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SUBJECT: Comments on WDOE's Proposed Recreational Water Quality Standards (WAC 173-201A)

Fecal indicator bacteria (FIB) tests are valuable indicators of fecal contamination in recreational waters impacted by sanitary sources or runoff from livestock operations and pet parks. However, EPA acknowledges that they are imperfect indicators when applied to waters primarily impacted by non-human or other environmental sources. EPA has reviewed numerous technical reports and studies to support its existing Recreational Water Quality Criteria (RWQC) recommendations, but also concluded that state regulatory agencies should have the flexibility to adopt alternate criteria or use alternate test methods on a site-specific basis in areas where environmental sources of FIB are predominant. Mills that do not treat sanitary wastes onsite would not be expected to have FIB levels associated with a sanitary contribution, but some fail to meet RWQC due to the presence of ubiquitous bacteria (NCASI 2016).

These comments are based on support documents in EPA's 2012 RWQC recommendations, EPA's recently published five-year review of the RWQC recommendations (USEPA 2018), and more than ten years of pulp and paper mill-specific data collection activities in which NCASI has participated. While these data suggest that *E. coli* is a better measure than fecal coliform for indicating the potential presence of fecal-borne bacteria, it is still an imperfect measure subject to false positive results. Addressing this problem requires the use of scientifically-defensible data and tools to assess the applicability of alternate criteria where levels exceed RWQC but fecal sources are not indicated.

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NCASI's detailed comments support several key findings including those summarized here.

- The option to develop site-specific alternate criteria in areas with environmental sources of bacteria should be retained.
- *E. coli* appears to be better than fecal coliform as an indicator of the potential presence of human pathogens but is still an imperfect indicator.
- Enterococci criteria can be poor indicators of fecal contamination in some discharges where there are no sanitary sources but where plant-derived bacterial species are predominant.
- Both *E. coli* and enterococci methods can be prone to interferences due to high background levels of ubiquitous, non-fecal borne bacteria.
- WDOE should consider providing guidance for collection of multiple discharge samples over a specified time interval if an FIB limit is exceeded to assist in determining permit compliance.

Comments Regarding Revision of WAC 173-201A-020 Definitions

Comment 1. Revise "fecal coliform" definition. The standard defines fecal coliform as "that portion of the coliform group which is present in the intestinal tracts and feces of warm blooded animals as detected by the product of acid or gas from lactose in a suitable culture medium within twenty-four hours at 44.5 plus or minus 0.2 degrees Celsius." Reference to the product of acid or gas form lactose should be removed because not all methods have this same endpoint. A more accurate definition is that portion of the coliform group that is thermotolerant and may include those present in the intestinal tracts and feces of warm blooded animals as detected by analytical methods using a suitable culture medium within twenty-four hours at 44.5 plus or minus 0.2 degrees Celsius.

Discussion 1. Revise "fecal coliform" definition. Some thermotolerant coliform bacteria are ubiquitous in the environment and will grow in suitable culture medium at the prescribed fecal coliform method temperature but are not associated with fecal matter. In addition, the notation that the analytical method is based on "the product of acid or gas from lactose" indicates that only the multiple tube fermentation procedures is applicable, when other methods have been approved for fecal coliform testing, including membrane filtration and enzyme substrate (Colilert) methods when conducted at 44.5 plus or minus 0.2 degrees Celsius (40 CFR, Part 136).

Comments Regarding Revision of WAC 173-201A-200 Fresh Water Designated Uses and Criteria

Section (2) Recreational uses

Section (2) (b) Water contact recreation bacteria criteria

Comment 2. *E. coli* Standards. NCASI agrees that *E. coli* test results correlate better with human illness rates than fecal coliform in waters with fecal sources of contamination. However, *E. coli* measurement methods are still imperfect indicators of fecal contamination in some waters with predominantly vegetative sources. Based on experiences at several mills, including some in Washington, NCASI is concerned about the potential for high-biased bacteria counts in discharges stemming from the presence of ubiquitous bacteria of non-fecal origin that can cause interferences. We suggest including provisions to consider alternate criteria developed using science-based assessment tools as provided herein (Discussions 2a, 2b, 2c).

