

U.S. Department of
Homeland Security

United States
Coast Guard



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JUL 12 2016

Trojan Technologies
Attn: Mr. Christian Williamson
3020 Gore Road
London, ON
Canada N5V 4T7

Dear Mr. Williamson,

I refer to your appeal dated February 12, 2016, on behalf of Trojan Technologies (Trojan). You requested a formal administrative appeal of the decision of the Marine Safety Center (MSC) denying Trojan's request for a testing alternative to be used in the Coast Guard's ballast water management system (BWMS) type approval process and Trojan's application for USCG type approval of the Trojan Marinex BWMS.¹

This is an administrative appeal of an MSC decision or action taken pursuant to 46 Code of Federal Regulations (C.F.R.) § 162.060-10 and is reviewed by my office under 46 C.F.R. § 159.001-2 and as provided in 46 C.F.R. § 1.03-15. In considering your request, I reviewed your appeal (including its appendix and the administrative appeals of DESMI Ocean Guard A/S, Alfa Laval Tumba AB, and Hyde Marine, Inc, incorporated by reference via page 5 of your appeal), the administrative record (including MSC's denial of your request for reconsideration), and applicable laws, regulations, and policy.²

Based on this review, I hereby deny your appeal, affirming MSC's decision to deny Trojan's request for testing equivalency and type approval under 46 C.F.R. § 162.060-10. This matter and your appeal are quite technical and detailed, and the issues raised will be discussed in more detail in this letter.³ To briefly summarize, during the development of the ballast water regulations, the Coast Guard explicitly rejected the use of BWMS that "may act to make organisms unviable or unable to reproduce rather than killing or removing them." This policy decision to reject "viability" was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations, and MSC lacked the discretion to accept your proposed

¹ Type testing and approval are used in equipment and manufacturing to determine that a specific "type" of equipment or process meets a minimum set of requirements. In this context, "type approval" is a vessel equipment approval process.

² I note in your appeal that Trojan reserves the right to "supplement" its appeal to address my response. That is unnecessary. This response constitutes final agency action on the issues raised in Trojan's appeal.

³ I have substantively responded to your appeal in the interest of transparency. This response does not waive any defenses the Coast Guard may have as to the timeliness of your assertions or failure to exhaust your administrative remedies.

testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). As your type approval application was based on testing that does not meet the regulatory standards and was not approved as an alternative, MSC was correct in denying your application for type approval of the Trojan Marinex BWMS. Your appeal is denied, and this decision constitutes final agency action.

Background

Marine environmental protection is one of the Coast Guard's core statutory and operational missions.⁴ As stewards of the marine environment, the Coast Guard maintains a robust environmental protection regulatory program and also assists other federal agencies in enforcing laws to protect, preserve, and remediate waters subject to the jurisdiction of the United States. The Coast Guard also leads and participates in initiatives at the International Maritime Organization (IMO), the intergovernmental organization specializing in commercial shipping safety, security, and environmental protection standards, to raise and standardize global shipping practices. These activities all have one desired end state: to help ensure the health and vitality of waters of the United States and its living marine natural resources.

The Coast Guard manages its marine environmental protection obligations through a well established network of Headquarters, regional and field offices. Within Coast Guard Headquarters, located in Washington, D.C., there are several organizations, or "programs," responsible for developing, promulgating, and enforcing marine environmental protection standards. The Office of Operating and Environmental Standards (CG-OES) and the Office of Design & Engineering Standards (CG-ENG) have the lead roles in promulgating and implementing (but not enforcing) Coast Guard environmental regulations, including requirements for approval of equipment installed on vessels. MSC's role focuses on regulatory compliance and policy development, generally related to plan reviews for domestic vessels and type approvals for vessel equipment. While MSC can be involved in the clearance process for rulemakings, MSC is not the lead office for environmental standards development. In other words, MSC applies environmental regulations but does not create them. All three programs are within the Directorate of Commercial Regulations and Standards (CG-5PS).

The Coast Guard's ballast water program is one of the Coast Guard's long-standing marine environmental protection programs. It is established under the authority of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, as amended by the National Invasive Species Act of 1996 (NANPCA/NISA).⁵ As mandated by NANPCA, the Coast Guard's program began in 1991 with voluntary ballast water management guidelines for the Great

⁴ See Section 888, Homeland Security Act of 2002, Pub. L. No. 107-296 (H.R. 5005), 116 Stat. 2135 (2002), as amended, *classified to* 6 U.S.C. § 468.

⁵ Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (NANPCA/NISA), Pub. L. No. 101-646 (H.R. 5390), 104 Stat. 4761 (1990), *as amended; codified in* 16 U.S.C. §§ 4701, 4702, 4711, 4712-4714, 4721-4728, 4741, 4751. Congress enacted NANPCA in response to the disruption and damage caused by the introduction of nonindigenous zebra mussels into the Great Lakes, likely released via discharges of ships' ballast water. NANPCA originally focused on ballast water operations in the Great Lakes but was later amended by NISA to cover waters nationwide.

Lakes.⁶ Mandatory requirements for the Great Lakes followed these voluntary guidelines in 1993.⁷ In 1996, NISA amended NANPCA to, among other things, cover all navigable waters of the United States.⁸ In the 1990s, the best method available for preventing the discharge of aquatic nuisance species from ballast water was an operational practice known as ballast water exchange. For this reason, NANPCA/NISA contained a specific requirement for certain vessels to conduct ballast water exchange.⁹ The Coast Guard updated its regulations to include nationwide, mandatory ballast water exchange requirements in 2004.¹⁰ However, ballast water exchange was an interim measure until more effective ballast water technology could be developed and verified. Under the NANPCA/NISA mandate to “ensure to the maximum extent practicable that aquatic nuisance species are not discharged into waters of the United States from vessels,” the Coast Guard collaborated with domestic and international partners to identify better methods and technology to prevent the introduction and spread of aquatic nuisance species. These efforts ultimately resulted in the Coast Guard’s 2012 Final Rule, Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters (2012 Final Rule).¹¹

NANPCA/NISA is a domestic legal authority and does not explicitly implement the international standard for ballast water discharges, which is found in the IMO International Convention for the Control and Management of Ships’ Ballast Water and Sediments, 2004 (BWM Convention).¹² The disconnect between U.S. domestic law and the international standard is partly timing: NANPCA was enacted in 1990, many years before the international community adopted the BWM Convention in 2004. Additionally, at this time, the United States is not a contracting government to the BWM Convention¹³ and the BWM Convention has not received enough ratifications to enter into force. The Coast Guard attempted to harmonize the 2012 Final Rule with the BWM Convention to the extent possible within its statutory authority.¹⁴ Ultimately, the

⁶ NANPCA/NISA, *supra*, n. 5, § 1101(a); *codified in* 16 U.S.C. § 4711(a). *See also* Ballast Water Management for Vessels Entering the Great Lakes, Final Rule, 58 Federal Register (Fed. Reg.) 18330 (April 8, 1993).

⁷ *Id.*

⁸ *See* NANPCA/NISA, *supra*, n. 5, § 1101(a) – (c); *codified in* 16 U.S.C. § 4711(a) – (c). This statutory bifurcation is the historical reason why Coast Guard ballast water regulations are promulgated in two subparts of 33 C.F.R. Part 151, despite there now being few differences between the ballast water management standards for the Great Lakes and those for the rest of the United States.

⁹ Ballast water exchange is a process by which a vessel replaces water, generally coastal water, in its ballast tanks. *See* definition of “exchange,” 33 C.F.R. § 151.2005(b).

¹⁰ Mandatory Ballast Water Management Program for U.S. Waters, 69 Fed. Reg. 44952 (July 28, 2004).

¹¹ 77 Fed. Reg. 17254 (March 23, 2012).

¹² IMO Doc. BWM/CONF/36.

¹³ If the United States became party to the BWM Convention, the Coast Guard could implement the convention through NANPCA/NISA even though there is no explicit reference. *See* NANPCA/NISA, *supra*, n. 5, § 1101(f)(3); *codified in* 16 U.S.C. § 4711(f)(3) (the Coast Guard “shall revise regulations...to make such regulations consistent with the treatment of a particular matter in any international agreement, *agreed to by the United States...*”)(emphasis added). However, this NANPCA/NISA authority is meaningless if the United States is not a party to the BWM Convention.

¹⁴ *See, e.g.*, 77 Fed. Reg. 17260. I note that the Coast Guard has made some statements which could confuse the issue of whether the Coast Guard ballast water discharge standard is “identical” to the BWM Convention standard. From the Coast Guard’s perspective, as discussed further *infra*, the BWM Convention’s use of “viable” is synonymous with the Coast Guard’s use of “living,” and in that sense the standards are the same. However, there are some minor differences between the two regimes. That is why the Coast Guard has used certain language, such as “align with” and “equivalent to,” to show that the Coast Guard regime is *not* identical to the BWM Convention regime.

BWM Convention is not a treaty of the United States, and the Coast Guard has a mandate to implement NANPCA/NISA as written.¹⁵

The Coast Guard's ballast water regulations are codified into Titles 33 and 46 of the C.F.R. The regulations in 33 C.F.R. Part 151 Subparts C (Great Lakes) and D (Nationwide) are operational or performance standards, and the regulations in 46 C.F.R. Subpart 162.060 are equipment standards.¹⁶ In this case, the Title 33 operational requirements apply to vessels, vessel owners or operators, or other persons associated with the vessel, and the Title 46 equipment requirements apply to BWMS manufacturers seeking equipment approvals. However, as discussed *infra*, the two are still interrelated and should be read together.

The 2012 changes to 33 C.F.R. Part 151 introduced a scheduled phase-out of ballast water exchange as an accepted operational measure to reduce the introduction and spread of aquatic nuisance species and provided the new option of using a BWMS to treat ballast water prior to discharging it.¹⁷ 33 C.F.R. Part 151 now contains a numeric discharge standard for the maximum number of living organisms in ballast water, a standard which all Coast Guard-approved BWMS must meet.¹⁸ The 2012 changes also added other ballast water management options, including the use of water from U.S. public water systems and discharge to a reception facility. For a vessel meeting the discharge standard by using a BWMS to be in compliance with 33 C.F.R. Part 151, its BWMS must have received type approval under the standards located in 46 C.F.R. Subpart 162.060.¹⁹

I now address the issues raised in your appeal, as well as the principal arguments raised by the other manufacturers in their respective appeals, which have been incorporated by reference, as follows:

1. MSC did not have the discretion to approve Trojan's request

¹⁵ As discussed further, *infra*, the Coast Guard's regulatory standard is "to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels..." NANPCA/NISA, *supra*, n. 5, § 1101; *codified in* 16 U.S.C. § 4711. The Coast Guard believes the "practicability" element of this standard requires the U.S. ballast water discharge standard to take into account the relevant IMO standard, as most vessels engaged on international voyages will likely need to comply with the BWM Convention once it comes into force. If the U.S. domestic standard conflicts with or frustrates an international standard, the result could range from increased vessel costs to preventing the vessel from calling on certain ports (implementing whichever standard the vessel cannot meet).

¹⁶ Generally, for the C.F.R., the reason vessel operational standards appear in Title 33 and vessel equipment standards appear in Title 46 is because many Coast Guard statutory authorities are contained either in Title 33 of the United States Code (Navigation and Navigable Waters) or Title 46 (Shipping). Title 33 authorities are generally more "operational," such as the Ports and Waterways Safety Act (Pub. L. No. 92-340 (H.R. 8140), Tit.I, 86 Stat. 424 (1972), *as amended*; *codified in* 33 U.S.C. §§ 1221 – 1232) and the Act to Prevent Pollution from Ships (Pub. L. No. 96-478, 94 Stat. 2297 (1980), *as amended*; *codified in* 33 U.S.C. §§ 1901 – 1911). On the technical side, the Coast Guard maintains broad authority to regulate inspected vessel equipment under 46 U.S.C. § 3306, and its vessel equipment approval processes are correspondingly located in Title 46 of the C.F.R. This is true even when the underlying statutory authority is contained in a different title. In this case, the Coast Guard has overlapping statutory authority to set BWMS equipment standards, under both NANPCA/NISA and 46 U.S.C. § 3306.

¹⁷ 33 C.F.R. § 151.1510(a); 33 C.F.R. § 151.2025(a).

¹⁸ 33 C.F.R. § 151.1511; 33 C.F.R. § 151.2030.

¹⁹ *See, e.g.*, 33 C.F.R. § 151.2025(a)(1).

I agree with MSC that it lacked the discretion to approve Trojan's 46 C.F.R. § 162.060-10(b)(1) request, which sought approval of a testing method that measured "viable" rather than "living" organisms in ballast water. The Coast Guard's type approval regulations exclude "viability" as an option. This was an environmentally conservative policy decision, based on best scientific information available, which went through the public notice and comment process. Therefore, MSC could not grant an alternative request that circumvented the text and policy position of the Coast Guard's ballast water regulations. Since Trojan's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying Trojan's type approval application.

