August 14, 2015

U.S. Environmental Protection Agency
Office of Water
Office of Science & Technology
Health and Ecological Criteria Division
1200 Pennsylvania Avenue, NW
Washington, DC 20460

RE: WEF Comments on EPA's REVIEW OF COLIPHAGES AS POSSIBLE INDICATORS OF FECAL CONTAMINATION FOR AMBIENT WATER QUALITY (820-R-15-098)[1]

The Water Environment Federation (WEF) appreciates the opportunity to offer comments on EPA's Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality. To present the water sector's unified response on the Literature Review, the WEF Bacteriophage/Virus Task Force, a WEF Task Force comprised of experts from nine different WEF committees, and the National Association of Clean Water Agencies (NACWA), have compiled, summarized, and included comments from the sector in this letter. WEF and NACWA are national leaders in environmental water policy representing the water sector.

The objective of these comments is to encourage EPA to consider all relevant scientific data in evaluating the need for a new indicator and in setting criteria for it, so that any large public expenditure, if needed, would be commensurate with the improved public health and environmental outcomes.

Furthermore, since we received a variety of comments ranging in scope and perspective, we included appendices in this letter: the first appendix covers additional relevant comments collected from individual stakeholders, organized by chapter; and the second appendix contains specific notes on the epidemiological studies and methods portions of the Literature Review.

WEF recognizes that some of the comments in this letter may be outside the scope of the Literature Review, but believe they present opportunities for further dialogue between EPA and the water sector. WEF requests that EPA place a higher value to studies by member agencies and subscribers of WEF, NACWA and WERF and other consensus-focused organizations that have attempted to study the behavior (incidence and attenuation rates; as well as relationship to pathogens) of current indicators in treatment processes and effluent waters, receiving waters and ambient waters. These and similarly structured studies should be considered by EPA for review and inclusion in an updated bibliography. Some of these studies are in the public domain[2] and may add value to this discussion going forward.

[1] Original Docket No. EPA-HQ-OW-2015-0300
[2] One example is this study funded by the State of California is accessible at: http://www.cclean.org/wp-content/uploads/2014/07/Prop50_Fecal_Pathogen_Final_Report.pdf.

Overall Major Concerns

We understand the Clean Water Act Section 304(a)(1) requires EPA to periodically revise criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of effects on health and welfare which may be expected from pollutants in ambient water, and reflecting the latest knowledge on the concentration and dispersal of pollutants.

WEF supports processes to evaluate and potentially replace or supplement the current list of indicators with more appropriate candidates, and this bibliography review is a step in that direction. However, while we support EPA's efforts to reduce risks of illness from exposure to recreational waters, the use of a new indicator for fecal contamination in ambient waters is a significant decision that has a potential large effect on the design, operation, and cost of disinfection processes at wastewater treatment facilities in the US. Depending on the indicator selected and on the concentration criteria established, the use of a new indicator could require large expenditures of public resources to change treatment processes at plants throughout the country. Consequently, we look forward to working with EPA to ensure a scientifically sound basis for any new criteria, and we respectfully request a continued dialog with the EPA regarding the concerns listed below in this document. This continued dialogue is essential because of the importance of the relationship between indicator phage, pathogenic viruses, human exposure and health which cannot be understated.

As we have discussed in our meetings with EPA, it is important to develop technically defensible 304(a) CWA criteria which will form the basis for defensible water quality standards and eventually NPDES permit limits. This relationship must be demonstrated and documented through rigorous scientific study to be reproducible across most surface waters where the criteria value will be applied and characterized by a statistically significant, quantitative and predictable relationship where health varies with exposure concentration. This review and continuous dialogue with EPA on this Literature Review and its attendant discussion is essential if the water sector and EPA are to continue to make progress in the objective of protecting public health and address what may be the lack of high correlation of disease incidence and indicator population levels of current bacteria indicators.