Discussion 2a. *E. coli* versus Fecal Coliform. EPA epidemiological studies showing a correlation with human illness rates were conducted primarily in areas with known human or livestock sources of fecal contamination where human pathogens were likely to be present, but there was less correlation when studies were conducted in areas with predominantly environmental sources (USEPA 2018). Although *E. coli*

is usually a better indicator than fecal coliform, it is not exclusive to fecal sources. One reason *E. coli* better correlates with illness rates than fecal coliform is the predominance of some ubiquitous thermotolerant coliform bacteria that test positive using the fecal coliform test but are of environmental origin. Figure 2a shows biochemical species testing results of 97 colonies from 16 fecal coliform membrane filters cultured using procedures in Standard Method 9222D of a final effluent from a pulp and paper mill. These data show that in this source only 11% of colonies were represented by *E. coli*, with the predominant species identified as *Klebsiella* spp. These results are typical of some pulp and paper effluent discharges (NCASI 2005).

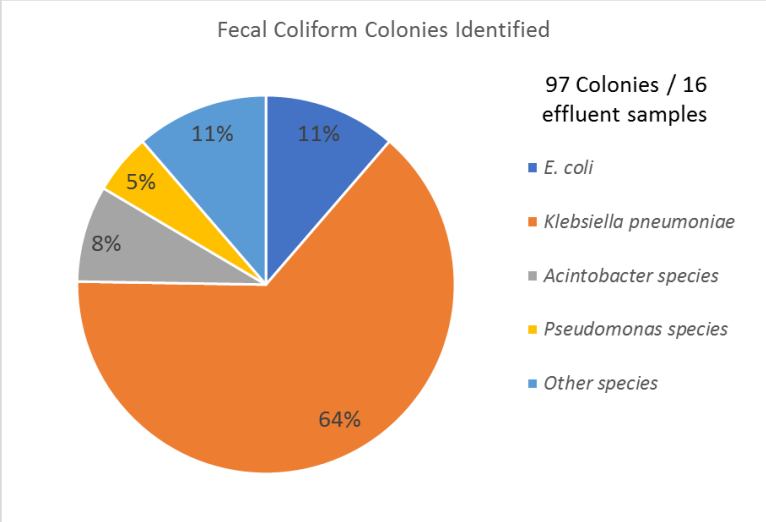

Figure 2a. Effluent Biochemical Species Testing Results from Fecal Coliform Membrane Filtration

Discussion 2b. *E. coli* Method 1603 Membrane Filtration Species Identification and False Positive Assessments. Mill studies have shown that *E. coli* false positive rates in pulp mill effluents can be high due to elevated background levels of other thermotolerant coliform bacteria (NCASI 2016), but contract laboratories rarely conduct additional biochemical species verification testing to assess the potential for misidentification. Section 12.0 of EPA Method 1603 for *E. coli* enumeration by membrane filtration specifies that "(v)erification of typical and atypical colonies may be required in evidence gathering and is also recommended as a means of quality control." This procedure uses biochemical species testing to establish false positive and false negative rates. A typical colony identified as *E. coli* is a positive confirmation. A typical colony identified as a species other than *E. coli* is a false positive. An atypical colony that is identified as *E. coli* is a false negative.

Several mill studies have used biochemical species testing to verify *E. coli* findings or characterize species composition. NCASI conducted a study to characterize the bacteriological species composition of woodyard samples and determine if this is a potential source of *E. coli*. Figure 2b shows that approximately 35% of 40 typical *E. coli* colonies tested were identified as other species (false positive) in pooled data from five woodyard runoff samples. (NCASI 2017). Those colonies confirmed as *E. coli* were flagged "abnormal" because their profiles were similar to, but not a match for, the strains in the clinical library used to conduct NCASI's woodyard study. *E. coli* strains in the library represent pathogenic strains commonly found in clinical settings. More recent mill studies have been conducted using a profile library with a broader list of species, including environmental strains. These data suggest that woodyard runoff can be a source of *E. coli* that is not of fecal origin, but rather is associated with plant decay.