Issue:

Trojan is a BWMS manufacturer of ultra-violet radiation (UV) based water treatment solutions, including BWMS. In the course of requesting Coast Guard type approval of its Trojan Marinex BWMS, Trojan submitted its application for a BWMS testing alternative under 46 C.F.R. § 162.060-10(b)(1). The crux of this appeal revolves around the type of tests that can be used, under the Coast Guard regulations, to validate the efficacy of UV BWMS. Specifically, Trojan requested to use a "Most Probable Number assay" (MPN) in lieu of 5-chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) direct staining methods to test for certain organisms to meet type approval requirements.²⁰ The simplified distinction between these measurement methods is that Trojan's preferred measurement method measures "viability" of an organism, while the regulatory requirement method measures whether an organism is "living." This appeal concerns the testing method being used and not whether the Coast Guard can or will grant type approval to UV BWMS as a class of system.²¹ While one appellant (Hyde) has characterized the question as "whether the measurement method – MPN – is reliable and accurate," the main question before me is whether the ballast water regulations allowed MSC to approve MPN as a measurement tool.²²

Applicable regulatory standards and the meaning of "living":

46 C.F.R. § 162.060-10(b)(1) provides:

If an evaluation, inspection, or test required by this section is not practicable or applicable, a manufacturer or independent laboratory may submit a written request to the Commanding Officer (MSC), Attn: Marine Safety Center, U.S. Coast Guard Stop 7410, 4200 Wilson Boulevard Suite 400, Arlington, VA 20598-7410, or by email to msc@uscg.mil, for approval of alternatives as equivalent to the requirements in this section. The request must include the manufacturer's justification for any proposed changes and contain full descriptions of any proposed alternative tests.

²⁰ Trojan appeal, APP. 2-1.

²¹ Not all UV BWMS are designed like Trojan's: some UV BWMS are designed to kill organisms rather than to render them unviable. Thus, my decision should not be interpreted to mean that the Coast Guard will not grant type approval to *any* UV BWMS under the current regulatory standards.

²² A follow-on question, answered in Section 3 of this response, is whether Trojan met all elements of 46 C.F.R. § 162.060-10(b)(1).

The Coast Guard applies 46 C.F.R. § 162.060-10(b)(1) by considering the following four elements:

1. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?
2. Is that evaluation, inspection or test not practicable or applicable?
3. Is the proposed alternative equivalent to the regulatory standard?
4. Does the request include a full description of the proposed alternative?

MSC's decision denying Trojan's application specifically addressed items 2 and 3 and found them both in the negative.²³ MSC found that Trojan's proposed alternative was not equivalent because

...it does not measure the efficacy of the ballast water treatment system to the performance standard required by the regulations. The regulations specifically require ballast water treatment systems to be evaluated based on their ability to kill certain organisms. Since the proposed MPN method assesses the viability of an organism to colonize after treatment, it measures to a different standard than that required by the regulations.²⁴

You contend for several reasons that this reasoning was unsound.

However, I believe MSC was correct that the Coast Guard's ballast water management regulations do not allow MSC to approve a method that measures viability in lieu of the regulatory standards.²⁵ I believe the reference, above, to the "performance standard required by the regulations" means the ballast water discharge standard contained in 33 C.F.R. Part 151.²⁶ As a general principle, a technical equipment standard in Title 46 of the C.F.R. would not be able to override a performance or operational standard in Title 33 of the C.F.R. In this case, the technical equipment standard and the operational standard are inextricably linked, and the meaning of "living" cannot be resolved by viewing a stark dichotomy between Titles 33 and 46 of the C.F.R.

To understand why, it is helpful to begin with the text of 46 C.F.R. § 162.060-10(b)(1). Trojan's request must meet the first prong of 46 C.F.R. § 162.060-10(b)(1), requesting an alternative to an "evaluation, inspection, or test required by this section." This is a reference to the requirements contained in 46 C.F.R. § 162.060-10(f) (emphasis below added):

A BWMS is eligible for approval if –

²³ Having found at least one of the elements in the negative, there was no need for MSC to opine on all of these elements. Additionally, I disagree that MSC "refus[ed] to consider" Trojan's application. (Trojan appeal, page 7).

²⁴ Letter dated December 14, 2015, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Trojan Marinex, "Request for Approval of the Use of the Most Probable Number (MPN) Method to Determine Biological Efficacy of the Trojan Marinex Ballast Water Management System (BWMS)".

²⁵ This is not a matter of literal "equivalency" of testing methods, which is discussed *infra*, Section 3.

²⁶ See Letter dated February 2, 2016, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Hyde Marine Inc., "Request for Review and Reconsideration of December 14, 2105 Decision of Marine Safety Center Denying Application for Approval of Equivalent Test Method Under 46 C.F.R. § 162.060-10(b)(1); ("Therefore, in order to demonstrate compliance with the BWDS as set forth at 33 CFR §§ 151.1511 and 151.2030, a BWMS must be evaluated on the basis of counting living/dead vice viable/unviable" organisms.").

(2) It is evaluated, inspected, and tested under land-based and shipboard conditions in accordance with §162.060-26 and 162.060-28 of this subpart, respectively, and thereby **demonstrates that it consistently meets the ballast water discharge standard in 33 CFR part 151, subparts C and D;**

(3) All applicable components of the BWMS meet the component testing requirements of §162.060-30;

(4) The BWMS meets the requirements of §162.060-32 of this subpart if the BWMS uses an active substance or preparation...

This provision clearly delineates the operational standard contained in 33 C.F.R. Part 151 from the equipment standard in 46 C.F.R. Subpart 162.060 and supports MSC's reasoning that a Title 46 alternative or equivalency for vessel equipment testing cannot be used to override a Title 33 performance standard. However, the actual text in 33 C.F.R. Part 151 shows that the two standards are inextricably linked (emphasis below added):

(a) Vessels employing a Coast Guard-approved ballast water management system (BWMS) must meet the following BWDS by the date in §151.1512(b) of this subpart:

(2) For organisms less than 50 micrometers and greater than or equal to 10 micrometers: discharge must include fewer than 10 **living** organisms per milliliter (mL) of ballast water.²⁷

There is no definition of "living" or any other regulatory text in 33 C.F.R. Part 151 that explains this important detail of the discharge standard. The only way a ballast water manufacturer can understand the "living" organism standard in 33 C.F.R. Part 151 is by referring to 46 C.F.R. Subpart 162.060 and its technical requirements and reading the preamble of the final rule. Thus, while the discharge standard in 33 C.F.R. Part 151 is an operational standard which applies to vessels, it is inextricably intertwined with the technical equipment standards contained in 46 C.F.R. Subpart 162.060 which apply to ballast water manufacturers.²⁸ The substance of "living" remains within 46 C.F.R. Subpart 162.060, and MSC was not solely bound by the indeterminate "living" language contained in 33 C.F.R. Part 151.

46 C.F.R. Subpart 162.060 also has no definition of "living,"²⁹ but this subpart contains extensive efficacy requirements which constructively define the term. Specifically, the Coast

²⁷ 33 C.F.R. § 151.1511, emphasis added.

²⁸ For vessels engaged on international voyages, this interlinkage between performance standards and technical standards is common, as typically a vessel's operation of a type approved or certificated piece of equipment satisfies the operational requirement unless a Coast Guard inspector or investigator has reason to believe that the equipment is not operating or being operated properly.

²⁹ A sole focus on the definition of BWMS (46 C.F.R. § 162.060-3) is misplaced. The BWMS definition alone does not set a performance or technical standard for BWMS. It merely identifies a category of equipment that the Coast Guard is regulating. The performance and technical standards in Titles 33 and 46, respectively, set the requirements for BWMS, and meeting the broad definition of BWMS does not necessarily mean that the BWMS meets all of the technical and performance standards in the regulations. Specific requirements control general terms. For an analogy, see the definition of "tank vessel" contained in 46 U.S.C. § 2101. The fact that a vessel may meet this broad definition does not mean that it can be certificated as a tank vessel under the inspection requirements in C.F.R.

Guard's testing regulations incorporate the Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV Protocol)³⁰ by reference.³¹ The ETV Protocol contains staining test requirements³² that evaluate the functioning of certain enzyme systems and cell membrane integrity of organisms, thereby defining "living" by virtue of these critical functions necessary for organisms to persist.³³ The ETV Protocol uses the term "viable," but defines it as "organisms and any life stages thereof that are living."³⁴ The ETV Protocol also explains why the ETV Technical Panel³⁵ decided to limit "viability":

Note that it is understood that many of the proposed regulatory discharge standards, and in fact the desired effect of BWTSs,³⁶ is that these technologies should render organisms unviable or incapable of reproduction. In other words, to "kill, remove or inactive" is technically unnecessary when the objective is to eliminate the organism's capability for reproduction. However, as the introduction of "viability" as a measure of efficacy significantly complicates the Protocol and test methods, and since "kill, remove or inactivate" is a conservative approach, the latter has been adopted as the measure of biological efficacy in this Protocol.³⁷

Title 46. The definition identifies a broad category of thing that the Coast Guard is regulating. The vessel would still need to meet the specific tank vessel inspection requirements to receive a tank vessel Certificate of Inspection.

³⁰ "Generic Protocol for the Verification of Ballast Water Treatment Technology," EPA/600/R-10/146, September 2010, available at https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=230926. The ETV Protocol is a document of the Environmental Technology Verification Program (ETV Program), funded in whole or in part by the U.S. Environmental Protection Agency.

³¹ See, e.g., 46 C.F.R. § 162.060-26(a), which refers to the Subpart's "Incorporation by Reference" section, § 162.060-5.

³² The ETV Protocol "requires" the determination of organism concentration, but it is true that the offered testing method – the dual stain method – is stated in voluntary terms. For example, the ETV Protocol states, "[t]his protocol recommends use a [sic] combination of two vital stains..." (paragraph 5.4.6.5). This is because the ETV Protocol recognizes that there are other stains and measurement methods that may be acceptable, as long as they measure the same thing: whether something is alive. If a manufacturer does not want to use the dual stain method to meet the ETV Protocol requirements, then a manufacturer uses the USCG approval process in 46 C.F.R. § 162.060-10(b)(1) to request an alternative for the purposes of complying with the USCG regulations.

³³ The appellants raise divergent arguments, sometimes claiming that the ETV Protocol trumps the regulatory text and other times claiming that the Coast Guard cannot rely on the final ETV Protocol at all because it was not properly incorporated into the ballast water NPRM. As to the latter argument, the Coast Guard fully explained in its response to comments the logical outgrowth basis of its decision to adopt the final ETV Protocol into the 2012 Final Rule. 77 Fed. Reg. at 17258. Additionally, FACA does not apply to collaborations or partnerships that gather and share information, provide individual or unsolicited advice, or that do not seek consensus. See Federal Advisory Committee Act of 1972, Pub. L. No. 92-463 (H.R. 4383), 86 Stat. 770 (1972), as amended, *classified to 5 U.S.C. App. §§ 1 – 16*.

³⁴ ETV Protocol, page xii.

³⁵ The ETV Technical Panel is a group "comprised of a subset of stakeholders and other individuals with a technical expertise in ballast water and environmental technology issues." Members include fresh water and marine biologists, environmental scientists, engineers, and ship architects. See ETV Protocol, page 4.

³⁶ The ETV Protocol uses the term 'Ballast Water Treatment System(s)' (BWTS); which the Coast Guard views as consistent with the term 'Ballast Water Management System(s)' (BWMS) in Coast Guard regulations. See, e.g., 33 C.F.R. § 151.1504.

³⁷ ETV Protocol, page 5.

The ETV Protocol is unequivocal on the viability issue.³⁸ “Living” does not mean “viable.”³⁹ The language of 46 C.F.R. § 162.060-10(b)(1) provides flexibility for the Coast Guard to accept a test method other than what is expressly enumerated in the ETV Protocol, but the alternative must be demonstrated to measure what the discharge standard requires.⁴⁰ A 46 C.F.R. § 162.060-10(b)(1) approval can be used to accept a different measurement tool (e.g., a 12 inch ruler versus a yard stick) but not to substitute the underlying measurement requirement (e.g., length versus weight).⁴¹ Thus, the efficacy standards in 46 C.F.R. Subpart 162.060 are a bar to MSC’s ability to accept the proposed viability measurement as a testing alternative.