Synopsis of Major Concerns

1. ***The current state of scientific knowledge may not yet be sufficient to establish that a viral indicator of fecal contamination is needed for ambient recreational water.***

2. ***The Literature Review did not demonstrate that phages perform any better than traditional bacterial indicators.***

Concern 1: The Literature Review focuses on bacteriophages as potential viral indicators of fecal contamination. However, the Literature Review cites some references that overestimate the *extent of health effects caused by viral pathogens in recreational waters. Therefore, the current state of scientific knowledge may not yet be sufficient to establish the benefit and or need for a viral indicator of fecal contamination in ambient recreational water.*

a) The Literature Review states on page 1: "It has been suggested that viral pathogens are the leading causative agents of recreational waterborne illnesses (Jiang et al., 2007; Sinclair et al., 2009)," and also on page 2,"…viruses are an important cause of recreational waterborne illness." WEF does not believe that these statements are supported by the supplied references in the review. The Jiang reference cited by EPA refers to a CDC report from 1991-1992, which states that viral pathogens might cause nearly fifty percent of waterborne illness from drinking water, not ambient water. The Sinclair reference cited by

EPA presents a summary of relative numbers of viral versus bacterial/parasitic recreational water-borne gastrointestinal disease outbreaks as reported to CDC from 1989-2002. These data indicated a trend toward increasing relative numbers of viral outbreaks in 1998-2002; however, many of these outbreaks were from pools rather than ambient recreational water.

b) Because the EPA literature review cites Jiang and Sinclair, which use CDC data as their source, it would be appropriate to include these CDC data as well. The CDC lists 114 and 266 reported cases associated with viral presence from 2009 to 2010 and from 2011 to 2012, respectively. Assuming a conservative estimate of 1,000,000 users of untreated recreational waters, this corresponds to an illness rate of 0.03%, which is significantly lower than the EPA's Recreational Water Quality Criteria (RWQC) level of 36 illnesses per 1000 (3.6%). Since the overall rate of viral illness is likely orders of magnitude lower than the current basis of the EPA RWQC, WEF would be interested in discussing with EPA the decision to pursue phage recreational water quality criteria.

c) One of the overall goals of using coliphages as indicators is because they appear to represent better surrogates for viruses. The CDC's web page on Healthy Swimming/Recreational Water lists the following top ten causes of recreational water outbreaks: *Cryptosporidium*, *Pseudomonas*, *Shigella*, *Legionella*, Norovirus, *E. coli*, *Giardia*, disinfection agents & their byproducts, Avian schistosomes, and *Leptospira* (http://www.cdc.gov/healthywater/swimming/). Since the CDC's list includes only one virus, WEF would be interested in discussing with EPA whether focusing on a viral surrogate is the best way to decrease illness when 9 of the 10 main contributors to illnesses in recreational waters are non-viral based.

Concern 2: The Literature Review did not demonstrate that coliphages perform any better than traditional indicators. The Literature Review states EPA is considering the use of F^+ specific and somatic coliphages as possible viral indicators of fecal contamination of ambient water. *But the review does not include a comparison of whether a coliphage indicator would be better, worse, or about the same as FIB as an indicator of fecal contamination. Therefore, if selected, phages may perform poorly compared to traditional bacterial indicators.*

a) This section should also review studies of correlations of fecal indicator bacteria to pathogens/illness to show whether those correlations are superior or inferior to potential correlations between coliphage and pathogens/illness. By just summarizing the coliphage work in isolation, we cannot unbiasedly evaluate the performance of phages compared to other fecal bacterial indicators in protecting public health and the environment.

b) Table 4, page 26, of the EPA's report suggests that male specific coliphages correlate with illness in five of the eight studies considered, but the somatic coliphages correlate with illness in only *one* of the eight studies reviewed. Why then consider somatic coliphages as viral indicators? Furthermore, how did coliphages compare to the traditional indicators in these studies? Did coliphages perform worse or better?

c) If "better" indicators of public health risk are needed for ambient water quality, did EPA begin the process by thoroughly examining the universe of possible approaches? Why were viruses singled out for investigation prior to looking at all possible approaches? Has EPA considered other novel approaches that may need to be put on the table and evaluated side-by-side with viruses?

d) The Boehm et al. 2009 study reviewed in Table 8, page 34, suggests that for the marine sewage impacted beach, there was no association between coliphages and adenoviruses or enteroviruses.

This suggests that for sewage impacted recreational waters, coliphages poorly correlate with enteric viruses. One possible explanation is that virus loads vary depending on the sickness of the human population, whereas phage populations may remain relatively constant.

