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RE: Performance Monitoring of BWMS (46 C.F.R. § 162.060-10(b)(1))

To Whom it May Concern :

I am writing to comment on the US Coast Guard's recent decision to disallow the use of MPN assays to determine microorganism viability following ballast water treatment.

By way of background, I am a past chair of the disinfection committees of both the American Water Works Association and the Water Environment Federation[1], and have been working on disinfection processes since 1974. I have no funding from any party of interest in the subject of ballast water treatment.

Most strongly, the use of a staining methodology to assess the performance of disinfection processes is a retrograde decision. I am familiar with how microbial quality is assessed in water treatment, wastewater treatment, and food safety, and in **none** of these procedures is a staining procedure used to assess treatment performance.

In the early days of concern for protozoan cysts and oocysts in drinking water, there were some studies published using staining techniques; however subsequent researchers discredited these techniques as being inadequate performance assessors. A number of review articles point specifically to viability as being the key criterion for definition of life or death in microorganisms, e.g.:

- Bogosian and Bourneuf[2] concluded that "The only validated operational test of bacterial viability is propagation in culture."
- Netuschil et al.[3] conclude "The nomenclature regarding 'viability' and 'vitality' should be used with appropriate care. *Per definitionem* no kind of stain used for bacteria can prove their 'viability'. Thus, such stains generally should be named 'vital stains'."

In the initial development of the LT2ESWTR (for drinking water) researchers considered the use of vital staining to assess protozoan inactivation efficiency. However it was concluded that this technique was fraught with concern, and therefore US EPA declined to develop quantitative methods

[1]This comment does not reflect an official opinion of either of these organizations, or my current institution, and is in my personal capacity.

[2]Bogosian, G., & Bourneuf, E. V. (2001). A matter of bacterial life and death. EMBO Reports, 2(9), 770?774. http://doi.org/10.1093/embo-reports/kve182

[3]Netuschil, L., T. M. Auschill, A. Sculean and N. B. Arweiler (2014). "Confusion over live/dead stainings for the detection of vital microorganisms in oral biofilms - which stain is suitable?" BMC Oral Health 14(1): 1-12.

for protozoa using such methods, and opted instead for a treatment technology regulation.

The MPN assay has been used in microbiology for well over a century. It can be reliably carried out by technicians with only a small degree of training, and is able to detect low concentrations of viable microorganisms. In contract, microscopic techniques require substantial training of laboratorians, and without concentration have a high detection limit; the need for concentration, which is often not required for the MPN assays, adds an additional source of variability to the assay — and may produce losses.

In my opinion, therefore, the best available assays for definitive determination of microbial viability are culture based methods, such as the MPN or membrane filter or plate count methods.

Sincerely,

Charles N Haas