Coast Guard policy and intent:

The preamble to the 2012 Final Rule is consistent with this ETV Protocol language. The Coast Guard responded to a comment directly on point regarding whether “living” meant or included “viable”:

One commenter stated that because some types of treatment processes, such as UV, may act to make organisms unviable or unable to reproduce rather than killing them outright, the Coast Guard should include viability as a criterion for determination of BWMS efficacy. The Coast Guard disagrees. This issue has been the point of much discussion both in the United States and internationally in association with the IMO BWM Convention. The Coast Guard has decided to use live/dead rather than viable/unviable, because the latter designations would require culturing potentially large numbers of different kinds of organisms to determine whether they were capable of reproduction. This would be made even more problematic by the fact that scientists are not able to culture many of the organisms in question. Finally, it is more conservative, and thus more protective, to base efficacy decision [sic] on the basis of live/dead, rather than viable/unviable.⁴²

This response to comment is unequivocal, and I find Hyde’s arguments, incorporated by reference, to discredit it unpersuasive. First, the commenter who submitted this UV-oriented, “viable/nonviable” comment was Hyde, a manufacturer of UV-BWMS and also an appellant of

³⁸ The ETV Protocol was developed independently from the USCG ballast water regulations. Its first two chapters describe an organizational process that is superseded by, or otherwise irrelevant to, the specific USCG equipment approval process contained in 46 C.F.R. § 162.060.

³⁹ This ETV Protocol language is a counterpoint to the argument that viability was only addressed in the 2012 Final Rule preamble, but not in the regulation text. The ETV Protocol was incorporated by reference into the regulation text of 46 C.F.R. Subpart 162.060. *See, e.g.*, 46 C.F.R. § 162.060-26(a).

⁴⁰ I note the existence of ETV Protocol paragraph 5.4.8, which provides for the use of “alternative and emerging methods.” This section is expressly limited to methods relating to “living” organisms, not organisms capable of reproduction, and even if it were not, the Coast Guard’s process set forth in 46 C.F.R. § 162.060-10 provides the relevant process for seeking alternatives. The specific alternative procedures created by the Coast Guard supersede the ETV Protocol’s generalized procedures. Additionally, if there is a conflict between the ETV Protocol and the regulation text, the two must be read such that the ETV Protocol does not render 46 C.F.R. § 162.060-10 meaningless. Finally, the Coast Guard disagrees with any interpretation of paragraph 5.4.8 which would effectively eliminate agency oversight of a federal regulatory program.

⁴¹ I disagree that MSC’s denial required MPN to be “identical” to the staining methods identified in the ETV Protocol. Hyde Appeal, page 26. MSC has approved other 46 C.F.R. § 162.060-10(b)(1) requests.

⁴² 77 Fed. Reg. at 17274.

the MSC decision under review here.⁴³ If Hyde believed that the standard clearly meant to include viable organisms, it would not have submitted this comment. In fact, it appears Hyde believed the opposite:

The characteristic “unviable” should be used in place of “dead” in determining the efficacy of BWMS... We wish to emphasize that the terms “kill” and “dead” should be replaced with “make unviable” and “unviable” throughout the proposed regulation.⁴⁴

At the time of this comment, the word “living” did not appear in the proposed discharge standard in either 33 C.F.R. § 151.1511 or 33 C.F.R. § 151.2030. The plain reading of the proposed discharge standard was that ballast water could contain only a maximum of *any* organism, living or dead. This is an extremely conservative and still practicably unachievable standard.⁴⁵ With such a conservative standard, it is understandable why a comment would be made to change the discharge standard. The upshot of the exchange is a clearly articulated statement of Coast Guard policy in the preamble to the Final Rule.

Additionally, this Coast Guard response is consistent with another section of the 2012 Final Rule preamble, which provided:

One commenter requested that the proposed BWDS include language necessary for differentiation between living and nonliving organisms. Another said that the standard should allow for the presence of nonliving organisms since some treatment technologies act to kill living organisms without necessarily removing them from the ballast water.

The Coast Guard acknowledges that the proposed BWDS is slightly different in this respect from the IMO discharge standard, which uses the term “viable” instead of “living.” It is important to note that, while the text of the IMO BWM Convention refers to “viable” organisms, the G8 guidelines define “viable” as “living.” Therefore, the Coast Guard has decided that this issue is best addressed in the BWMS approval process, and will not alter the standard as suggested by these commenters. We note that the standard and approval process do allow for the presence of nonliving organisms. Additionally, we corrected a technical error present in the NPRM, which mistakenly omitted the term “living” from the proposed 33 CFR 151.1511(a). This final rule corrects that omission.⁴⁶

⁴³ Letter dated December 3, 2009, Hyde Marine, Inc. to U.S. Department of Transportation, “Reference: Docket # USCG-2001-10486.”

⁴⁴ *Id.* at page 5.

⁴⁵ In response to comments, the Coast Guard corrected the intended discharge standard by adding the word “living.”

⁴⁶ 77 Fed. Reg. 17266. Trojan argues that this language shows an intention by the Coast Guard to apply two different discharge standards to the Great Lakes and nationwide. However, the record shows that the Coast Guard intended both subparts (33 C.F.R. Subparts C and D) to have the same discharge standard, which was meant to align with the BWM Convention discharge standard. *See* 77 Fed. Reg. 17260 (“We corrected the BWDS in both subparts C and D to align with the IMO BWM Convention.”). If the nationwide discharge standard is read without the “living” modifier, the standard becomes *stricter* than the Great Lakes discharge standard. Such a result is nonsensical.

Like the ETV Protocol, this response explains that “viable” means “living” and not vice versa. As discussed, *supra*, the IMO BWM Convention provides the international standard for organisms discharged from vessels’ ballast water. The Coast Guard’s ballast water discharge standard is nearly identical to the BWM Convention discharge standard. One difference is that the IMO standard contains the adjective “viable” rather than “living” to modify “organisms.” This Coast Guard response shows that its deviation from the IMO standard text was deliberate. This response also tries to articulate that, from the Coast Guard’s perspective, the two standards are substantively the same.⁴⁷ The BWM Convention does not define “viable.” However, one BWM Convention guidance document, the Guidelines for approval of ballast water management systems (G8 Guidelines),⁴⁸ defines “viable” to mean “living.”⁴⁹ While Trojan flips the definition around, so that “living” means “viable,” that is not reflected in the text of the G8 Guidelines. Instead, the G8 Guidelines provide a more narrowed interpretation of the BWM Convention text, consistent with the Coast Guard’s discharge standard.⁵⁰ That is why there was no need to alter the proposed discharge standard in 33 C.F.R. Part 151 to harmonize with the IMO standard.

The Coast Guard’s response goes on to fully respond to the comments by stating that “this issue” is “best addressed in the BWMS approval process...” This means the Coast Guard’s intent was to leave “living” in 33 C.F.R. Part 151 undefined while relying on the technical efficacy requirements in 46 C.F.R. Subpart 162.060 to set the standard by defining how the standard would be measured. Subpart 162.060 contains a myriad of equipment requirements, but its efficacy requirements incorporate the ETV Protocol, which does not allow viability as a measurement of efficacy.⁵¹ “[B]est addressed in the BWMS approval process...” does not mean

⁴⁷ I note the argument set forth by DESMI and Alfa Laval claiming that the Coast Guard failed in its rulemaking to consider the extra cost to foreign flag vessels which would be barred from using foreign-approved UV BWMS using an MPN-based test method. This argument confuses an environmental analysis with a Regulatory Analysis. The Coast Guard properly considered and calculated the costs to foreign flagged vessels in its Regulatory Analysis as a sensitivity analysis. Coast Guard developed a range of cost for a ballast water treatment systems based on potential technologies, calculating total costs based on the low end of the system cost estimates. Since UV BWMS are not the lowest cost option, the fact that some are not available for use in U.S. waters does not impact the Coast Guard’s analysis. Additionally, the reference to “more stringent measures” is to the “phase-two” ballast water discharge standard proposed in the 2009 NPRM but not promulgated as part of the 2012 Final Rule.

⁴⁸ Guidelines for approval of ballast water management systems, Resolution MEPC.174(58), adopted October 10, 2008. (Titles of IMO guidance documents are typically not capitalized except for the first word.) IMO guidelines are non-legally binding documents and are applied under the individual discretion and interpretation of the relevant flag administration.

⁴⁹ *Id.* at paragraph 3.12 (“Viable Organisms are organisms and any life stages thereof that are living.”). This language should be read as it is written and not reversed, so that living means viable. At the most recent meeting of the IMO Marine Environment Protection Committee (MEPC), this specific issue – whether the G8 Guidelines should be amended to remove or change the definition of “viable” – was raised and considered. MEPC did not reach a conclusion either way (i.e., the international community is not agreed that the G8 Guidelines allow BWMS to be type approved for viability rather than live/dead). See IMO Report of the Marine Environment Protection Committee on its Sixty-Ninth Session, MEPC 69/21, May 13, 2016, paragraph 4.39.

⁵⁰ Because “living” is broader than “viable,” the term covers more organisms, and thus a discharge standard incorporating the broader term into a maximum allowable concentration means that fewer organisms can remain in the discharged ballast water. That is why it is a more protective standard, and a “more narrowed” interpretation, despite “living” being a broader term than “viable.”

⁵¹ See, e.g., 46 C.F.R. § 162.060-26(a) and 46 C.F.R. § 162.060-28(j). I note the Coast Guard’s use of “viability” in § 162.060-28(j). While it is true that the Coast Guard often uses “viable” and “living” synonymously, it is not in a favorable way for Trojan. Both the ETV Protocol and the G8 Guidelines define “viable” as “living” and not the other way around. It should also be noted that the Coast Guard’s use of the term “viable” or “viability” has changed

that 46 C.F.R. § 162.060-10(b)(1) can be used as a back door to insert viability into the discharge standard after it was deliberately excluded via rulemaking. It means that the ETV Protocol, or the regulation text, can be amended or updated to include viability as a measurement option once better scientific and technical capabilities are discovered. A new version of the ETV Protocol would still need to be incorporated by reference into the Coast Guard's rulemaking, via the public notice and comment process.⁵² At a minimum, the Coast Guard's policy decision on viability was established through public notice and comment and would need to go back through public notice and comment to change.

Clarification of the administrative record:

I acknowledge some confusion in the administrative record regarding the interactions between Trojan and the Coast Guard on accepting viability as part of the discharge standard. I think more blame for this confusion falls on Trojan than it does on the Coast Guard. After the Coast Guard rejected the request to include viability in the 2012 rulemaking, the appellants then voluntarily launched campaigns to change the Coast Guard's position on the matter.⁵³ These campaigns were not initially for an alternative to the new, existing standards but for the Coast Guard to reopen the rulemaking it had just completed and *change the discharge standard*. The Coast Guard was clear from the beginning that it was very unlikely to open up the rulemaking any time soon.⁵⁴ The appellants persisted in their campaigns, however, and the drawn out process that followed is merely the result of a government agency trying its best to hear and try to accommodate a member of the regulated public, within the bounds of law and policy. Many of MSC's procedural recommendations were in direct response to their insistence to be heard, by any means possible.⁵⁵ There were no guarantees that the process would work in the manufacturers' favor. Additionally, the Coast Guard does not prohibit the submission of applications even when a Coast Guard employee anticipates it will be denied on the merits.⁵⁶

over time. In the 1990s and early 2000s, the Coast Guard used the term viability because of the state of available science. As technology and knowledge advanced, the Coast Guard developed a more narrow view of "viability" given the unknowns involved with trying to verify it. An agency is allowed to refine its policy position over time and is not bound to archaic or outdated ideas, particularly if the agency has a statutory mandate to base decisions on best scientific information available. I agree that the Coast Guard has considered UV BWMS as part of a suite of BWMS options for a long time, but under the current regulations, a UV BWMS must meet the prescribed technical requirements.

⁵² 46 C.F.R. § 162.060-5(d)(1) incorporates a specific version of the ETV Protocol, and so this reference would need to be updated.

⁵³ I disagree with the characterization of the appellants' interaction with the Coast Guard in late 2012, which paint a picture of dealing with an agency with no corporate knowledge of its own regulations. The Coast Guard had just completed what was a very labor intensive, very high profile rulemaking in which the viability issue was directly considered and addressed. In bureaucratic terms, "the ink wasn't even dry yet." The fact that there may have been internal Coast Guard uncertainty over how to procedurally address persistent requests is understandable considering the rulemaking – specifically the type approval program – was in the process of initial rollout and implementation.

⁵⁴ See, e.g., HYDE APPX-000002.

⁵⁵ See, e.g., MSC email, dated 12 February 2015, HYDE APPX 000417. This email begins, "Please submit your request for alternates as equivalent as stated. We'll review your request and determine if a meeting is warranted." This is in direct response to an email from Hyde, dated 10 February 2015, which ended "Alternately, please let us know of your continued refusal of this meeting and we will submit our official 162.060.10(A) and 162.060.10(B) letters by the end of the week." HYDE APPX-000416. MSC's remarks were not an endorsement but an attempt to be responsive to a direct Hyde demand.