Furthermore, if the one study on sewage impacted beaches suggested that coliphages poorly correlate with human viruses, and to the best of our knowledge, no other studies contradict this, WEF would welcome a discussion on why is the EPA proposing to develop criteria that will specifically affect wastewater facilities when there could be many non-point sources that contribute coliphage and viral loads to beaches? This study seems to argue against the use of coliphages to indicate fecal contamination, at least for marine beaches.

Conclusions

To address WEF's two major concerns with the EPA's Literature Review, WEF requests that the EPA address the following items:

- WEF requests that EPA revisit or re-engage in a dialogue related to the assumption that *viral pathogens* are the leading cause of recreational waterborne illnesses in the US.
- WEF requests that EPA review the most recent CDC reports of the causes of waterborne disease from untreated recreational waters in the US, and the relative contribution of viral pathogens.
- WEF requests that EPA review how phages compare to current FIB as indicators of fecal contamination and of illness risk in recreational waters – are they scientifically proven to be better indicators?
- WEF requests that EPA provide additional explanation of why somatic coliphage could be a desirable viral indicator, when it was correlated to viral illness in only one of eight studies.

Finally, a general comment on timing, as WEF has discussed this issue with EPA in the past, we want to try to avoid various revisions over a period of time (similar to what happened with UVDGM 2003 then 2006, and NWRI 2003 then 2012 Guidelines). This is a significant issue for suppliers who have to commit resources and capital to validate their systems and for utilities that may need to invest significant public funding and time into designing and constructing infrastructure to meet new criteria; therefore, it is extremely important that any new criteria have a strong basis, with few future changes. WEF would also welcome a discussion with EPA on whether it expects any impact on the practice for filtration and/or biological processes? If so, we recommend a broader group of stakeholders/experts to be included in the early stages of this effort.

Again, we thank the EPA Office of Water, Office of Science and Technology, for the opportunity to provide input in this important phase of your criteria review and hope to continue the open and transparent discussion EPA has established. WEF and NACWA's Committees and staff look forward to further dialogue on this important matter. Please contact me at (703) 684-2416 or at cternieden@wef.org should you have any questions or welcome follow up discussions.

Sincerely,

Claudio H. Ternieden
Director of Government Affairs
Water Environment Federation

APPENDIX A – ADDITIONAL SPECIFIC COMMENTS FROM INDIVIDUAL STAKEHOLDERS

Chapter 1 - Introduction

Section 1.3 – the authors lay out a list of general attributes of ideal indicators of fecal contamination. I feel the list needs to be justified - there may be good indicator options that do not meet the criteria listed. For instance, what is the reason for these?

- the indicator should be a member of the intestinal microflora of warm-blooded animals (see Section 2);
- the indicator should be nonpathogenic (see Section 2);
- the indicator should be specific to a fecal source or identifiable as to source of origin (microbial source tracking [MST] is not included in this review).

Chapter 2 – Bacteriophage Characteristics

Section 2.2.1 – in intro sentence, add "and genome type" in the last sentence

Section 2.2.1 – 3rd paragraph – add discussion of the confounding factors involved in purification of viral stocks in studies

Chapter 4 – Occurrence in the Environment

In the introduction portion of this Chapter, in page 27, a number of factors are discussed which impact the methods for sampling when comparing coliphages and viruses, one aspect not listed is that it also includes the soil-water interface.

Table 5, page 28, in reviewing Silva et al (2010), should be noted that the correlation between virus detection and FIB is poor in part due to the re-growth of FIB in the environment.

Section 4.1, Table 7, page 30, Important to note related to the advantages of qPCR/RT-qPCR is that these are Semi-quantitative data (versus "quantitiatve data" as noted in the Literature Review) since factors such as DNA/RNA extraction methods and environmental inhibitors can skew quantification. In addition, when listing advantages of ICC-PCR and ICC-RT-PCR, important to note that it would be more accurate to say "less time rather than half time." General comment: It seems that EPA is arriving at quantitative conclusions that it believes are reliable but may not be defensible. First, when qPCR was used, this is not possible because qPCR cannot differentiate viable DNA from non-viable DNA. We noted that EPA is or may be using information based on qPCR to document quantitative relationships. Second, it seems that EPA is assuming log removal rates based on concentrations that are not quantitated. WEF would welcome clarification on this issue.