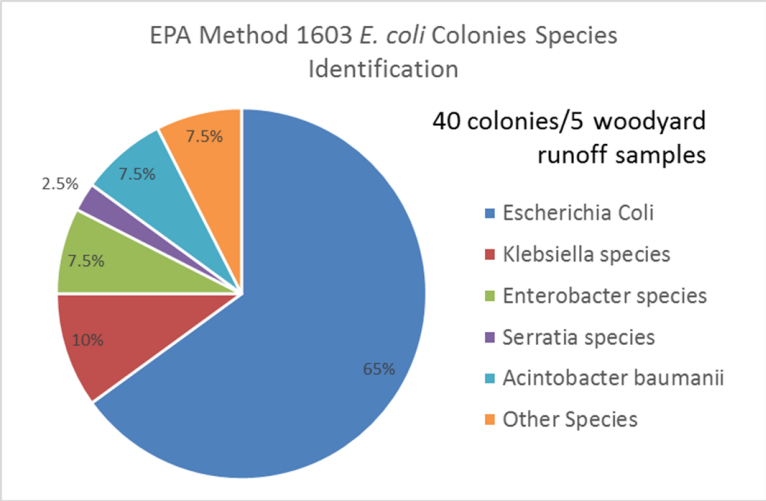

Figure 2b. Woodyard Runoff Biochemical Species Testing Results from Method 1603 Membrane Filtration

Discussion 2c. Colilert Enzyme Substrate Species Identification and False Positive Assessments. The approved enzyme substrate Quantitray (Colilert®) *E. coli* method does not provide instructions for determining false positive or false negative rates. However, NCASI has worked in conjunction with IDEXX (the Colilert supplier) and a contract laboratory conducting biochemical species testing to evaluate the potential for false positive results using the method. To conduct this test, an aliquot of sample from multiple positive wells (typically 10) is plated on an agar that is selective for *E. coli*. Biochemical species tests are conducted on "typical" colonies from the agar plate. Results of biochemical species testing from the same woodyard runoff samples shown in Figure 2b are illustrated in Figure 2c. The same clinical profiles as those for membrane filtration were used and these *E. coli* strains were flagged as "abnormal." The figure shows that 45% of colonies tested from *E. coli* positive Colilert wells were not identified as *E. coli*. This suggests that background levels of these bacteria may be elevated enough to be causing false positive test results (NCASI 2017).

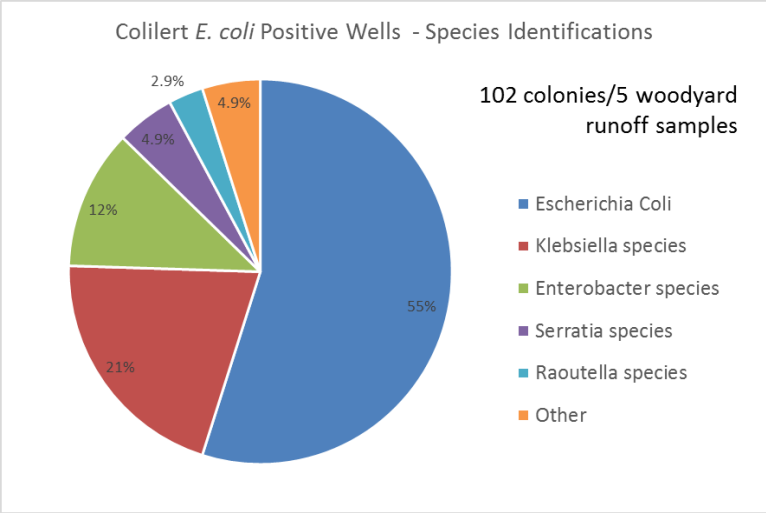

Figure 2c. Woodyard Runoff Biochemical Species Testing Results from Colilert *E. coli* Positive Wells

Although these examples are specific to woodyard runoff, species testing has been conducted in pulp and paper wastewaters with similar results, depending on the sample source (NCASI 2016). These examples

illustrate the importance of a rigorous laboratory certification program that includes species verification and the ability to apply scientifically defensible additional testing or alternative tests in cases where the presence or amount of FIB is suspect.

Comment 3. Enterococci Standards. NCASI believes enterococci measurement methods are not suitable for monitoring fecal sources of contamination in most mill effluents because pulp and paper discharges are predominantly characterized by a plant derived species of *Enterococcus* (*E. casseliflavus*) even when sanitary sources are not present. Pulp and paper discharges do not have a salinity similar to marine or estuarine waters; therefore freshwater alternatives such as *E. coli* may be a viable option for managing the potential for fecal contamination.

