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National Council for Air and Stream Improvement, Inc.

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Dear Andrew Edwards and Relevant SCDES Staff,

NCASI appreciates the opportunity to provide input on South Carolina's proposed Water Quality Standards through triennial review process.

NCASI is an independent, non-profit research organization that focuses on environmental topics of interest to the forest products industry. NCASI conducts research and technical studies on behalf of forest products companies across the US, and its members represent over 80% of the pulp and paper production and two-thirds of wood panels produced nationwide. In its capacity as a research organization, NCASI has a long history of working to inform the science needed to address numerous environmental topics related to the forest products industry.

As the triennial review commences, we wish to comment on the use of fecal indicator bacteria (FIB) for non-POTW industrial dischargers. The following comments are provided to help ensure that development and implementation of bacteria WQS are based in the best available science, the challenges and limitations of such an approach are sufficiently described, and guidance is available on addressing shortcomings.

Specific Comments

1. Test methods used for enumeration of FIB are prone to bias and interference

Methods approved by EPA for regulatory testing for FIB (i.e., Colilert® for fecal coliform and *Escherichia coli*, and Enterolert® for enterococci) are susceptible to interference from physical, chemical, and biological constituents that impact their ability to accurately enumerate bacteria, and cannot differentiate between fecal and non-fecal sources. In pulp and paper mill wastewater, effluent color and solids and chemicals used in the manufacturing process, such as optical brighteners and detergents, can interfere with the color change or fluorescent endpoint that is the basis of quantifying FIB. A non-target bacterial species (i.e., *Bacillus licheniformis*) that has been measured in pulp and paper mill effluent (Bailon-Salas et al., 2018) causes similar interference that hampers accurate FIB enumeration (Peperzak and van Bleijswijk, 2021). Further, several bacterial species ubiquitous in the environment and not associated with fecal contamination are measured by FIB tests, resulting in inaccurate conclusions regarding fecal contamination. For example, *Klebsiella* spp. are common, non-fecal, environmental bacteria that are measured in the fecal coliform test, and often predominant in pulp and paper mill wastewaters (Gauthier and Archibald, 2001). Similarly, the enterococci test method is not specific to the two strains associated with fecal contamination (i.e., *Enterococcus faecalis* and *E. faecium*) but measures several enterococcus species, including *E. casseliflavus,* which is associated with plants (Mundt and Graham, 1968) and is dominant in forest products industry wastewaters (Niemi et al., 1993; Ragsdale

and Flinders, 2026). Because of interference from typical effluent constituents in FIB tests and their quantification of several non-fecal bacteria common in pulp and paper mill effluents, outcomes from these tests are unreliable for determining human fecal contamination in forest products industry wastewaters.

2. ***Test-associated 95% confidence intervals and lab variability results in considerable uncertainty in measured FIB***

Colilert and Enterolert methods estimate bacterial density based on the color change or fluorescence across 97 test wells (49 large and 48 small), with the number of positive wells necessary to exceed regulatory thresholds depending on the FIB measured (i.e., fecal coliform, E. coli, and enterococci), the relative number of positive small and large wells, and sample dilution. Associated 95% confidence intervals are also provided by the test manufacturer, with methods specifying that the dilution factor should be applied to both the test result and the 95% confidence intervals if samples are diluted to overcome the physical, chemical, and biological interferents discussed previously and that test sensitivity is greatest when 68-78 wells are positive. This test-related variability introduces uncertainty in reported FIB concentrations in the context of regulatory thresholds because assurance that FIB concentration in a sample exceeds or is within the regulatory limit occurs only when the 95% confidence intervals are above or below the regulatory threshold. For example, confidence that the regulatory enterococci threshold (35 MPN/100 ml) of an undiluted sample has been exceeded occurs only when FIB ≥ 45 MPN/100ml because, at this concentration, the lower 95% confidence interval is also above the regulatory threshold (Figure 1a). Notably though, most mill effluents require a 1:10 dilution to overcome test interference, meaning that enterococci will exceed the water quality standard if >3 wells (out of 97) are positive. Critically, if no wells fluoresce indicating that enterococci were not detected (with results reported as <10 MPN/100 ml), the 95% confidence limits are 0-37 MPN/100ml and overlap the regulatory threshold. Documented variability across contract labs adds additional uncertainty in reported FIB results, particularly for enterococci. Contract lab-associated 95% confidence intervals calculated using performance testing data associated with lab certification indicate confidence that the regulatory enterococci threshold of an undiluted sample has been exceeded occurs only when FIB ≥ 53 MPN/100ml (Figure 1b).