4.1.1 Coliphage – Virus Associations in Freshwater, page 31, when discussing the Payment and Locas (2011) study, the Literature Review states:

> "[they] used 20 years of sampling data from their laboratory to examine the association between pathogens and multiple microbial indicators, including coliphages, in sewage, surface water, and groundwater. Although the authors review data for several water types, coliphage associations with pathogens were investigated in groundwater. Their analysis of 242 samples from 25 municipal groundwater well sites indicated that somatic and F-specific RNA coliphages were not predictive of virus presence or absence. *This was due in part to the low numbers of coliphages present in the samples and their infrequent detection* (Payment and Locas, 2011)."

WEF adds that this is so possibly due to the detection limit of the assay. Was 100mL or 1-L of groundwater analyzed? Also may be in part due to the method-cell culture vs. genetic method.

Table 8, pages 33 through 36, of the EPA's report suggests that the association between phage indicators and viral pathogens is stronger in brackish and saline waters than in freshwaters. If coliphages poorly correlate with pathogens in fresh waters, WEF would welcome some clarification on why EPA is considering coliphages for use in setting recreational water quality criteria in fresh waters?

Chapter 5 – Environmental Factors and Fate

Section 5.1 – studies may have artifacts because of conformational changes of virus capsids (e.g. poliovirus undergoes a conformational change with change in temperature which can cause it to be non-culturable but still viable); review should mention this and indicate the type of detection methods that were used in the study (e.g. culture methods); in addition, in this section, the Literature Review states that "In dechlorinated water at 4°C and 25°C, MS2 survived three times longer than both E. coli and FCV, whereas they had similar survival rates at 37°C (Allwood et al., 2003)." Important to note that under chlorinated conditions the survival rates should be even lower. Finally, regarding coliphage persistence, recent studies performed at Texas A&M University using pyrosequencing reported similar findings; and because coliphages may generally persist longer than most viruses, therefore further studies may be needed to determine their utility with treatment efficacy and in with chlorine residual.

Section 5.2 – explain terms endogenous direct, endogenous indirect, and exogenous indirect

Table 9 – very different rates for the same virus type (but different studies) indicate that only the relative rates within a single study are meaningful, not the absolute rates. There may be artifacts due to sample preparation that impacts results. The text should emphasize this.

Section 5.3 Salinity – need to mention some findings that Norovirus GI and GII show opposing adsorption behavior in the presence of different ions (see cited paper da Silva et al., 2011) and therefore it is difficult to pick an indicator that would behave like a range of pathogenic viruses with regards to sorption; in addition, there are other studies that have demonstrated that decay has been increased at higher temperatures and at higher concentrations of sodium hypochlorite, which is more commonly used as an disinfectant. Regarding the comment in the Literature Review related to the Hurst and Gerba (1980), decay rates can be influenced by adsorption to microbial mats or by natural organic matter. In the environment, viruses are often associated with mats or organic matter.

Section 5.3 "Synergistic effects" – do these studies control for clumping of viruses? Were the authors able to do something to demonstrate whether the cell culture methods used were plating single viruses or aggregates?

Section 5.4 "Effects of predation and…" – did the studies control for sorption and aggregation of viruses? In addition, usually microorganisms are present in too low of a concentration in groundwater to affect virus concentrations. Finally, in waste stabilization ponds, adsorption, predation, and biofilms can affect inactivation of viruses and affect presence/absence assays.

Section 5.5 – isoelectric point may NOT govern sorption – see cited paper da Silva et al, 2011; in page 50, related to the statement "[i]n four of the five natural waters, the inactivation rate of poliovirus type 3 was significantly slowed relative to a clear, buffered control." Should be noted that the concentration of

radicals can also influence virus inactivation rates.

Transport properties – while there's generally thorough discussion of factors governing the survival of coliphage in the environment (with respect to degradation), there's generally not much discussion about factors involving transport of coliphages versus pathogens and FIB.

Chapter 6 – Wastewater Treatment

Section 6 – in the initial discussion of treatment efficacy, it is important to note that chlorination is dependent on the types of chlorine species produced during disinfection and if shock treatment or residual chlorine is present during disinfection. See studies done by Trussell et al. On pages 56-57, rather than listing the "type of chlorination" as a factor affecting disinfection efficacy, a more accurate phrase would be "the disinfectant species." Free chlorine may be added to a wastewater but form chloramines (see bullet below); in this case, the" type of chlorination" may be ambiguous, but the species are clearly chloramines.