Discussion 3. Enterococci Standards. Analytical test methods for enterococci do not differentiate between different species of enterococcus. As noted in WDOE's Preliminary Regulatory Analysis, enterococci has been linked to vegetative sources. Common species of enterococci found in the intestinal tracts of warm blooded animals are *E. faecalis* and *E. faecium*, which are not typically predominant species in pulp and paper discharges. Most pulp and paper mills do not discharge to brackish waters, but those that do will probably have difficulty meeting enterococci monitoring requirements even when they do not have a sanitary source because of the presence of *E. casseliflavus*. One such facility tested its effluent using EPA Method 1600 (a single-step agar method) and conducted biochemical species testing from the typical colonies on the membrane filtration plates. During 23 sampling episodes 174 typical colonies were tested. Results from all sampling episodes were pooled and the species distribution is shown in Figure 3. The results show a 7.5% false positive rate due to non-enterococcus species identification and identify 87% of colonies as *E. casseliflavus*. *E. faecalis* and *E. faecium*, common species in sanitary sources and some warm-blooded animals, represented 2.3% of the species identified.

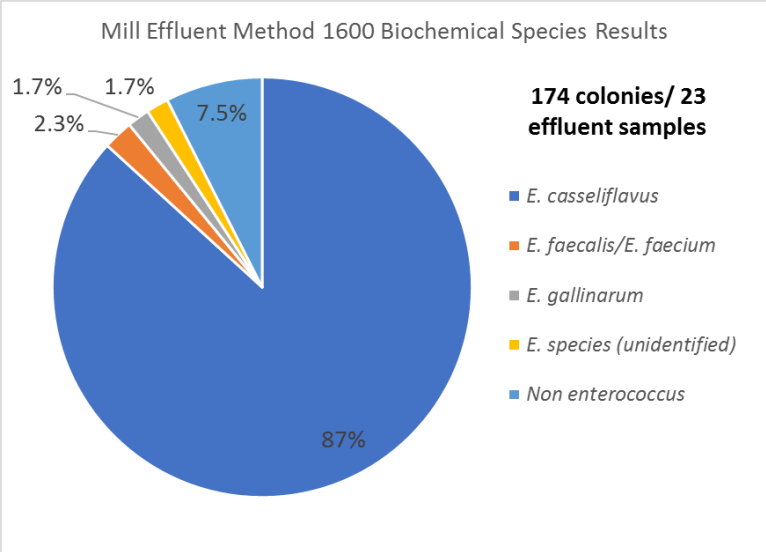

Figure 3. Mill Effluent Biochemical Species Testing Results from Method 1600 Membrane Filters

Enterococcus qualitative polymerase chain reaction (qPCR) testing at this facility also showed that *E. casseliflavus* was the primary species identified. Similar results were obtained in an earlier study at a different mill, where *E. casseliflavus* was the predominant species present using both biochemical species testing and qPCR (NCASI 2016). *Bacteroides* testing was also conducted at both mills and was non-detect for human markers. In the earlier study, the state implemented alternate requirements at this facility for biennial *Bacteroides* human markers monitoring.

Comments Regarding Revisions to Section (2) (b) (i) Averaging

Comment 4. Averaging. Collection of only three samples during the 90-day averaging period for ambient waters is insufficient for assessing the frequency of exceeding the STV over the same averaging period. This is important for determining if a waterbody is impaired.

Discussion 4. Averaging. Although the proposed minimum sample requirement is valid for calculating a geometric mean, this low sampling frequency is insufficient to assess how frequently the STV may be exceeded during the same averaging period. When assessing if a waterbody is impaired, at least 10 samples are needed over the sampling period to determine if the STV is exceeded in more than 10% of samples.

Comments Regarding Revisions to Section (2) (b) (ii) Multiple Samples

Comment 5. Multiple Samples. Consider revising this section to clarify the purpose of collecting multiple samples and how the data will be used to make water quality or beach closure decisions. The section states, "multiple samples should be arithmetically averaged together (to reduce concerns about low bias when the data is later used in calculating a geometric mean) to reduce sample variability and to create a single representative data point." It is unclear why an arithmetic mean would be used when the water quality standard is based on a geometric mean value that was determined to be protective of human health. This section also does not address how collection of multiple samples may be applied in NPDES permits if a single sample result indicates an exceedance of the standard.

Discussion 5a. Multiple Samples. Changes to the section are not currently proposed, but the section recommends using arithmetic averages to reduce concerns with low bias. Standard Methods states, "Microbial distributions are not necessarily symmetrical. Bacterial counts often are characterized as having a skewed distribution because of the many low values and a few high ones. These characteristics lead to an arithmetic mean that is considerably larger than the median." The RWQC are based on geometric mean values, and corresponding STVs were developed by using log-transformed data to address this distribution. Therefore, it is unclear why an arithmetic mean value would be used. WDOE's rule could be made more flexible if similar language were included.

