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Re: Opinion on the use of the MPN method in evaluating disinfection technologies

Dear US Coast Guard and other stakeholders interested in ballast water treatment:

As an environmental engineering professor with a long history of water treatment research, and specific expertise in the area of water disinfection, the topic of ballast water treatment and disinfection is an interest of mine that I look toward contributing knowledge to. I have published extensively on inactivation of viruses, bacteria, protozoa and my laboratory has performed the assays to evaluate disinfection effectiveness. I was surprised to read about the US Coast Guard's decision to not consider the use of the most probable number (MPN) assay as an acceptable method for evaluating the effectiveness of a technology (Coast Guard Maritime Commons, December 14, 2015 "Coast Guard decision on use of Most Probable Number method"). Indeed, the MPN method is a long established standard method used daily in the compliance practices of water and wastewater utilities across the United States. The method is a fixture in the Standard Methods for the Analysis of Water and Wastewater manual used by water researchers worldwide.

When deciding on a method of analysis for evaluating disinfection effectiveness, one must consider both the pathogen or target microbes of interest and the mechanism of action of the disinfectant. For a chemical disinfection method that relies on an oxidation process that may typically damage a cellular membrane, the use of methods such as vital dye staining may be appropriate as a surrogate to a culturing method. So for a method such as chlorination where damage to the cellular membrane is the mechanism, an approach such as dye inclusion/exclusion assay may correlate well with actual cellular viability and ability to reproduce. For a physical disinfection method like ultraviolet light inactivation, where targeted damage to the nucleic acids is the mechanism for disinfection, the use of a staining technique would be of no value and provide false results, because the UV light does not impact the cellular membrane but solely targets the organisms genetic code. In either case, the use of a culture based method, which could be demonstrated by direct counting methods or an MPN approach, would work equally well.

A case in point regarding the importance of pairing an appropriate assay method with a specific mechanism of inactivation for a disinfection process, is the discovery in 1998 that UV light was very effective for inactivation of *Cryptosporidium parvum*. Before 1998, the two methods of assaying the disinfection of protozoan (oo)cysts were either inclusion/exclusion vital dye staining or expensive animal infectivity assays. Based on vital dye staining, which appeared to be a good enough method for chlorination and ozonation, it appeared that a UV dose of over 8000 mJ/cm<sup>2</sup> would be required for disinfection of water containing *Cryptosporidium*. For years, public health engineers were under the impression from these results that UV light technology was ineffective for inactivation of this pathogen. During a study of innovative UV technologies that utilized the "gold standard" of assays – animal infectivity – the UV technology performed surprisingly well in preventing infection from occurring in the neonatal mice. Subsequent studies with standard UV lamp technologies confirmed that UV was very effective for protozoan cysts and oocysts, and UV is now considered a best available technology for water disinfection for these pathogens.

Given the history of the misunderstanding by scientists and engineers of using the proper assays to evaluate the effectiveness of certain technologies, the clear commonality among different technologies is invariably the ability of an organism to reproduce following exposure to a disinfectant. Therefore the use of culturing methods, including an approach like the MPN test, is the safest common denominator for

assessing technologies utilized in ballast water treatment. In my professional opinion, the use of a culturability or infectivity assay that incorporates an MPN approach, adds credibility to the technology evaluation process and indicates specifically what we want to know – does the organism reproduce after the treatment – much more confidently than a dye inclusion/exclusion-based assay such as vital dye staining. I encourage the USCG to take a new look at this issue in regards to the above facts. UV technology has many positive attributes compared to other disinfection technologies and the field of ballast water treatment as well as the ecological health of the US waterways can be effectively promoted by UV disinfection. Please feel free to contact me using the above coordinates to discuss these points further or for provision of any scientific literature in support of the opinions expressed herein.

Sincerely,

A handwritten signature in cursive script that reads "Karl Linden".

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