On page 57, the review states that "coliphages can be resistant to some chlorination practices," but the practices that lead to resistance are not specified. The relevant references for this statement show that coliphage are resistant to chloramines but sensitive to free chlorine. It would be more accurate to simply state that "coliphages have been found to be resistant to chloramines but sensitive to free chlorine, which provided up to 6-log removal (Havelaar, 1987; Sobsey, 1989; LACSD, 2013)."

Section 6 – discussion of WERF study on page 58 – how did the 2 studies that looked at the same data (Rose et al., 2004 and Harwood et al., 2005) come to opposite conclusions regarding the correlations between enteroviruses and coliphages? This should be explained further. In addition, FIB are generally found in greater numbers, therefore, log removal of FIB are greater and more accurate than of coliphage. Also, growth-based detection of coliphage requires concentration of the sample/greater volume of the wastewater sample (true of F-specific coliphage).

Section 6 – the Literature Review stated "Levels less than 10 coliphage PFU per 100 mL (either F-specific coliphages, or F-specific combined with somatic coliphages) were indicative of effluents with *no detectable cultivatable enteroviruses* (Rose et al., 2004). While Rose et al. (2004) reported log10 reductions, they did not provide detailed information on the treatment processes. Harwood et al. (2005) evaluated the same data reported in Rose et al. (2004)" - Due to even less numbers of viable enteroviruses compared to coliphage, therefore, this generalization is most likely treatment dependent. See Trussell et al studies.

Section 6 – discussion of Aw and Gin (2010) on page 58 – did the authors look at correlations between coliphages and pathogens in the effluent (in addition to the reported results regarding correlations in the influent); in addition, since NoV concentrations were determined by qPCR, it is difficult to determine if the approximate 2-log reduction is an overestimate measurement of NoV viability. Figure 1, in page 59, "shows example reductions for three WWTPs in Singapore (secondary effluent - activated sludge). Somatic and F-specific coliphages had on average 2.4-log10 reduction, and were *reduced at a similar rate as enteric viruses, adenovirus, and astrovirus. NoV reductions were less,* but assays were based on qPCR results evaluating both viable and nonviable NoV (Aw and Gin, 2010)" – again since NoV concentrations were determined by qPCR, it is difficult to determine if the approximate 2-log reduction is an overestimate measurement of NoV viability.

Page 60, Table 14 "shows the log10 reductions for wastewater treatments used by the South Australian and Victorian Departments of Health. *Note that coliphage removals are more similar to human virus*

removal than E. coli or bacterial pathogen removal for many treatments" - this is most likely due to higher concentrations of E. coli in raw sewage.

Page 61, the Literature Review states "[i]n a study of WWTPs in Switzerland, Baggi et al. (2001) found that three WWTPs with mechanical, biological, and chemical processes provided 0.6- to 0.8-log10 reductions for F-specific and somatic coliphages. A fourth WWTP with mechanical, biological, and chemical processes, plus *sand filtration provided 1- to 4.4-log10 reductions for F-specific and somatic coliph*ages (Baggi et al., 2001)" – WEF commenters ask whether this is so most likely due to adhesion?

Page 62, Section 6.2 Secondary Treatment, states, "NoV is the leading etiological agent of gastrointestinal illness in the United States, and of an estimated 36.4 million cases of domestically acquired gastrointestinal illness, NoV caused an estimated average of 20.8 million cases annually (Scallan el al., 2011)." Scallan's paper concerns foodborne illness, not illness acquired by contact with untreated recreational water. For clarity, this statement should indicate that these 20.8 million illnesses are from foodborne viruses, not waterborne. The reader is likely to assume this statistic applies to recreational water because it is cited in the section on wastewater treatment. For perspective, the CDC reported approximately 72 known or suspected norovirus illnesses per year for untreated recreational water from 2009-2012.

Page 62, the Literature Review states "[a] comparison of influent to secondary effluent found that mean culturable F-specific coliphage densities were reduced by 2.13-log10, NoV GI gene copy densities were reduced by 0.8-log10,NoV GII gene copy densities were reduced by 0.92-log10, and E. coli densities were reduced by 1.49-log10 (Flannery et al., 2012)." WEF commenters note that this study compared reductions of coliphage concentrations as determined by growth-based assay and compared to reductions by qPCR.

