenyl-indole and propidium iodide) for *Cryptosporidium* sp. oocysts. Water Research 33 (13):3037-3039

**Clancy, J.L**., T.M. Hargy, M.M. Marshall, and J.E. Dyksen. 1998. Inactivation of *Cryptosporidium parvum* oocysts in water using ultraviolet light. J. Am. Water Works Assoc. 90 (9):92-102. **Received the 1999 AWWA Water Quality & Technology Division Best Paper Award.**

Dyksen, J.E., A. Gera, M.M. Marshall, and **J.L. Clancy**. 1998. Cost of advanced UV for inactivating *Cryptosporidium*. J. Am. Water Works Assoc. 90 (9):103-111.

**Clancy, J.L**., T.M. Hargy, M.M. Marshall, and J.E. Dyksen. 1998. Innovative electrotechnologies for *Cryptosporidium* inactivation. CR-111090. EPRI, Palo Alto, CA.

**Clancy, J.L**., M.M. Marshall, and J.E. Dyksen. 1997. Inactivation of *Cryptosporidium parvum* oocysts in water using advanced ultraviolet irradiation. In C.R. Fricker, J.L. Clancy, and P.A. Rochelle (eds). Proc.1997 International Symposium on Waterborne *Cryptosporidium*. AWWA, Denver, CO.