Discussion 5b. State Use of Multiple Samples in Discharge Permitting. In some cases a single sample result will exceed a discharge limit, which may be due to a random highly variable result. To address this issue, some state water quality standards include provisions to collect multiple samples that demonstrate an unusual occurrence. Examples from Oregon and South Carolina follow.

Oregon Department of Environmental Quality (OR-DEQ):

> (C) No violation will be found for an exceedance if the permittee takes at least five consecutive re-samples at four-hour intervals beginning as soon as practicable (preferably within 28 hours) after the original sample was taken and the geometric mean of the five re-samples is less than or equal to 126 organisms per 100 mL of E. coli. However, if the Department finds that re-sampling within the timeframe outlined in this section would pose an undue hardship on a treatment facility, a more convenient schedule may be negotiated in the permit, provided that the permittee demonstrates that the sampling delay will result in no increase in the risk to water contact recreation in waters affected by the discharge;

South Carolina Department of Health and Environmental Control (SC-DHEC):

> 12) Provided the permittee verifies in writing to the Department that conditions (12)i. through (12)iv. below have been met, the permittee would be in compliance with the daily maximum

bacterial requirement. However, nothing in this regulation precludes the Department from taking action, depending on the individual circumstances to protect public health and/or the environment.

i. If the facility exceeds the permitted Daily Maximum bacterial limitation listed above (for E. coli, enterococci or fecal coliform) but two (2) additional samples collected within 48 hours of the original sample result do NOT exceed the required Daily Maximum limit; and

(A) For all waters not involving shellfish protection (regardless of the specific water classification), the individual bacterial sample result has not exceeded 800 MPN per 100ml, and for those waters involving shellfish protection, the individual bacterial sample result for fecal coliform has not exceeded 200 MPN per 100ml; and

(B) There is neither an existing Consent Order nor Administrative Order associated with the facilities operation of their disinfection system; and

(C) Either:

1. For facilities that routinely collect ten (10) bacterial samples per month (or 120 or more samples per calendar year), there were no more than four (4) total bacteria samples exceeding the daily maximum limit in the previous twelve (12 months); or

2. For facilities other than those listed in (C) 1. above (e.g. smaller facilities or those that do not routinely collect 10 samples or more per month), there was no more than one (1) bacterial sample exceeding the daily maximum limit in the previous twelve (12 months); and

ii. The permittee verifies that all disinfection equipment was fully functional, and the solids handling system was fully functional during that monitoring period; and

iii. Any additional bacterial sampling collected during the monthly monitoring period when the daily maximum exceedance occurred was reasonably distributed in time while maintaining representative sampling; and

iv. The permittee must provide sufficient laboratory data sensitivity (e.g., dilutions) to accurately represent the effluent bacterial concentration to utilize this procedure. Effluent bacterial results reported as greater than (>) do not meet this criteria, since the actual results are unknown.

Comments Regarding Revision to Section (2) (b) (iv) Alternate Indicator Criteria

Comment 6. Removal of Alternate Indicator Criteria. Consider reinstating this section: "Where information suggests that sample results are due primarily to sources other than warm-blooded animals (e.g. wood waste), alternative indicator criteria may be established on a site-specific basis by the department." Elimination of the option to consider alternate criteria restricts the permit writer's and WDOE's ability to make science-based, site-specific water quality decisions.

Discussion 6a. Removal of Alternate Indicator Criteria. A finding of *E. coli* in freshwater or enterococci in marine water does not always indicate whether the source is anthropogenic or connected to a sanitary source without additional science-based data. Use of a combination of alternate criteria or science-based tools in conjunction with FIB testing can provide strong evidence characterizing sources and help to inform choices about the need to control discharges when no sanitary source is expected to be present. Therefore, the provision to allow alternate indicator criteria should not be eliminated. EPA's 2012 RWQC recommendations permit states to develop site-specific criteria and provide tools and guidance for

determining if site-specific criteria should be applied. As noted (Discussion 3), *E. cassiliflavus* is a species of enterococci commonly found in nature that is associated with plant decay. It has been found to be the predominant species measured in some pulp and paper wastewaters where there are no sanitary connections. In addition, a high rate of false positive results has been demonstrated using some *E. coli* indicator test methods when applied to industrial sources (Discussions 2b, 2c). Removal of a provision to consider alternative indicator criteria on a site-specific basis where environmental sources may be predominant limits WDOE's ability to utilize scientifically defensible data to assess water quality and implement RWQC in permits.