Pages 62-63, the Literature Review states "[s]ome of the studies reviewed in this section evaluated correlations between coliphages and enteric viruses to determine the usefulness of coliphages as *surrogates for human viral presence in non-disinfected secondary effluent*. Gantzer et al. (1998) showed a significant correlation between the density of coliphages and infectious enteroviruses in secondary effluent and the correlation between the density of somatic coliphages and the presence of the enterovirus genomes (p-value <0.0001)." WEF Commenters note that to determine the usefulness of coliphage as a surrogate, comparisons should be made using growth-based assays for both coliphage and human viruses.

Section 6.3 – add mention of study showing that settling is not a dominant removal mechanism in waste stabilization ponds (da Silva et al, 2008 – cited in references but not discussed here)

Section 6.4 – The authors should add discussion of biofiltration as an alternative to reverse osmosis for advanced water purification. (Top of page 64)

Section 6.4 – Depth filtration – it would be good to note whether the studies summarized were operating under 'biological' filtration or not, because the biofilms change the mechanisms of removal/degradation dramatically compared to non-biological filtration; in addition, the Literature Review states *"[t]he removal efficiency of MS2 was more sensitive to the coagulant dose, compared to the indicator bacteria*. In an experimental rapid sand filtration setup, virus size (based on ΦX174, MS2, and T4 coliphages) was the only factor that influenced retention and *the larger the virus, the greater the retention* (Aronino et al., 2009)." WEF commenters note that the "removal efficiency of MS2 was more sensitive to the coagulant dose, compared to the indicator bacteria" and the "the larger the virus, the greater the retention" was possibly

due to the ability of FIB to settle and adhere to the coagulants and during sedimentation.

Page 64-65, the Literature Review states that "in general, log10 reductions of indicator bacteria (coliforms, enterococci, and Clostridium) was 2-to 9-fold greater than the log10 reduction of pathogens, suggesting that monitoring with bacterial indicators may *over predict pathogen reductions*." WEF commenters note that Rose et al. demonstrated that viruses were generally not present in samples with less than 10 FIB/100mL.

Page 66, Section 6.5 Disinfection. The Section focuses on 4 main disinfection technologies – chlorine, chloramines, UV and ozone – but there is no information on "emerging" technologies such as peracetic acid (PAA) or pasteurization. The effectiveness of peracetic acid (PAA) on coliphages should be included in this section on Wastewater Treatment. For example, PAA was approved by the EPA for municipal disinfection, and several US cities are using PAA, or are moving toward full-scale use of PAA, for wastewater disinfection.

In the free chlorine section, page 66 states that "Chlorine (Cl-) is the most widely used wastewater disinfectant (Asano et al., 2007)." It is true that chlorine, either in the gaseous form as Cl2 (not Cl-) or in aqueous solution as NaOCl, is the most commonly applied disinfectant at water resource recovery facilities. However, as also noted in Asano et al. (2007), "the effluent from most water reclamation plants also contains significant amounts of nitrogen, usually in the form of ammonia." This ammonia reacts with the added chlorine to form chloramines. Therefore, chloramines (not free chlorine) are the most commonly used disinfectant at water resource recovery facilities in the US, and the above statement would be more appropriate in the combined chlorine section.

In the free chlorine section on page 67, the Rose et al. (2004) study is discussed. The Literature Review states that four of the six WWTPs used chlorine but only one had ammonia levels low enough for free chlorine disinfection; the cited report lists five WWTPs using chlorine with only one having low ammonia levels. The Literature Review states that the data from all plants were combined and "that on average, 300 minutes of contact time with chlorine (or combined chlorine) in secondary effluent resulted in a 3-log10 reduction of enterococci, whereas 500 minutes of contact time were required for a 3-log10 reduction of enteroviruses." Because the data are mostly for plants using chloramines, this statement would be more appropriate in the combined chlorine section.

On page 67, the Literature Review states that "More studies that compare chlorine inactivation of FIB, indigenous F-specific and somatic coliphages, and enteric viruses in nitrified effluent are needed." These studies are difficult to conduct because, as noted above, the effluents of most WWTPs contain ammonia. Although nitrification is increasingly common to meet nutrient limits, it is extremely challenging to continuously achieve ammonia concentrations that are low enough for free chlorine disinfection. Some of these issues are detailed in Huitric et al. (2015)[3] for a facility that is switching from primary reliance on chloramines to free chlorine for disinfection; this facility is a scalping plant that was able to reduce the influent flow to provide low ammonia levels, but most water resource recovery facilities must accept all of the flow that comes to them and do not have the flexibility to control influent flow rates. The practical implication is that implementation of free chlorine disinfection would likely be difficult and costly for most WWTPs.