The regulatory implication of test- and lab-associated uncertainty is reflected in measurements of enterococci in sample duplicates by different certified contract labs. Split sample data (n=69 samples) compiled in a robust NCASI database of FIB in pulp and paper mill effluent and runoff from wood storage areas (e.g., woodyards and wood chip storage areas) showed that the relative percent difference (RPD) between reported enterococci concentrations was as high as 193% (mean RPD=74%) with 33% of samples having different regulatory outcomes depending on the contract lab (Figure 2). Taken together, there is considerable uncertainty that FIB in a sample exceeds a regulatory threshold, particularly for enterococci, due to the test uncertainty reported by the manufacturer of approved regulatory tests, disconnect between the number of positive wells resulting in an exceedance and those necessary to achieve the best test sensitivity, complex nature of pulp and paper mill effluents and need for sample dilution to overcome interference, and variability among FIB contract labs.

3. Risk to human health based on reported FIB may be over-estimated in the presence of environmental and non-human bacterial sources

The key purpose of testing fecal indicator bacteria (FIB) is to protect recreational waters and human health from harmful pathogens. EPA acknowledges that a limitation of the currently recommended FIB methods is their inability to distinguish bacterial sources, which may indicate different risk levels to human health. FIB from non-human sources (e.g. those derived from plant or animal sources) are also present in recreational waters and often pose a lower risk to human health (USEPA, 2018), making information on fecal pollution sources essential for management and protection of public health in recreational waters (USEPA, 2018).

Because FIB tests cannot differentiate between sources, the presence of bacteria from non-fecal and animal fecal sources can result in FIB values that exceed RWQC (USEPA, 2007) even though the human health risk from these bacteria is generally lower than risk from human fecal sources (USEPA, 2010, 2024). For example, the risk of illness from non-human fecal sources has been shown to be 28-6,000 times lower than that from human fecal sources (Soller et al., 2015) (Figure 3). The use of FIB as indicators of fecal contamination may be further complicated by the presence of naturalized populations of *E. coli* and enterococci, which are measured by FIB tests but do not carry the same human health risk as fecal-derived populations (Byappanahalli et al., 2012; Devane et al., 2020). Elevated FIB attributable to non-human environmental bacteria was prevalent in pulp and paper mill effluents and wood-associated wastewaters (Ragsdale and Flinders, 2026). Microbial source tracking showed that human markers were absent 73–100% of samples with elevated FIB and, among those samples, fecal bacteria from ruminants or birds were detected in fewer than 11% of samples, although bacteria from non-quantified sources may be present. For enterococci tests with speciation data, elevated bacteria were attributable to non-fecal, environmental enterococci species in 96% of samples. These outcomes highlight that elevated FIB, likely due to the test interference and bias and test and lab variability described in previous sections, are not necessarily indicative of a health risk associated with human fecal sources.