⁵⁶ See HYDE APPX-000417. "If manufacturers do not want to wait until the ETV technical panel process plays out, they can submit a -10(b)(1) proposal for acceptance of an alternative method...however, that request would have to

How can the Coast Guard precisely know what the applicant is requesting if it does not see the actual request? In fact, if the Coast Guard refused to allow the appellants to submit an alternative request, that would have exposed the agency to claims of arbitrary and capricious behavior. The record shows that the appellants were persistent in trying to change the Coast Guard's decision on viability, and the efforts Coast Guard employees went through to provide an answer to them, at their insistence, should not be held against them now.

I will also clear up some confusion over the "ETV process," as Hyde's appeal, incorporated by reference, refers to it. The "ETV process" can mean different things. One "ETV process" is the process by which Independent Laboratories (ILs) use the ETV Protocol to conduct testing pursuant to the ballast water regulation requirements.⁵⁷ A different "ETV process" is the process by which the ETV Technical Panel considers new developments in ballast water treatment technology and whether to update or amend the ETV Protocol. In this case, the ETV Technical Panel convened to consider general issues relating to the ETV Protocol, including whether the use of viability as a measurement and the MPN assay as a measurement tool was acceptable, independently of the appellants' 2012 campaigns.⁵⁸ Since MSC knew that this work was underway, it is reasonable that MSC (or CG-OES) would mention it to them and suggest that they wait for the ETV Technical Panel to conclude its work.⁵⁹ If the ETV Technical Panel found in their favor, that decision would provide the basis for future Coast Guard action, including updating the regulations to include viability. It would also be very difficult for the Coast Guard to come to an independent conclusion on viability, as the Coast Guard does not have the same scientific and technical resources as the ETV Panel. Additionally, the Coast Guard never suggested that the ILs or the ETV Technical Panel could decide or approve a 46 C.F.R. § 162.060-10(b)(1) request. However, information from either "ETV process" can certainly be submitted to support a 46 C.F.R. § 162.060-10(b)(1) request.

Finally, I reject Trojan's characterization of the Coast Guard's *Shipboard Technology Evaluation Program* (STEP) program and development of its current ballast water requirements as implicitly accepting the MPN method. As I mentioned earlier, the Coast Guard has regulated ballast water for decades. The Coast Guard ballast water requirements began with a Congressionally-mandated ballast water exchange requirement, discussed *supra*, certain best management

meet the -10(b)(1) requirements..." This is an objective, likely palliative, instruction that does not take into account specific facts or presuppose that the request will be granted. MSC's following comments provide the specific warning that a "-10(b)(1)" request for MPN would not be simple. Even after receiving a favorable opinion from the ETV Technical Panel (which has not occurred), Hyde would still need to come back to the Coast Guard for further consideration.

⁵⁷ See, e.g., 46 C.F.R. § 162.060-42.

⁵⁸ "Currently, the MPN remains an unapproved method for determining the biological efficacy of the BWMS. The method remains under review by the EPA tech panel and we have no outlook on when an answer may be reached or any indication as to what that answer may be. I would be cautious of conducting testing prior to approval of this method if your system will rely solely on this method to meet the discharge standards...MPN data may be accepted as existing data following testing provided that...the MPN method is accepted as an approved method." MSC email, dated 6 February 2015, HYDE APPX-000413. This warning from MSC is categorical.

⁵⁹ I also note that all four applicants relied on the recent work of the ETV Technical Panel in their respective 46 C.F.R. § 162.060-10(b)(1) requests. While the appellants characterize their MPN-based method as being singular (i.e., "the MPN method") and in use for decades, the record shows that the "MPN" method they submitted for alternative approval is the preliminary draft MPN-based method developed through the work of the ETV Technical Panel, which has yet to be validated (i.e., the specific submitted method was developed after the testing was conducted for their foreign type approvals).

practices, and reporting and record keeping requirements. During this time, and in accordance with its statutory mandate,⁶⁰ the Coast Guard considered and reviewed various technological alternatives to ballast water exchange. These alternatives included BWMS based on UV technology. The STEP program is “intended to facilitate the development of effective [BWMS] technologies, to create more options for vessel owners/operators seeking alternatives to ballast water exchange...vessel owners/operators have expressed a reluctance to invest the resources to install and operate an experimental treatment system that might not meet discharge standards mandated by future regulations.”⁶¹ In other words, the point of the STEP program is that those BWMS are experimental. The fact that the Coast Guard, in 2008,⁶² accepted a UV BWMS for use on a STEP-enrolled vessel does not mean that the particular UV BWMS, regardless of its efficacy, *meets the regulatory discharge standard*.⁶³ At the end of the day, MSC’s actions were to uphold a regulatory discharge standard in light of a proposed alternative test method and were not an opinion on UV BWMS efficacy.⁶⁴

In sum, I find that MSC did not have the discretion to approve viability as an alternative measurement to the regulatory standards under 46 C.F.R. § 162.060-10(b)(1). The ballast water discharge standard in 33 C.F.R. Part 151 must be read together with the BWMS type approval requirements found in 46 C.F.R. Subpart 162.060 to understand the meaning of the word “living” in the Coast Guard’s ballast water regulations. The type approval requirements do not allow “living” to be substituted with “viable,” and therefore MSC did not have the discretion to approve a testing alternative that would insert viability into the discharge standard. While Trojan argues that certain Coast Guard employees agreed that 46 C.F.R. § 162.060-10(b)(1) could be used to insert viability into the discharge standard, I do not believe the administrative record definitively or specifically proves this assertion. Additionally, the Coast Guard’s regulations and viability policy decision went through the public notice and comment process, and those decisions cannot be changed without returning to the public notice and comment process.

2. The Coast Guard has the statutory and regulatory discretion to reject alternative proposals

⁶⁰ NANPCA/NISA, *supra*, n. 5, § 1101(e); *codified in* 16 U.S.C. § 4711(e).

⁶¹ Navigation and Vessel Inspection Circular (NVIC) 01-04. The STEP program predates the 2012 Final Rule.

⁶² Letter dated October 31, 2008, from M. L. Blair, Captain, U.S. Coast Guard, Office of Operating and Environmental Standards, to Princess Cruise Lines, no. 33.151.2035.0040. This acceptance pre-dated the 2009 NPRM’s publication.

⁶³ A similar reasoning dismisses the appellants’ assertions that the Coast Guard violated the National Environmental Policy Act, Pub. L. No. 91-190, §2, 83 Stat. 852 (1969), as amended, *codified in* 42 U.S.C. §§ 4321, 4331-4335, 4341-4346, 4346a, 4346b, 4347. The purpose and need of the Coast Guard’s 2012 Final Rule Final Environmental Impact Statement (FEIS) is to provide “an assessment of the potential environmental impacts associated with the proposed establishment of a ballast water discharge standard. The standard would be used to approve alternative ballast water management methods that are effective in preventing or reducing the introduction of nonindigenous species via discharged ballast water into the waters of the United States.” FEIS Appendix F, which lists BWMS enrolled in the STEP program, is meant to provide a “rational basis” that BWMS exist that could achieve the discharge standard. The FEIS was not intended to prove that any particular system met the type approval standards established in 46 C.F.R. Subpart 162.060. Additionally, it is clear from the description of each of the alternative concentration levels considered as being “living organisms (per volume)” and in the description of UV BWMS assessed, that the Coast Guard examined the impacts of the regulatory discharge standard in the context of killing organisms (*see, e.g.*, Pages 2-5 and 2-6 and Appendix F).

⁶⁴ In fact, the Coast Guard still considers UV BWMS a valid ballast water treatment technology and believes that UV BWMS can be type approved under the existing regulatory requirements.

Trojan and the other appellants contend that MSC's rejection of their applications for regulatory alternatives was tantamount to failing to consider them and that such failure was arbitrary, capricious, and inconsistent with law. As an initial matter, I believe Trojan confuses "to consider" with "to approve." It is clear from the record, including MSC's rejection letter of December 14, 2015, that MSC considered Trojan's application. As discussed *supra*, MSC found that Trojan's application failed to meet the second and third prongs of 46 C.F.R. § 162.060-10(b)(1). The fact that MSC ultimately denied Trojan's application does not mean MSC did not consider it. If there were any defect in MSC's consideration,⁶⁵ I cure it now by independently finding that Trojan's application fails to meet the requirements of 46 C.F.R. § 162.060(b)(1). That reasoning is provided in Section 3, *infra*.

Trojan and the other appellants make various legal arguments asserting various levels of Coast Guard discretion or mandate to accept alternatives to the Coast Guard regulatory requirements. The Coast Guard does not dispute that it has the discretion, in theory, to accept viability as a BWMS efficacy measurement.⁶⁶ The Coast Guard also has not permanently rejected MPN as a BWMS measurement tool by a regulatory change. The 2012 Final Rule and its 2009 Notice of Public Rulemaking (2009 NPRM)⁶⁷ were very clear that the 2012 Final Rule is an interim phase of ballast water treatment and management. At that time, the Coast Guard did not have sufficient information to include viability as an approval criterion. However, NANPCA/NISA contains a mandate which requires the Coast Guard to periodically review and revise its ballast water regulations based on the best scientific information available.⁶⁸ If and when the Coast Guard has such information, it can reconsider whether to include viability. As this criterion would differ from the existing regulatory text and policy established through public notice and comment, this change would need to go through public notice and comment rulemaking.

While I agree, in principle, about the Coast Guard's discretion to accept viability, there are some very important statutory and regulatory limitations that these arguments raise that must be addressed within this response.

⁶⁵ MSC should have waited for the final results of the independent analysis before denying the application. We now have the final results, and my decision is based on those results.

⁶⁶ As a counterpoint to the appellants' arguments that NANPCA/NISA mandate that the Coast Guard accept viability by virtue of the NANPCA/NISA definition of "nonindigenous species" including the word "viable," Congress enacted the assumption that vessel water treatment systems should "kill" aquatic nuisance species: "provide an exemption from ballast water exchange requirements to passenger vessels with...treatment systems designed to kill aquatic organisms in ballast water..." NANPCA/NISA § 1101(c)(2)(K), 16 U.S.C. § 4711(c)(2)(K) (emphasis added). "Viable" is used in this definition to cover things like viruses, which are not universally considered to be "living." In any event, NANPCA/NISA is based on a precautionary rather than prescriptive framework. That means that the Coast Guard has the authority, under "maximum extent practicable," to regulate the concentration of both indigenous and nonindigenous, as well as invasive and noninvasive, species in ballast water in order to prevent the introduction and spread of aquatic nuisance species. For example, ballast water exchange does not discriminate among "viable" or "nonviable" organisms.

⁶⁷ See, e.g., Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters, Notice of Proposed Rulemaking, 74 Fed. Reg. 44632 at 44634 – 44635 (Aug. 28, 2009).

⁶⁸ NANPCA/NISA, *supra*, n. 5, § 1101(e)(1)(D); *codified in* 16 U.S.C. § 4711(e)(1)(D). The Coast Guard also committed to a regulatory obligation to conduct a practicability review for a more stringent standard, initiating a rulemaking by 2017 if appropriate. See, e.g., 33 C.F.R. § 151.1511.

The Coast Guard interprets NANPCA/NISA Section 1101 (16 U.S.C. § 4711) differently than Trojan and the other appellants. In particular, the Coast Guard disagrees with the applicability and interpretation of Section 1101(c)(2)(D) and believes Section 1101(e)(1) expressly or constructively “trumps” Section 1101(c)(2)(D).

Section 1101 can be difficult to understand if it is not read in the context in which it was originally enacted and subsequently amended.⁶⁹ When NANPCA was amended to include voluntary guidelines for the entire United States (Section 1101(c)(2)), the specific mandates in Section 1101(c)(2)(D) were for those initial voluntary guidelines:

The voluntary guidelines issued under this subsection shall-

- (D) direct a vessel that is carrying ballast water into waters of the United States after operating beyond the exclusive economic zone to
 - (i) carry out the exchange of ballast water of the vessel in waters beyond the exclusive economic zone;
 - (ii) exchange the ballast water of the vessel in other waters where the exchange does not pose a threat of infestation or spread of nonindigenous species in waters of the United States, as recommended by the Task Force under section 4712(a)(1) of this title; or
 - (iii) use environmentally sound alternative ballast water management methods, including modification of the vessel ballast water tanks and intake systems, if the Secretary determines that such alternative methods are at least as effective as ballast water exchange in preventing and controlling infestations of aquatic nuisance species...