Section 6.5.1 Free chlorine - suggest changing "the CT (disinfectant concentration times contact time)

[3] Huitric, S.J., Tang, C.C., Ackman, P., Munakata, N. 2015. Full-Scale Implementation of Sequential Chlorination to Produce California Title 22 Recycled Water. To be presented at WEFTEC in Chicago, IL, September 28-30, 2015.

required for NoV inactivation was not significantly different from other viruses even though the molecular methods likely overestimate the CT needed" to "the CT (disinfectant concentration times contact time) required for NoV inactivation was not significantly different from other viruses, based on PCR results."

Section 6.5.4 UVC – "the reactive properties of the organic matter don't affect the UV, as happens with chemical disinfectants" – is this unique to UVC? In the sunlight discussion, the authors point to evidence that organic matter influences degradation rates. This should be clarified.

Section 6.5.4 UVC – Can the authors update the text on page 73-74 to include more guidance from the 2015 ULTRAVIOLET DISINFECTION FOR WASTEWATER – LOW-DOSE APPLICATION GUIDANCE FOR SECONDARY AND TERTIARY DISCHARGES book published by WEF?

Pages 72-73 – the Literature Review stated that "[I]n general, coliphages have been found to be *more resistant to UVC light than FIB*. For example, Gehr et al. (2003) demonstrated that GI F-specific RNA coliphage MS2 is more resistant to UV inactivation than fecal coliforms in effluent." WEF commenters note that Following UV exposure viability should be measured as oppose to presence/absence since genetic particles may still be detected but are not viable or infective.

Page 74 – the Literature Review states that "[i]n contrast, other studies have found that coliphages are *more resistant to UV than human viruses*." WEF commenters note that Adenovirus is an exception. Also, commenter notes that they are more resistant than FIB.

While PCR may overestimate viable viruses, if the viruses are exposed to chlorine, it is likely that capsid damage happens first, followed by viral damage. Therefore, if there is intact viral RNA, it is probable that the capsid is intact, though not provable. This distinction is important because it may not be true that PCR results always dramatically overestimate viable viruses – it depends on the degradation mechanism.

WEF knows from other discussions with EPA that it is still unclear which virus(es) is/are going to be the target, e.g rotavirus, poliovirus, adenovirus or norovirus. Studies seems to indicate that noroviruses (NoV) may be responsible for the large majority of viral-based gastrointestinal illnesses; assuming the choice of a coliphage will be made based on the target virus, the selection of such virus(es) will have a significant impact on the technology selected AND the size of its system, with significant economic consequences. It is unclear which coliphage(s) seem the most promising. It is critical to note that, depending on the disinfection technology, a coliphage may under or overestimate the performance of a disinfection system. There are missing data on the efficiency of some technologies for some coliphages; for example, how does the sensitivity to UV for the somatic coliphages λ and ΦX174 compare to the sensitivities of T1, T7 and Qβ?

There is a difference in sensitivity between laboratory and indigenous species. For example, "laboratory propagated F-specific RNA coliphage MS2 is inactivated by UV at a rate that is twice that of indigenous F-specific coliphages".

In addition, while WEF understands the information provided in the document is trying to correlate data from various biological treatment systems and bacteriophage we believe needs additional work. The removal of micro-organisms through biological treatment systems is a function of a number of operating factors like SRT which were not discussed. We believe that a discussion of operating parameters related to removals needs to be added.

The discussion on various disinfection alternatives seems different than literature considered standard. A discussion needs to address how the standard disinfection criteria (like the Ten States Standards) can

achieve removals of various bacteriophages.

Data seemed to focus on removals using UV. WEF suggests many UV systems have been validated with a dose that provides 2 to 4 log removal of bacteriophage. Information on ozone and chlorine seems to be a somewhat different than previously published.

It would seem that additional studies need to be conducted to validate performance. Chemical Doses (either chlorine or ozone) described in the literature would result in significant formation of Disinfection By-products so that need to addressed as well.