4. Approaches to address uncertainty associated with FIB

EPA allows States to use scientific approaches to propose alternative site-specific water quality criteria (WQC) that are scientifically defensible, and protective of the recreational designated use (USEPA, 2014). EPA acknowledges that "there are waterbodies with conditions that differ from those studied and used to inform 2012 RWQC" and that "water quality managers may wish to consider WQC reflective of specific conditions or may want to use a different indictor of fecal contamination and/or enumeration method" (USEPA, 2014). The flexibility to distinguish human/non-human sources and their associated risk to human health when evaluating attainment of RWQC are described in the Code of Federal Regulations (i.e. 40 CFR 131.41(c)(1) footnote e; 40 CFR 131.41 (c)(2) footnote c).

Given the potential for FIB test interference, test- and lab-associated variability, and source-specific bacterial risk to human health, it is appropriate to consider provisions where a non-POTW can provide scientifically defensible data demonstrating that non-anthropogenic sources are responsible for elevated levels of FIB, and that there is no connection to sanitary sources in order to assess the need for permit limitations. Demonstration of reasonable assurance would be site-specific based on the unique configuration of treatment processes at the facility, and the NPDES permit may include effluent

limitations for both industrial and sanitary parameters in the combined discharge. Demonstrations could include monitoring for *E. coli* at internal locations that meet the freshwater definition, chemical analyses documenting treatment of sanitary contributions, or a combination of other methods to characterize identified bacteria sources as predominantly of non-human origin.

Some states have already taken this approach and have incorporated language into their water quality standards that allow a discharger to provide scientifically defensible data demonstrating that elevated levels of these indicator bacteria are not associated with human fecal sources. For example, the Florida Department of Environmental Protection included a memorandum as part of the record in its revision of recreational water quality criteria that recognizes the limitations of these tests and affords dischargers the opportunity to demonstrate that bacterial assay results are not indicative of the presence of bacteria linked to sanitary wastewater (FDEP, 2015). Similarly, the state of Oregon has acknowledged that pulp and paper mill effluent may contain bacteria that are detected as *E. coli* or enterococci but are not pathogenic and do not indicate the presence of fecal contamination (Borok, 2016). As such, Oregon WQS specify that requirements apply to bacteria in effluent associated with fecal sources[1], which allows flexibly to entities that can demonstrate that their discharge does not come from a fecal source.

We appreciate the opportunity to offer comment on your current triennial review of WQS and would be happy to respond to questions concerning these comments.

Best,

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[1] 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.