In other words, there was no existing nationwide standard, and Congress provided an initial *minimum* or *floor* for the Coast Guard⁷⁰ to meet. The Coast Guard’s initial guidelines were based on the framework contained in Section 1101(c)(2)(D) and included guidance on conducting ballast water exchange for vessels carrying ballast water into waters of the United States after operating beyond the U.S. exclusive economic zone.⁷¹ Eventually, the Coast Guard converted its voluntary guidelines to mandatory, regulatory requirements under the mandate contained in Section 1101(f)(1).⁷² These regulations were also based on the framework contained in Section 1101(c)(2)(D), as ballast water exchange remained the best available ballast water management option.

However, Section 1101(e)(1) requires the Coast Guard to consider revising its ballast water regulations no less than every three years. After conducting this periodic review, the Coast Guard is required under Section 1101(e)(1) to amend its ballast water regulations if, based on the

⁶⁹ Please refer to the background section of this response for this discussion.

⁷⁰ The Act refers to the “Secretary,” defined as the Secretary of the department in which the Coast Guard is operating. For simplicity, I will instead refer to the Coast Guard, as the properly delegated entity of the Department of Homeland Security.

⁷¹ See Implementation of the National Invasive Species Act of 1996 (NISA), Interim Rule, 64 Fed. Reg. 26672 (May 17, 1999).

⁷² See Mandatory Ballast Water Management Program for U.S. Waters, Final Rule, 69 Fed. Reg. 44952 (July 28, 2004).

best scientific information available, the existing guidelines and regulations implementing Section 1101(c) do not effectively reduce the introduction and spread of aquatic nuisance species by vessels. The Coast Guard's 2012 Final Rule was the result of an on-going review that began almost immediately after promulgation of the initial voluntary guidelines for ballast water exchange, and continued through participation in the development of the IMO ballast water management convention and development of test protocols for BWMS, and the best scientific information available showed that some BWMS were more effective than ballast water exchange in reducing the introduction and spread of aquatic nuisance species. While the Coast Guard initially characterized its new requirement of BWMS as an approval of an "environmentally sound alternative ballast water management method" under Section 1101(c)(2)(iii),⁷³ the Coast Guard later explained its reasoning that subparagraph (c)(2)(D) merely set forth initial ballast water requirements for certain vessels and it was acting under the broader mandates found in paragraphs (a) and (e).⁷⁴ To read Section 1101 as permanently binding the Coast Guard to the initial floor set by Section 1101(c)(2)(D) would render Sections 1101(c)(2)(A) and 1101(e)(1) meaningless.⁷⁵ The BWMS manufacturers' argument that the Coast Guard is bound to implement all of Section 1101(c)(2)(D) is even more perplexing considering it would mean the Coast Guard has no discretion to phase out ballast water exchange in favor of BWMS.⁷⁶ The Coast Guard has moved beyond the initial mandate contained in Section 1101(c)(2)(D) to the more stringent mandate contained in Section 1101(c)(2)(A), which requires the Coast Guard to ensure, to the maximum extent practicable, that aquatic nuisance species are not discharged into the waters of the United States from vessels.⁷⁷

⁷³ See, e.g., 2009 NPRM, 74 Fed. Reg. 44633.

⁷⁴ 77 Fed. Reg. 17282, 17286. This analysis also explains why the Coast Guard believes it has the authority to require all vessels equipped with ballast tanks – and not just those that have operated beyond the exclusive economic zone – to comply with its ballast water management requirements. The Coast Guard has previously, and publicly, rejected the legal argument that Section 1101(c)(2)(D) contains specific requirements which control "broader" requirements in Section 1101.

⁷⁵ I note Trojan's argument that NANPCA/NISA mandates the Coast Guard use "best science available," which Trojan evidently believes requires the Coast Guard to perpetually amend its equipment standards without going through a public notice and comment rulemaking. The Coast Guard properly incorporated the ETV Protocol into its regulations, as it does for many other vessel equipment standards such as those from the International Organization for Standardization. To suggest that the Coast Guard can "undo" a proper regulatory incorporation by reference without a notice and comment rulemaking is untenable. Additionally, the Coast Guard used "best science available" at the time it promulgated the 2012 Final Rule, four years ago. The Coast Guard is now completing its NANPCA/NISA-required periodic review, in which it is considering any new, properly validated scientific information. That information will inform whether to amend the Coast Guard regulations. Vessels must be able to keep up with changing equipment standards or the Coast Guard would not be maintaining NANPCA/NISA's "maximum extent practicable" mandate.

⁷⁶ Even reading Section 1101(c)(2)(D) alone, without reference to any other parts of NANPCA/NISA, cannot support this conclusion. Section 1101(c)(2)(D) is formed in the disjunctive, allowing the Coast Guard to choose all or only one of the options under it, while it was still operative. DESMI and Alfa Laval may have used a Coast Guard preambular statement out of context on page 12 of their respective appeals. The quoted statement, from the ballast water NPRM, meant that any alternatives to ballast water exchange must be approved by the Coast Guard, not that the Coast Guard was required to approve all alternatives to ballast water exchange.

⁷⁷ For this reason, Trojan's argument about accepting a BWMS as an alternate management system (AMS) fails. There is a difference between the initial standard of "at least as effective as ballast water exchange," which is consistent with the purpose of AMS – a bridging strategy between ballast water exchange and BWMS – and an evolved "maximum extent practicable" standard. Type approved BWMS must meet the *discharge standard*, not meet the floor of "at least as effective as ballast water exchange."

Thus, while I generally agree with the appellants' arguments that NANPCA/NISA gives the Coast Guard the discretion to consider and accept viability, I do not agree with all of their interpretations and analysis of NANPCA/NISA § 1101.⁷⁸ NANPCA/NISA does not "mandate" that the Coast Guard accept viability unless and until viability falls within the Coast Guard's responsibility to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels. Given the current state of science and technology, discussed *infra*, live/dead is the most environmentally conservative and practicably achievable standard, and viability does not yet fall within this mandate.

3. Trojan's application fails to meet all elements of 46 C.F.R. § 162.060-10(b)(1)

I find that Trojan's application did not meet the minimum requirements in 46 C.F.R. § 162.060-10(b)(1) and confirm MSC's denial of Trojan's alternative request.

This decision is based, in part, on the technical review and conclusions of the Naval Research Laboratory (NRL),⁷⁹ whom the Coast Guard contracted to review the technical aspects of the alternative request submitted by Trojan.⁸⁰ The NRL review resulted in a comprehensive evaluation and the report includes more detailed descriptions and comments, not all of which were considered pertinent to the narrow issue of the 46 C.F.R. § 162.060-10(b)(1) requirement. I find the NRL report persuasive, and summarize my agreement with it as follows:

The applicants request that Coast Guard approve as equivalent an alternative to the required test specified in the ETV Protocol for determining the concentration of living organisms in the 10-50 micrometers (μm)⁸¹ size range in samples of water during type approval testing. The proposed alternative method is composed of two separate procedures, one (based on "viable") for autotrophic (photosynthetic) organisms and one (based on "living") for heterotrophic organisms.

The autotroph procedure is based on using a "grow-out" approach, wherein samples are serially diluted, and replicate tubes at each dilution are cultured for a period of time, and then assayed for population growth of phytoplankton by detecting changes in the concentration of chlorophyll. The pattern of tubes with and without positive population growth is then used to estimate the probable original concentration, through a calculation termed MPN. The MPN statistical

⁷⁸ I also reject Trojan's argument that NANPCA/NISA does not apply to non-reproductive organisms, and therefore the Coast Guard lacks the authority to promulgate the existing discharge standard. (Trojan appeal, page 45 – 46). NANPCA/NISA's reference to aquatic nuisance species, of course, concerns the species prior to treatment in a BWMS.

⁷⁹ NRL, "Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(B)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{M}$ and $<50 \mu\text{M}$," Feb. 10, 2016, 3900 Ser 6130/1622.

⁸⁰ The NRL reviewed only one 46 C.F.R. § 162.060-10(b)(1) application: Trojan. Nonetheless, the NRL report on Trojan is relevant to all four applicants. Three of the four applicants (Trojan, DESMI, and Alfa Laval) submitted a base set of the same six documents containing a description of the proposed test, an overview of the use of the most probable number approach for evaluating BWMS, and results of various experiments conducted during development of the method, including several in which the alternative and required methods were compared. Hyde submitted only three of the six documents. Trojan also submitted additional documentation. Thus, Trojan's application was the most comprehensive of the four and covered all of the evidence provided by all four applicants. If Trojan's application fails to meet 46 C.F.R. § 162.060-10(b)(1), then all four fail. I have also included a discussion of arguments (vice documentation) of the individual applicants.

⁸¹ A micrometer equals 0.001 millimeter.

approach has been used for over a century to quantify monocultures (single species) of bacteria and phytoplankton, and several automated “calculators” are available for use. Significantly, the applicants propose to use MPN as the basis for quantifying concentrations of mixed assemblages of phytoplankton, composed of numerous different species in varying relative abundance.⁸² This is a “new” use for MPN as a statistical approach under a regulatory context and has not been adequately validated for such purpose.

The heterotroph procedure, for heterotrophic organisms, uses microscopy to count numbers of non-photosynthetic organisms that are motile, where movement is the criterion for determining an organism is alive. This method is conceptually similar to the required method, in that direct counts of “living” (not “viable”) organisms are made using a microscope. However, the specific procedures for determining whether an organism is “living” and the type of microscope are different than specified in the required method.

Compliance with 46 C.F.R. § 162.060-10(b)(1):

A. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?

I find that Trojan meets the first prong of 46 C.F.R. § 162.060-10(b)(1). Trojan’s request to use the MPN assay is a request for an alternative to the testing requirements of the ETV Protocol, incorporated by reference into 46 C.F.R. § 162.060-26 and 46 C.F.R § 162.060-28, which are referred to in 46 C.F.R. § 162.060-10(f)(2).

B. Is that evaluation, inspection or test not practicable or applicable?

I find that Trojan has not provided sufficient justification that the testing requirement is not practicable or applicable.⁸³ Three applicants argued, in various ways, that the required method was not practicable and/or not applicable for evaluating the efficacy of UV-based BWMS. Some of the arguments were not always clear whether they were being made on the basis of practicability or applicability, and so for this review I consider the two issues to have been combined. The applicants essentially make a two-part argument: first, that the phrasing of the discharge standard should be in terms of, or include, “viable” (or reproductive) organisms instead of, or in addition to, “living” organisms as currently phrased in the discharge standard; and second, that the required method for enumerating “living” organisms in the type approval requirements is not applicable to BWMSs intended to render organisms non-viable.

Of the appellants, only Trojan offered any objective basis to argue that the required method was not practicable (Miller 2015d). Trojan essentially argued that it is too expensive to use UV to kill organisms rather than render them non-reproductive, and so UV should be used to render organisms non-viable. This, according to Trojan, would provide an equivalent level of protection for the environment as killing them, because non-viable and dead organisms

⁸² I will refer to the applicants’ approach as the “alternative method.” I note that there is no globally accepted “MPN method” for BWTS type approval. While the applicants used the same MPN-based method, it appears that MPN approaches vary across flag administrations.

⁸³ I agree that MSC’s conclusion on practicability was not sufficiently justified. For that reason, I vacate the practicability decision of MSC and reach my own decision.

represented the same level of risk reduction with respect to biological invasions. Rather than supporting an argument that the required method is not practicable for evaluating numbers of living organisms, I view this as an argument that UV is not practicable for killing organisms in ballast water. Under the current type approval requirements, BWMS are required to be tested to demonstrate efficacy in reducing numbers of ‘living’ organisms below concentrations in the discharge standard, and to do so using specific test methods that measure numbers of ‘living’ organisms. As explained, *supra*, the Coast Guard cannot grant a waiver to the ‘living’ requirement under the current regulations.

Furthermore, the proposed alternative method includes an assessment of ‘living’ organisms in the case of heterotrophic organisms, which undermines the contention that the required method, which assesses ‘living’ organisms is impracticable or inapplicable. In the proposed alternative method, a culture-based viability assay is used to assess viable autotrophic organisms (organisms capable of synthesizing their own food from inorganic substances using light or chemical energy; e.g., plants and algae) while a ‘living’ assay is used for heterotrophic organisms (organisms that cannot manufacture their own food and instead obtain food and energy by taking in organic substances, usually plant or animal matter; e.g., animals and fungi).⁸⁴ Trojan does not explain why it is practicable and applicable to evaluate ‘living’ organisms when assessing the efficacy of UV systems in treating heterotrophic organisms, but it is not when the focus is autotrophic organisms. This inconsistency must be explained.⁸⁵

I address the following specific points raised by Trojan (Miller 2015d):

- i. *The existing method is not practicable for evaluation of UV-based BWTS. The current requirement for analysis of the organisms in the 10-50µm size class in the ETV protocol requires the use of FDA/CMFDA stains to categorize organisms as being alive or dead. The stains evaluate the functioning of an organism’s esterase system as a proxy for cell death. Treatment with UV irradiation, causes damage that prevents cell replication (and thus precludes invaders from colonizing), but esterases are not directly affected at UV doses typically employed for disinfection, and thus effective treatment by UV is not evaluated by the existing method.* Trojan asserts that the required method is not practicable for evaluation of UV-based BWMS that are intended to render organisms non-reproductive rather than dead. I do not dispute that the staining methods evaluate the functioning of an organism’s esterase system rather than the ability of an organism to reproduce, the issue is that the discharge standard is

⁸⁴ The required method is used to enumerate both of these two components of the size group.