Chapter 7 - Conclusions

Page 78, Section 7 Conclusions of EPA's report. Regarding the bacteriophage host, E. coli CB390, since somatic coliphages poorly correlate with illness in recreational waters, why would a host that simultaneously quantifies both somatic and male specific coliphages be appropriate?

EPA's Table 23 comparing the attributes of coliphages to FIB show coliphages equivalent to FIB in many respects, likely more costly to quantify in the lab, and potentially superior as an indicator of viruses. But given the CDC data showing viruses are not a major cause of waterborne illness in ambient recreational water, does the potentially improved correlation with viruses warrant introduction of a new indicator?

Please add these overarching conclusions: a. aggregation and sample treatment is a confounding factor in some of the studies, b. regarding infectivity, while PCR may overestimate viable viruses, some of the studies cited in the Literature Review show that cell culture and PCR-based methods yielded the same results under certain conditions. The "overestimation of PCR results" issue may be overemphasized in general in this line of research.

APPENDIX B – SPECIFIC NOTES ON EPIDEMIOLOGICAL STUDIES AND METHODS

Epidemiological Studies

In the Literature Review, there are summaries of eight studies that evaluated the relationship of coliphages and gastrointestinal (GI) illness from exposure to recreational water. Five of these eight studies found a statistically significant relationship between coliphage and GI levels, although not all of these studies used quantitative methods for measuring coliphage. As a result, as WEF and NACWA understand it, EPA is conducting additional meta-analysis of the 2010 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology NEEAR (EPA, 2010) and Southern California Coastal Water Research Project (SCCWRP) data during the summer 2015. This information will be used to inform a Quantitative Microbial Risk Assessment (QMRA) approach to criteria development. QMRA is a formal process, analogous to chemical risk assessment, of estimating human health risks due to exposures to selected infectious pathogens (Haas et al., 1999; NRC, 1983). To the greatest possible extent, the QMRA process should include the evaluation and consideration of quantitative information; however, qualitative information is also used when appropriate (WHO, 1999). WEF, NACWA and WERF are exploring conducting a specific review of the epidemiological portion of EPA's literature review and will provide that analysis to EPA. The information is expected to be available in the Fall of 2015 or early Spring of 2016.

Bacteriophage Methods

The EPA Literature Review clearly identifies challenges with coliphage methods. For example, there are currently methods available for drinking water quality monitoring, however, while these current methods (EPA 1601 and EPA 1602) are useful, they have not been validated in ambient waters or wastewater. It is of note that EPA Method 1601 is only a qualitative method, as written, however, EPA is in the process of assessing two culture methods that include a membrane filtration method and a cartridge method. The EPA will investigate additional methods including rapid methods, single host methods, and microbial source tracking methods as these are developed and validated. Further, EPA is conducting additional work to validate a quantitative culture method for ambient waters, and conducting evaluations at four Great Lakes beaches in the summer of 2015.

WEF notes that qPCR-based rapid methods are not viability methods and require additional steps to determine infectivity. qPCR methods can be used for initial screening but infectivity and viability should be confirmed by growth-based assays such as Method 1601. QMRA may be an option for a state to develop site-specific criteria but the Literature Review and EPA should be cautious not to infer that this tool is acceptable for developing 304(a) criteria.

In addition, WEF notes that the rapidity of test to data outcomes (Turn-Around-Time) in the selection of candidates to replace current indicators, is one of the strongest attributes of some of the easiest methods for coliphage enumeration (within 12 hours), and as candidates for use in evaluating ambient waters. This attribute is lacking in enumerative methods used for the current indicators, and has led a number of WEF members to continue the investment in qPCR technologies which are far more expensive than the more common methods used to enumerate the indicators for compliance monitoring. This may be an area EPA might conduct additional research and/or partner with others to conduct such research.

Finally, WEF notes that the selection of indicators that would correlate better with pathogens needs to be informed of exposure risks to the testing community. Coliphages, even when they are molecularly similar to highly infectious viruses in water and wastewater (eg MS2 relative to poliovirus), are highly restrictive in their host range because of their genetic structure and physical configuration. Therefore, scientifically-based efforts to find appropriate indicators with these characteristics are welcome, since such a candidate would be protective of the professional workforce who must evaluate and enumerate them.