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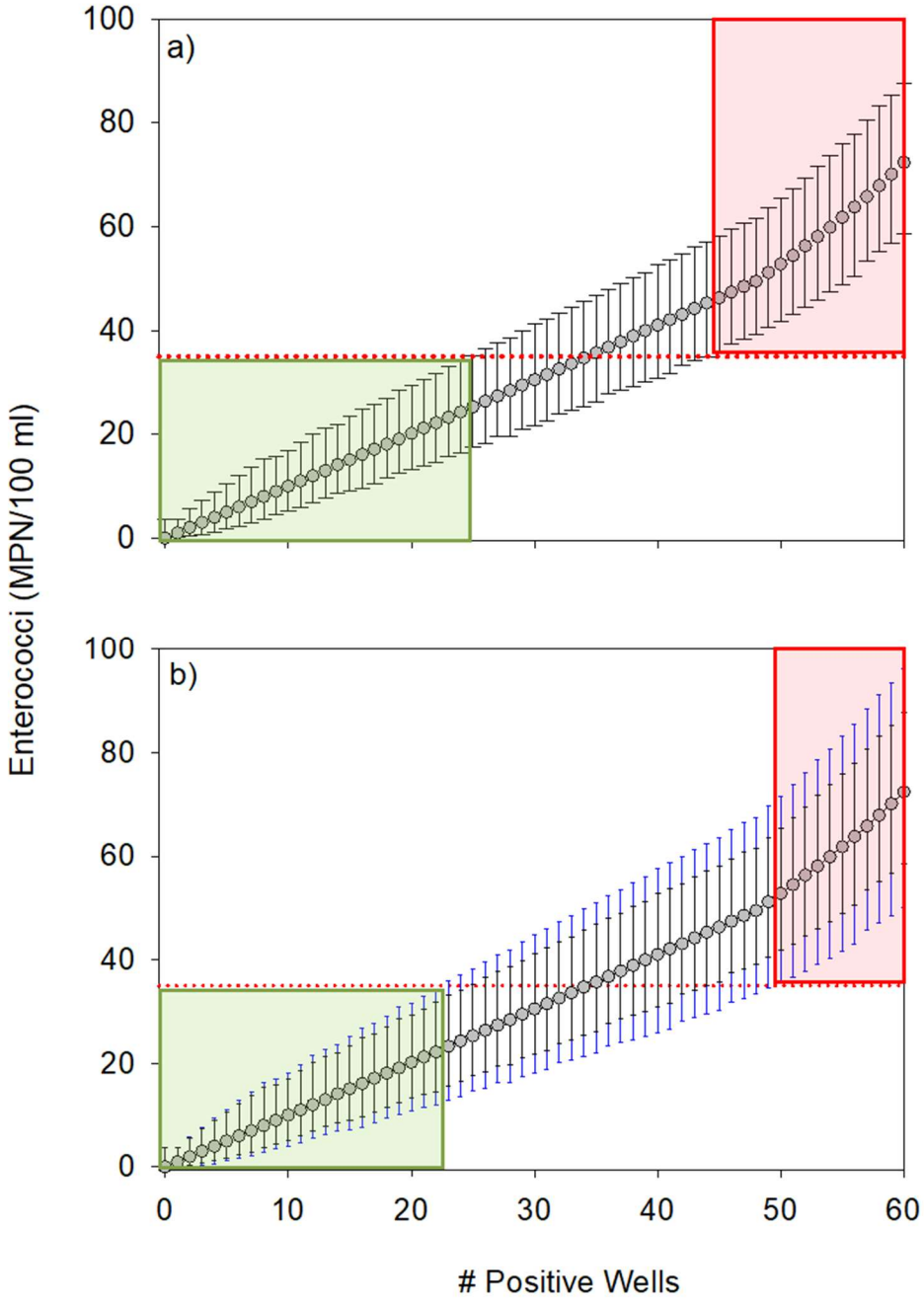

Figure 1. Enterococci concentrations (most probable number (MPN)/100 ml) and associated 95% confidence intervals with respect to (a) test and (b) test (black error bars) and laboratory (blue error bars). Red line indicates regulatory limit of 35 MPN/100 ml. Green and red boxes indicate where there is confidence that enterococci concentrations are below or above the regulatory threshold, respectively (because 95% confidence intervals are also below or above the threshold value). Values outside of the colored boxes indicate samples where confidence in regulatory compliance is lacking.