⁸⁵ DESMI and Alfa Laval make a similar argument against the Coast Guard, stating that for organisms in the <10 µ size class, tests for only 3 indicator species are allowed. This argument conflates the ETV Protocol with the overarching Coast Guard regulatory standard, found in 33 C.F.R. §§ 151.1511 and 151.2030. There is no “<10 µ size class.” The Coast Guard regulations set forth specific requirements for those three indicator microorganisms, and they are not ‘indicators’ for a size class. DESMI and Alfa Laval also point to the Coast Guard’s incorporation of the option to use water from a U.S. public water system for ballast water management as evidence that MPN is acceptable. However, this point proves the opposite: the Coast Guard *limited* the option to *only* U.S. sources because of the difficulties in determining water quality from foreign sources. In the case of the United States, the regulatory requirements for these facilities are known, uniform, and verifiable. Additionally, water treatment facilities use MPN-based methods to estimate abundances of *specific* well known species, not the thousands in ballast water.

not phrased in terms of “non-reproductive” or “non-viable” organisms, but instead is phrased in terms of “living” organisms. At the time of the 2012 Final Rule, the Coast Guard believed that there were significant difficulties associated with determining the reproductive ability of the thousands of species of organisms found in ships’ ballast water. Because of those difficulties, the Coast Guard determined that it was more practical and protective of the environment to phrase the standard in terms of living organisms. Concurrently with establishing the discharge standard, the Coast Guard established the required methods by which numbers of living organisms would be determined during type approval testing. The required method for organisms in the 10-50 μm size class is the FDA/CMFDA fluorescent marking method specified in the ETV Protocol. The applicant has not provided an adequate argument that the required method is not practicable for evaluating the number of *living* organisms in ballast water.

- ii. *A BWTS designed for UV doses equivalent to ten (10) times the current UV doses employed in the industry would require 10X the number of UV lamps, and would therefore be approximately 10X the footprint and require 10X the electrical power for operation. This increase would render UV systems impractical for implementation on board vessels.* Rather than making an argument that the required method is not practicable for enumerating living organisms after UV treatment, Trojan essentially argues that the required method is not applicable to UV treatment when such treatment is intended to render organisms non-reproductive. In essence, Trojan does not present an argument that the required method is not practicable, but rather that it is not practicable to use UV treatment to achieve the required discharge standard. Trojan argues that the UV doses commonly used in BWMS that are intended to render organisms non-reproductive are not great enough to induce mortality during type approval testing. Increased UV doses would not only prevent reproduction or cellular division, but if sufficient, also induce mortality that is detected following treatment using the required method. Thus, the required method would indicate treatment efficacy in meeting the “live” discharge standard because the dead cells would not fluoresce green. An alternative method would not be needed.
- iii. The applicant asserts that it is not practicable to increase the UV dose so treated cells are dead (as determined by the required method) rather than not viable (as determined by the alternative method). The applicant provides no data on the actual UV dosages necessary to kill the many species of organisms found in ballast water. Using derived ratios of UV doses needed to kill to doses needed to render organisms non-reproductive (no actual doses or dose-response curves were provided) from a study using 12 species of algae, the applicants argued that a UV dose sufficient to damage cells’ non-specific esterases—the foundation of the required method—and reduce concentrations of algae by 100-fold (from 1000 cells mL⁻¹ to 10 cells mL⁻¹) would be “extremely high”. The study found that for the 12 species, on average, a 10-fold increase in UV dose was needed to kill cells using the required method compared to the dose needed to show cells were non-viable using a culture-based viability assay. However, no data on actual dosages required to kill organisms or the power required

to achieve those dosages were provided, so it is not possible to objectively evaluate this claim.

I also address the following specific points raised by Hyde (HMI 2015):

- i. *“The inherent nature of the proposed staining technique measures the capability of organisms to hold fluorescence rather than to provide a measurement of “living/dead”...”* The fluorescent markers in the required method, as well as many other fluorescent markers specific for other biochemical characteristics of living organisms, are widely used in biology to differentiate living from dead.
- ii. *“...all scientific definitions of the word “living” include the ability to reproduce”.* This assertion is a gross mischaracterization of the definition of “living” in the context under discussion, which is how one can differentiate between a living organism and a dead organism. The context for the assertion is the higher-level issue of differentiating between “living” things (i.e., organisms) such as animals, plants, fungi, bacteria, etc and “non-living” things (e.g., rocks, water, fire, etc), wherein living things are defined in part as those things that exhibit reproduction at some time. Such an overarching definition is not pertinent to the issue at hand, as the required method is used to differentiate “living” organisms from “dead” organisms, not organisms from rocks (although the fluorescent “signal” from the marker does assist in seeing living organisms within the jumble of nonliving material such as bits of rock, shell fragments, and plant detritus.) Many “living” organisms are not capable of reproduction (adult humans who have undergone sterilization procedures; sterile castes in social insects, mules, female mammals that have reached menopause, etc). Viable organisms are by definition included in the larger sub-set of ‘living’ organisms (because an organism must be alive to reproduce). If a reliable procedure can be identified by which viable individuals of thousands of different species can be consistently discriminated from non-viable individuals, then such methods could be included in the approved methods for determining numbers of living organisms, through amendment of the regulations. To avoid reducing the protectiveness of the discharge limits, any such viability assessment must also have levels of accuracy and precision equivalent to that of the method used to determine the number of living organisms.
- iii. *“UV as a disinfection product sterilizes organisms by disrupting their DNA and interrupting their ability to reproduce”.* The use of UV as a disinfection process is not the issue. The issue is whether UV has been, or can be, demonstrated to be an effective disinfection process for the vast number of different species in the 10-50 μm size group found in ships’ ballast water. UV is generally used to render organisms non-reproductive because it is cheaper than using UV to “kill.” Using UV to render organisms non-reproductive, rather than to kill, depends on a good understanding of the specific UV dose necessary to render specific organisms incapable of reproduction. This has been done for a relatively small subset of organisms that are either human pathogens (e.g. *Vibrio cholera*, several species of the genus *Cryptosporidium*, *Hepatitis A*, etc) or indicators of poor water quality due to

unsanitary conditions (e.g., *Escherichia coli* and several species of *Enterococcus*) that can be associated with the presence of such pathogens). By contrast, there are many thousands of species carried in ships' ballast water, and little or nothing is known about the specific UV doses required to render these permanently incapable of reproduction, nor is there a good understanding of how these many species could be consistently cultured in the laboratory to detect viability.

- iv. *In order not to eliminate UV disinfection in ballast water, a reproductive measurement assay must be used.* This is an argument based on economics, not the question of whether the required method is applicable to testing whether UV-based technologies are effective in achieving the limits on concentrations of living organisms.
- v. *"...in any disinfection enumeration, looking at the reproductive capability of the organisms studied is a more accurate representation of effectiveness than looking simply at their ability to hold a stain".* This statement is only true for those disinfection processes intended to render organisms non-reproductive. For a process intended and designed to kill organisms, the appropriate approach in measuring effectiveness would be to identify the numbers of living organisms. The assertion that the required method "simply" looks at the ability of organisms to "hold stain" is a gross over-simplification and ignores completely the broad use of vital markers to identify "living" cells.
- vi. *"The international testing community and US EPA have historically used reproductive assays in lieu of staining to demonstrate the effectiveness of UV systems in ballast and other applications".* The "international testing community," meaning the test facilities conducting tests of BWMS for foreign administrations, may have used, collectively, a range of methods that involved reproductive assays, but these have not been made generally available to the public, including in particular the documentation demonstrating the careful validation of culture-based methods. Several different "methods" have been used by different facilities, and the validations that these methods were able to measure what they were intended to measure, or the comparisons among methods, have never been made public; indeed, at least one test facility has long asserted that the details of its methods are "proprietary". When the ETV panel first began to consider the issue of whether an acceptable viability assessment could be identified for use in testing BWMS, the various test facilities and manufacturers were unable to provide or point to any specific validations, and had to subsequently conduct much of the validation work submitted (after much testing of BWMS had been completed) in support of the method being developed within the ETV Program, a draft of which was submitted to the Coast Guard for consideration under the -10(b)(1) provision.

I address the following specific points raised by DESMI:

- i. *UV treatment of ballast water does not outright kill organisms in the water, but destroys their DNA and RNA making them incapable of reproduction.* This is a

decision made by treatment system manufacturers on the basis of economic considerations, and reflects the specific, and differentiated from shipboard, circumstances of drinking water, food safety and waste water concerns. UV can have a biocidal (i.e., killing) effect. Currently the type approval testing requirements specify measurement of numbers of living organisms as determined by the specific method described in the ETV protocol. This method is as appropriate for enumerating living organisms in UV-treated water as it is for the same purpose in water treated by other means.

- ii. *Reproductive capability is central to the definition of invasive species laid out by the US Federal Aquatic Nuisance Species Task Force in 2012. It follows that organisms incapable of reproduction cannot become invasive species.* A 46 C.F.R. § 162.060-10(b)(1) analysis does not consider the definition of “invasive species” or whether organisms that are incapable of reproduction cannot become invasive species. Rather, this section is concerned with the method(s) accepted by the Coast Guard under these equipment type approval regulations.
- iii. *Destruction of organisms’ ability to reproduce therefore grants the same protection against aquatic invasive species as is obtained by killing the organisms.* The crux of the matter is the degree to which “ability to reproduce” can be determined for all of the different kinds of organisms in ballast water. I do not believe that there is currently an acceptably validated method that can be used to consistently assess whether organisms in treated ballast water are capable of reproduction.
- iv. *The FDA/CMFDA staining method prescribed in the ETV protocol for testing of ballast water treatment does not show whether an organism is capable of reproducing or not.* I do not dispute this assertion. However, if it is not practicable to adequately determine whether living organisms in treated water are able to reproduce, then limiting the number of “live” organisms includes both viable and non-viable individuals, and so provides a conservative, more protective limit.⁸⁶
- v. *Therefore, the FDA/CMFDA method is not well suited for assessment of the effectiveness of a UV based ballast water treatment system.* The relevant question is whether the required FDA/CMFDA method in the ETV Protocol is suitable for assessing effectiveness of a BWMS in meeting the “live” criterion in the USCG discharge standard, not whether it is well suited for a BWMS meeting some other discharge standard.
- vi. *On this basis it is concluded that:*
 - a) *The method contained in the ETV protocol for organisms in the 10-50 micron size category is not practicable or applicable to evaluate the performance of UV based ballast water treatment systems.* I disagree. DESMI frames practicability in terms of measuring something (“viable”) that is not pertinent to the actual

⁸⁶ 77 F.R. 17274 (“Finally, it is more conservative, and thus more protective, to base efficacy decision on the basis of live/dead, rather than viable/unviable.”).

standard, which is framed in terms of “live” organisms. Again, the required method is practical for enumerating the number of living organisms in a sample of ballast water. The applicant desires to be held to a different end-point – not “living” but instead “able to reproduce”.

- b) *The proposed alternative test method provides same level of protection against invasive species, and is equivalent to existing requirements in terms of accuracy.* This is irrelevant to the issue of whether the required method is practical or applicable in the case of assessing the effectiveness of a BWMS in meeting the discharge standard. This point is addressed, *infra*, in regard to whether the proposed alternative is equivalent to the requirement.

Consequently, I find that none of the applicants provided an adequate justification that the required method was not practicable and/or not applicable for its intended purpose when used to evaluate the efficacy of BWMS that used UV to treat organisms in the 10-50 μm size range in ballast water to meet the discharge limits set in the Coast Guard’s March 2012 Final Rule.