Discussion 6b. EPA's 5-Year RWQC Review. In 2018, EPA released its 5-year review of the 2012 RWQC. As part of this effort, EPA examined recent literature (peer-reviewed scientific papers published after 2010) to assess advances in the state of the science supporting the 2012 RWQC. One section of the review examined the relationship between non-point sources of fecal pollution (sites with no identified point source of human fecal contamination) and the risks posed to human health. The review found that, "studies with non-point, non-human, diffuse and sporadic sources have often failed to identify significant associations between FIB density and illness" (USEPA 2018). It concluded that additional health studies pertaining to the basis for the 2012 RWQC provided confirmation that waters affected by some non-human sources could pose less risk than human fecal contamination.

Citing works by Soller et al. (2010) and McLellan and Eren (2014), the EPA document states that, "fecal contamination from human and animal sources contribute different pathogens to ambient waters resulting in variable relationships between FIB, pathogen, and illness outcomes" (USEPA 2018). For example, Soller et al. found that while GI illness risks associated with exposure to recreational waters impacted by fresh cattle feces may not be substantially different from waters impacted by human sources, risks associated with exposure to recreational waters impacted by fresh gull, chicken, or pig feces appeared substantially lower than those in waters impacted by human sources. Additionally, a 2009 EPA study conducted at sites with no identified point sources of human fecal contamination found no strong or consistent associations between levels of FIB and swimming-associated illness (Wade et al. 2010 as cited in USEPA 2018). The EPA review also references the World Health Organization recreational water quality guidelines (WHO 2003), which highlight the pollution source risk differential and incorporates a water classification scheme that emphasizes fecal contamination from humans (USEPA 2018). Findings from EPA's review continue to support the viewpoint that scientific methods that identify and characterize sources of FIB are essential to accurately assess risk to human health and inform decisions related to management of recreational waters. Some of the scientific methods and techniques reviewed included: (a) improvements to qPCR *enterococcus* and *E. coli* species testing; (b) identification of new sanitary survey tools; (c) implementation of microbial source tracking (MST) tools (e.g., human *Bacteroides*); and (d) exploration of development of RWQC for Coliphage.

Discussion 6c. State Provisions for Alternate Criteria. Several states have adopted provisions for alternative criteria in their Water Quality Standards or Implementation Documentation, including Oregon, Florida, Virginia, and South Carolina. Examples from Oregon and South Carolina follow.

OR-DEQ recently revised its Recreational Water Quality Criteria. An accompanying issue paper (Borok 2016) discusses its position regarding non-fecal sources, in which it notes the following provision:

> This change acknowledges that certain non-fecal discharges, such as pulp and paper effluent, may contain bacteria that are detected as *E. coli* or enterococcus, but are not pathogenic and do not indicate the presence of fecal contamination. (Gauthier and Archibald 2001; Degnan 2007; Croteau, et al. 2007). Due to the potential interference of plant-based bacteria in enterococcus tests, it may be difficult for pulp and paper mills to achieve compliance with enterococcus

> criteria even if the discharge poses little risk to public health due to the lack of pathogenic bacteria in the discharge. The proposed provision will allow flexibility to entities that can demonstrate to DEQ that their discharge does not come from fecal sources. DEQ would require such entities to demonstrate through biochemical species identification techniques that the effluent contains non-fecal based bacteria species. Once the demonstration is made, DEQ would include appropriate effluent limits in the permit to ensure that public health is protected.

SC-DHEC R.61-68, WATER CLASSIFICATIONS & STANDARDS includes the following language regarding site-specific criteria:

> (7) Site-specific permit effluent limitations and alternate criteria less stringent than those derived in accordance with the above requirements may be derived where it is demonstrated that such limits and criteria shall maintain the existing and classified uses, adequate opportunity for public participation in such derivation process has occurred, and the effluent shall not cause criteria for human health to be exceeded. Where a site-specific permit effluent limitation and alternate criterion has been derived, such derivation shall be subject to EPA review as appropriate. Also, at a minimum, opportunity for input in derivation of a site-specific permit effluent limitation and alternate criterion shall be provided via public notice in NPDES permit notices.

Reference

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