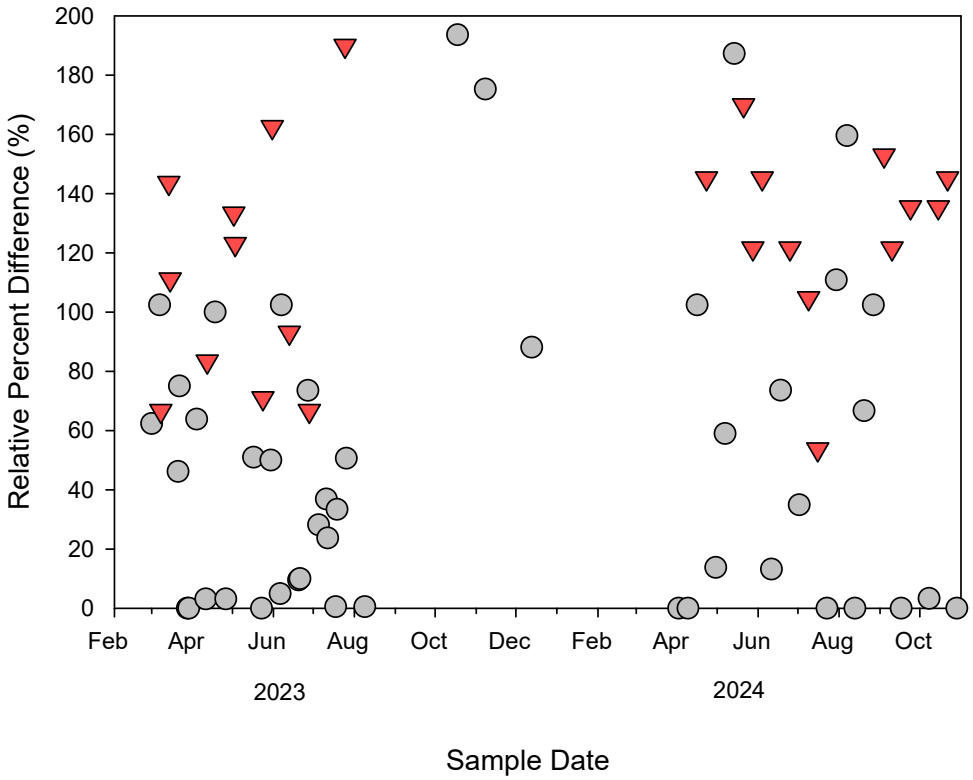

Figure 2. Results of split-sample analysis of enterococci using Enterolert. [Red triangles indicate samples with different regulatory outcomes attributable to laboratory]

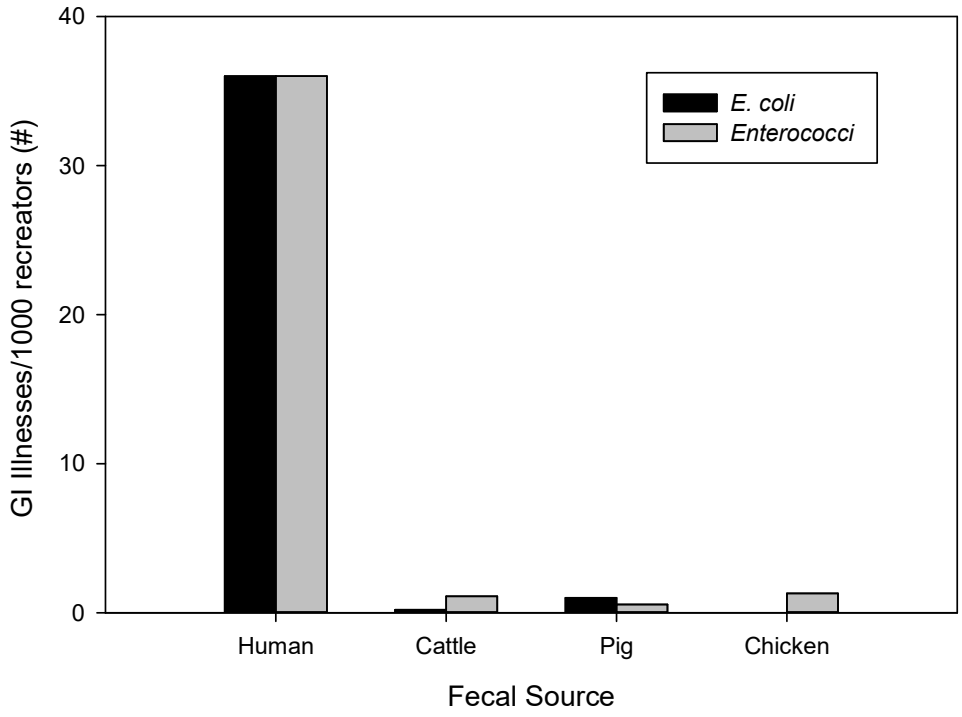

Figure 3. Human health risk from fecal sources. GI=gastrointestinal. [Figure adapted from Soller et al. 2015]