C. Is the proposed alternative equivalent to the regulatory standard?

I find that the applicants have not justified the proposed alternative’s equivalency to the requirement. “Equivalence” among testing methods, in a literal sense, entails different methods of achieving the same measurement. A simple example would be measuring the length of an object by using a ruler in a specific manner or taking a photo of the object and analyzing length using an image analysis software program (in which case the program converts pixels along the delineation into length, based on a user-supplied calibration between pixels and length). In such a case, the methods result in measurement of the same thing (in the example, length). In a more technical sense, “equivalence” among testing methods⁸⁷ means that the methods must have the same or very similar accuracy (closeness to true value) and precision (degree of repeatability under unchanged conditions).⁸⁸

I have already found that the proposed alternative is not “equivalent” in the literal sense, and MSC did not have the discretion to use 46 C.F.R. § 162.060-10(b)(1) to rewrite the discharge standard. Even if the Coast Guard could accept viability without going through public notice and comment, which it cannot, the UV applicants’ alternative would still fail for technical reasons.

To determine if the alternative “viable” and required “live” methods are equivalent, a proposal for the alternative must demonstrate equivalent accuracy and precision of measurement – even if

⁸⁷ I will assume for the sake of argument that “viable” and “living” relate equivalently to the risk of biological invasion. However, I do not reach a conclusion on that issue.

⁸⁸ Neither Hyde nor Alfa Laval included any additional arguments for accuracy or precision in their respective alternative requests, outside of the included documentation. DESMI did not provide direct examination of either accuracy (agreement) or precision, outside of its included documentation. DESMI presented information on the relative potential for “false negative” results in the required and proposed alternative approaches. While false negatives in the required method (living organisms that are not motile and do not stain) and proposed alternative (viable autotrophic organisms that do not reproduce under the provided conditions and living heterotrophic organisms that are not motile) are contributors to potential differences in agreement and precision, they are not the sole sources of error.

the actual parameters being measured are different. Precision can be assessed within a method, but accuracy is usually evaluated through comparison with a “true” value.

Accuracy or Agreement

“Accuracy” means that the two methods should return the same result in terms of closeness to a known or “true” value. In this case, we have no way of knowing the “true” concentration that is independent of the methods being evaluated. In other words, how do we count the organisms without using the methods under consideration? In the absence of a way to evaluate accuracy, several approaches were taken to evaluate the degree of agreement between the proposed alternative and the required method in determining the number of living and viable organisms.⁸⁹

The applicants analyzed three subsamples of each of three concentrations of the cultured phytoplankter *Tetraselmis suecica*, using both the required and the autotroph (MPN) methods (Miller and Petri 2015).⁹⁰ In this experiment, the cultures of the alga were healthy and robust, and it could be expected that all or most of the living cells were likely also viable. To evaluate agreement between the two methods, the applicants calculated the mean of the three subsamples using the autotroph method divided by the mean concentration determined using the required method, multiplied by 100 (the required method was considered the benchmark for this comparison). The value (converted to a percent) would be 100% if both methods yielded the exact same concentration. Because the “true” concentration is unknown, this metric actually quantifies the agreement between the required and alternative methods (rather than accuracy, which is the term used in the proposal).

In this test, where most if not all the cells were both living and viable, the percent difference in measured concentration between the required and alternative methods ranged from 38% difference at a concentration of 1000 cells/mL to 412% difference at the lowest concentration of 10 cell/mL. In other words, in a circumstance where one would expect the results to be the same, the result using the alternative was at best 38% different, and at worst 412% different than the result using the required method. It is particularly concerning that the percent difference was so great at the lowest concentration, which is essentially at the level of the discharge standard. However, due to the high degree of variability in results (6 of 9 means for the required test had standard deviations less than 10, while 6 of the 9 means for the alternative test had standard deviations greater than 46), it is difficult to draw any further conclusions from this test regarding how agreement between methods might vary as a function of concentration.

⁸⁹ “Agreement” means that the proposed alternative method provides at least the same result when circumstances are such that the results should be the same. In the supporting material, the applicants provided results from an experiment in which the two methods were used to measure the concentration of samples in which all cells were likely both living and viable. In such case, the two methods should result in the same numbers. If, as in this case, accuracy cannot be evaluated because there is no independently derived “true” value against which to assess the methods, then agreement can be used to evaluate equivalency.

⁹⁰ *MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 µm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology*. Only autotrophs were used; no heterotrophs were used in this case. Since all organisms were phytoplankton and presumably the phytoplankton were capable of growth (since they were in culture, under favorable light and nutrient conditions), there was no need to perform the heterotroph method in addition to the autotroph method to determine the total number of organisms.

The applicants also evaluated the agreement between the alternative method and the required method when all of the organisms in the samples were heterotrophic. Cultures of the rotifer *Brachionus plicatilis* were evaluated using the heterotrophic component of the alternative method and direct counts using a stereomicroscope; in both of which, movement of organisms was the parameter used to assess whether the organisms were living. There was no difference between the two methods in the concentration of rotifers. However, in this experiment, the alternative heterotrophic method was not compared to the actual required method, which uses epifluorescence microscopy to detect positive reactions of two vital stains and cell movement. For a legitimate comparison, both the heterotroph and required methods must be used.

When using the two methods to assess samples of ambient communities (Miller *et al.*, 2015b), the applicant examined the agreement between methods using a calculated “factor of agreement” (FOA, calculated as the average alternative concentration/average required concentration). The FOA varied widely among different experiments, from 0.47 to 33.8. Half of the 16 comparisons had an FOA of less than one, and half had values greater than one; six of the latter values were greater than 5.0. It is difficult to draw any conclusions from this experiment, given the possible sources of confounding effects: the MPN counts could have been reduced due to the presence of viable but non-culturable species, or increased due to the presence of organisms smaller than 10 μm , while the stain counts could have been depressed by the presence of non-motile, non-staining organisms that were nonetheless living (false negatives). These potentially confounding effects would have to be carefully partitioned in experiments using ambient organisms, and that would entail a significant amount of work. Alternatively, consistently culturable species known to take up the fluorescent stains of the required method and larger than 10 μm in size could be used, in laboratory tests using mono- and mixed cultures, to evaluate the degree of agreement between the two methods. The need for statistical adjustments for the presence of viable but non-culturable species would need to be evaluated and possibly developed and incorporated into the method, and the effect of contamination by organisms less than 10 μm in size would also need to be addressed. Even with pre-filtration to remove organisms less than 10 μm , some contamination is unavoidable in samples of natural assemblages, and controls for this source of error will need to be developed.

Given the findings discussed above, I cannot agree that the proposed alternative has been reasonably shown to be equivalent to the required method in terms of agreement.

Precision

A measure of equal precision means that the two methods should demonstrate the same result in terms of the variability in the measurement (e.g., variance around the mean). Unlike accuracy, the variance in result can be examined without having to know the “true” concentration. Repeatability, even if “wrong”, is the key. Precision can be examined systematically for known cultures, both singly and mixed, as well as for a variety of ambient assemblages, although the assumption that all of the living organisms are also viable gets more tenuous when samples of organisms from the environment are used, rather than organisms from known cultures in a healthy phase of population growth.

Problems arise when the ability to get organisms to consistently reproduce sufficient for detection by a method is unknown. When organisms are obtained from culture collections, these are, to a degree, “predisposed” to growing under culture during an MPN-based method. In those cases, organisms are likely to be detected by serial dilution and culturing. For ambient organisms collected from the environment, one would have to run repeated culture experiments: either by selecting organisms and culturing from an initial “seed” individual, or by somehow demonstrating that every time organisms of that species are present in an ambient sample, they demonstrate increased concentration under the provided culture conditions.⁹¹ In the experiments conducted by the applicants, this is at least partly examined when taxonomic analyses are conducted for the post-incubation dilution tubes. However, the focus in the analysis was on species shown to increase in population size at least some of the time, rather than on species that were shown to be culturable in all tests. For a repeatable standard method, consistent culturability would be critical.

Laboratory experiments with monocultures of the autotrophic organism *Tetraselmis* were conducted (Miller and Petri 2015) to compare the precision of the autotrophic component of the alternative method to that of the required method.⁹² The results from this test showed the precision was better in the required method than in the alternative method: the coefficients of variation (CVs, a measure of precision, with larger CVs indicating lower precision; calculated from the data in Miller and Petri 2015) were 1-26% (average = 13%) for the required method and 20-135% (average = 50%) for the proposed alternative. The means of the CVs resulting from the two methods were significantly different ($p < 0.05$), when compared using a non-parametric Mann-Whitney U-test. In all but one case, the CV of the required method was lower than the CV of the alternative method. Thus, by this comparison, the required method has greater precision than the proposed alternative.

The applicant also compared precision between the heterotrophic component of the alternative method and a similar microscopical method in experiments using monocultures of the rotifer *Brachionus* (Miller and Petri, 2015). In this comparison, there was no difference between the results produced by the two methods. However, while the two methods were shown to have similar precision (CVs of 23% and 30% respectively), the method against which the alternative was compared was not the required method. Thus, the comparison is invalid and irrelevant for proving equivalent precision with the required method.

Precision was not specifically examined in the experiments conducted with ambient assemblages at two test facilities (Miller *et al.*, 2015b).⁹³ However the experiments did report the results of comparisons between concentrations measured with the required method and with the proposed alternative method at the two locations and on two separate dates, providing 4 measurements

⁹¹ This would potentially be confounded by inhibition or encouragement of reproduction by other organisms that would not be in effect if the target species was present as one individual in a MPN dilution tube.

⁹² In this case, all of the organisms were autotrophic, so the heterotrophic component of the alternative method was not pertinent.

⁹³ MPN Method Development Report No. 5; Miller, A. F. Norlin and B. Petri; 2015. These experiments were focused on evaluating the best culture conditions and in examining the Factor of Agreement (FOA) between the two methods.

each for five methods.⁹⁴ The applicant did not provide raw data for the results from the experiment for the required method. However, a summary figure was produced showing average concentrations of organisms obtained using the five methods, and also showing some measure of the variance around the averages as “error bars.”⁹⁵ The degree of variance as indicated by the error bars is clearly greater for the alternative method results than for the required method. Additionally, the CVs for the measurements using the alternative method are provided in a table. Interestingly, the range of CVs was 19-145 for the 14 tests where “0” variance due to method problems was not an issue, and the average CV was 58. This is very similar to the range and average observed for the alternative method when used to measure concentrations of the single autotroph *Tetraselmis* (range: 20-135 and average: 50), as discussed above.

On the basis of these comparisons, the Coast Guard cannot agree that required and alternative methods are equivalent with respect to precision.

D. Does the request include a full description of the proposed alternative?

Trojan’s request does not contain a full description of the proposed alternative. Overall, the proposed alternative method is clearly described and understandable. However, in several specific sections there are ambiguities or potential errors that must be addressed:⁹⁶

While the MPN statistical approach is a common microbiological assay for calculating concentrations based on observations of population growth in replicate serial dilution cultures, Trojan has failed to justify why it is appropriate to use for BWMS.

Many microorganisms, such as bacteria, fungi, and algae, can reproduce via asexual cell division, and under the appropriate conditions for growth, a single cell can reproduce to concentrations that are easily detectable. For autotrophic organisms such as phytoplankton, a suspension containing a single organism, given ideal conditions (e.g., nutrients, substrate, temperature, and light), will undergo exponential population growth. After sufficient time for such population increase, bulk metrics can be used to indicate the presence of that population, indicating that at least one reproductive organism must have been present in the original suspension. Changes in common bulk metrics, including chl-a fluorescence for algae and turbidity for bacteria, between initial measurements and measurements after a period of culturing, denote the presence of at least one organism in the initial diluted sample capable of undergoing reproduction.

MPN’s success under these circumstances does not mean that it is appropriate for all circumstances, particularly for use with ambient, mixed assemblages of phytoplankton rather than a monoculture. In a given water body, the number of species varies geographically and seasonally, but it is on the order of dozens, if not hundreds, of species.⁹⁷ Applying MPN to such diverse communities may violate the assumptions of the method.

⁹⁴ The 5 methods were the required method and four combinations of the proposed alternative method using two media and two temperatures.

⁹⁵ It is unclear what the “error bars” represent. They could be standard deviations, standard errors, or CVs.

⁹⁶ These issues are separate from the problems related to lack of equivalence identified *supra*.

⁹⁷ For example, over a thousand phytoplankton species have been identified in the Chesapeake Bay estuary.

The following assumptions, which were written in reference to bacteria but are also applicable for phytoplankton, are “necessary to support the MPN method” and are stated up front in all specific versions of the MPN statistical approach:⁹⁸

- a) The organisms are distributed randomly within the sample.
- b) The organisms are separate, neither clustered together nor repelling each other.
- c) Every replicate (tube, plate, etc.) whose inoculum contains even one viable organism will produce detectable population growth or change.
- d) The individual tubes of the sample are independent.

A full description of the method must explain why apparent violations of these assumptions are either not critical, or are otherwise accounted for.

The most critical assumption of MPN as a statistical approach is that each viable cell is capable of reproducing, so its original presence in the diluted subsample is detectable through population growth and an increase in the measured parameter over the course of the MPN incubation. The ability of an organism to reproduce must be independent of other organisms, inhibitory factors, such as toxicity due to the presence of trace metals and viruses in the cultures. If every cell may not be capable of being cultured in MPN tubes, and in turn, may not be detected, then the mathematical underpinnings of the MPN calculations are not applicable and procedures that incorporate the MPN methodology will not return accurate estimates. One of the most important aspects of test procedures that incorporate MPN as a statistical approach is that all of the necessary conditions for reproduction and population increase be present during the incubation period. If this is not the case, the method will not detect the presence of organisms that do not reproduce sufficiently to result in a detectable population increase, and the procedure will not result in an accurate measurement of original concentration.

With the above fundamental requirement in mind, it is important to recognize that the information submitted in support of the proposed method includes data showing not all species of phytoplankton are capable of being consistently cultured, at least under the conditions that were used, during all tests. This point is not addressed in the application, other than to assert, without substantiation, that it will present a small bias. The applicants argue that the important issue is whether all species have been observed to reproduce under the provided conditions at least once, rather than consistently (i.e., every time culturing is attempted), and the species that are not known to have reproduced at least once approaches 0% when a long-term historical record is considered. However, the key issue is not whether a viable organism of a species has been observed to reproduce at least once in the past, but instead whether it is known to reproduce consistently whenever present during a test. Thus the percentage of organisms that may be viable but non-culturable is greater when the ability to grow in each test is considered.

The data submitted with the proposed alternative indicate that the proportion of species observed to consistently demonstrate reproduction and population increase varied between the two locations where the issue was examined. At one site, 20-44% of species present in the samples had consistently demonstrated population growth in culture, while at the other the range was 56-

⁹⁸ See, e.g., US Food and Drug Administration, *BAM Appendix 2: Most probable number from serial dilutions*, Washington, DC, (2010).

89%. Hence, the proportion of species present that had not been observed to reproduce consistently in tests intended to detect organisms capable of reproduction was 56-80% and 11-44% at the other. The conclusion is that the test is not capable of detecting whether members of these species are capable of reproduction.

The assumption that organisms do not aggregate is clearly called into question for mixed assemblages of phytoplankton because of the existence of many species that naturally occur as multi-celled colonies. It may be that this problem could be ameliorated by gentle agitation of the sample to break up the colonies into individual cells, but many of the colonial types adhere quite strongly, and the ability to disaggregate the colonies without causing mortality would need to be established for all the colonial species that might occur in test waters.

The ability of every cell to be cultured is a fundamental tenant of MPN, and a significant shortfall in meeting that criterion calls into question the use of the proposed alternative method. This critical shortfall needs to be resolved.⁹⁹

Additionally, there is further uncertainty regarding the estimated MPN in the proposal. Typically, the upper and lower values defining the confidence interval (CI) are reported with MPN estimates. Standard methods for MPN analyses, including methods published by the EPA (U.S. EPA 1978), the U.S. Food and Drug Administration (U.S. FDA 2010), and other scientific authorities (APHA et al. 1999), include tables that list the corresponding 95% CIs. These values must be included when reporting the outcome of an MPN analysis to assure data quality.¹⁰⁰ Likewise, the calculators evaluated in the submitted documents all report CIs. However, the proposed method does not require reporting the CIs, nor explain why. As for all calculations of standard errors (SE), of which the CI is an example, larger sample sizes (n) generally result in smaller SE. Take for example, a single dilution MPN with a sample volume of 0.1 mL and an original undiluted concentration of 2.23 viable organisms per mL. Varying the numbers of tubes in an MPN, while holding the percentage of sample tubes positive for growth in each test at 20%, results in a wide range of confidence interval sizes around the MPN. Using 5, 10 or 20 tubes per test results in confidence intervals of 0.31 – 15.9; 0.56 – 8.9; and 0.84 – 5.96, respectively. The precision of the estimate of a 5-tube MPN was limited: the range of CI spanned two orders of magnitude (from 0.31-15.9 organisms mL⁻¹). Higher numbers of sample tubes yield narrower CI ranges, and thus greater confidence in the calculated MPN value.

The proposed method stipulates an MPN matrix should consist of 3 dilutions x 5 tubes per dilution. As shown from the above example, a larger number of tubes must be used to ensure that an MPN value generated from an MPN table does not have a large CI. If the 5 tube case described above were observed in an approval test, even though the MPN is 2.23 viable organisms per mL, a value below the discharge standard, the upper confidence limit is 15.9 viable organisms, a value above the discharge limit.

⁹⁹ This is and remains a central issue of discussion within EPA's ETV Technical Panel formed to consider, among other things, the acceptability of an MPN-based viability assay. The data presented in support of the proposed alternative method have been the basis for many of the panel's discussions, and a generally accepted resolution has not yet been identified.

¹⁰⁰ See, e.g., EPA, *Soil sampling quality assurance user's guide*, Report number EPA/600/S4-84-043, Washington, DC.

Of further concern is that the proposed alternative method does not account for the CIs generated by MPN tables. The CI can be relatively large (as noted above), and excluding the CIs can potentially result in a BWMS being considered to meet the discharge standard on the basis of the MPN, whereas the BWMS may not meet the discharge standard if the upper CI was taken into consideration. The lack of consideration of the CI in the alternative method is not explained, other than by a statement that they are not used. The CI, and its meaning with respect to the calculated MPN, must be explained in the alternative method and it should be reported with all results.

Additionally, several sections of the method description require clarification, as discussed below:

- i. **Sampling:** The sampling scheme described in the method allows for two options: "...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken." Having two options allows for unnecessary and potentially disruptive differences among practitioners. Furthermore, the latter option (one sample with 3 subsamples) could greatly affect the outcome of the sampling and is inappropriate for use in this circumstance. This option results in "pseudoreplication,"¹⁰¹ in which the "replicates" are actually "subsamples" that violate the assumption of independence among samples. If the latter option were used, the statistical analyses would be flawed, potentially yielding results that were wrong. Thus, there must be only one recommended sampling scheme, it should be the first option (three replicate samples).¹⁰²
- ii. **Autotrophs – Filtering:** The proposed alternative method directs that samples for the autotroph method be filtered onto 10- μm filters. However, if organisms $<10\ \mu\text{m}$ are retained on the filter, as could be expected, estimates of organism numbers from the MPN analysis will be artificially inflated, because the final MPN number would include organisms that are regulated by the Coast Guard ($\geq 10\ \mu\text{m}$ and $<50\ \mu\text{m}$) as well as those that are not regulated by Coast Guard ($<10\ \mu\text{m}$). Similarly, organisms $\geq 50\ \mu\text{m}$ could also be retained on the filter, again, artificially inflating the MPN estimate. The applicant asserts this bias would be small, but in any case, it would be prudent for each test facility using this approach to examine the potential for these circumstances to bias the estimates from the MPN analysis. According to the method description, the filter may or may not be left in the MPN tube during the grow-out period. It is unclear if the practice of leaving a filter in the tube affects the potential for population growth due to smaller organisms entrained on the filter being included in the MPN tubes, or if the presence of the filter itself affects the fluorescence reading. The alternative method must provide unambiguous direction, and if the direction is to leave the filter in the tube, data showing that the practice does not affect the results must be presented.

¹⁰¹ Hurlbert SH, *Pseudoreplication and the design of ecological field experiments*, Ecol Monogr 54:187–211 (1984).

¹⁰² This does not apply to sample tubes used in the MPN approach; in such case, water for all dilution tubes (i.e., an array consisting of 3 dilutions, each with 5 replicates) should be drawn from the same population. Tubes are inoculated with water from a single, original sample.

- iii. Autotrophs – Measurements: To determine if reproduction and population growth has occurred in an MPN tube, the proposed method specified a minimum threshold fluorescence of four times the standard deviation (SD) of fluorescence measurements from method blanks (tubes containing no chlorophyll). In order for this threshold to be uniformly applied across laboratories, the fluorometers would need to be calibrated following the same, standardized procedure, and demonstrate consistency in measuring the threshold value. This specification must be included in the proposed method.
- iv. Heterotrophs – Detection: The heterotroph component of the proposed alternative method relies partly on the ability to detect the red autofluorescence of chlorophyll-a (chl-a) containing organisms using epifluorescence microscopy. However, the optical filter set specified in the method is optimized to detect the fluorescence from fluorescein, not chl-a; according to the wavelengths of the filter set specified in the heterotroph method (B-2E/C), the red autofluorescence of chl-a would not be visible (i.e., the optical filter would not serve to identify organisms with chl-a). Potentially, all organisms detected would not fluoresce, and would appear to lack chl-a and, therefore, if they were motile, they would be scored as heterotrophs. Filter sets are available that allow chl-a fluorescence to be detected and must be used. Additionally, the procedure used to quantify living heterotrophic organisms scores cells that are moving and do not show red autofluorescence (i.e., they do not contain chl a) as living heterotrophs. However, if organisms do not exhibit fluorescence and are not viewed with another light source (one is not stipulated in the proposal), they will, whether moving or stationary, be, at best, dimly illuminated by light and difficult to see. This potential problem should be addressed.
- v. Data Analysis: It is unclear how the uncertainties around the counts resulting from the autotroph and heterotroph components of the proposed alternative method are applied. Each set of replicate measurements will result in an estimate of the variance around the mean. If the variance estimates are to be added together, as are the means to arrive at a total number of organisms, there is no statistical justification provided for doing so. Instructions, and justifications for such, should be provided.

Conclusion

In sum, I deny your appeal and affirm MSC's decision denying Trojan's request for a testing equivalency and type approval under 46 C.F.R. § 162.060-10. When promulgating its ballast water regulations, the Coast Guard explicitly rejected "viability" as part of its ballast water discharge standard. This policy decision was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate.

MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations. MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1).

This decision constitutes final agency action on the issues raised in your appeal.

Sincerely,



Linda L. Fagan
Rear Admiral, U. S. Coast Guard
Deputy for Operations Policy and Capabilities

Technical References

1. (Miller 2015a) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Supplemental Information 02 MAR 2015
2. (Miller 2015b) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Updated Documentation 06 MAY 2015
3. (Trojan Marinex 2015a) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples 31 JAN 2015
4. (Trojan Marinex 2015b) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples, 05 MAY 2015
5. (Petri 2015a) Evaluating the MPN Dilution-Culture Method for the Enumeration of Viable Phytoplankton Cells
6. (Maurer and Welschmeyer 2015a) Flow Cytometric Analysis of the Relative Abundance of Heterotrophs and Autotrophs in the Regulated 10-50 μm Size Class
7. (DHI 2014) MPN Assay – Analyses of Algal Regrowth for Performance Evaluation of Ballast Water Management Systems Primary Validation
8. (Petri 2015b) MPN Method Development Experiments 1 to 3 Inter-Lab Comparison of the MPN Dilution-Culture Method and Fluorescein-Based Staining Methods for the Enumeration of Viable or Living Phytoplankton Cells
9. (Miller et al. 2015a) MPN Method Development Report Experiment 4
10. (Miller et al. 2015b) MPN Method Development Report Experiment 5
11. (Miller and Petri 2015) MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 μm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology
12. (Cullen and MacIntyre 2015) On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment
13. (Maurer and Welschmeyer 2015b) Rationale for the Use of Most Probable Number (MPN) Technique in the Evaluation of UV-based Ballast Water Management Systems
14. (MacIntyre et al. 2015) Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water

15. (Miller 2015c) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 03 JUN 2015
16. (Miller 2015d) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 30 JUN 2015
17. (Miller 2015e) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 23 JUL 2015
18. (Miller 2015f) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 26 JUL



5800

JUL 12 2016

The Boutique Firm PLC
Attn: Matthew D. Melewski
3928 Xerxes Ave S
Minneapolis, MN 55410

Dear Mr. Melewski,

I refer to your appeal dated February 11, 2016, on behalf of DESMI Ocean Guard A/S (DESMI). You requested a formal administrative appeal of the decision of the Marine Safety Center (MSC) denying DESMI's request for a testing alternative to be used in the Coast Guard's ballast water management system (BWMS) type approval process and DESMI's application for USCG type approval of the DESMI RayClean BWMS.¹

This is an administrative appeal of an MSC decision or action taken pursuant to 46 Code of Federal Regulations (C.F.R.) § 162.060-10(b) and is reviewed by my office under 46 C.F.R. § 159.001-2 and as provided in 46 C.F.R. § 1.03-15. In considering your request, I reviewed your appeal (including its appendix² and the administrative appeals of Hyde Marine, Inc., Alfa Laval Tumba AB, and Trojan Technologies, incorporated by reference by your appeal, footnote 30, the administrative record, and applicable laws, regulations, and policy.³

Based on this review, I hereby deny your appeal, affirming MSC's decision to deny DESMI's request for testing equivalency and type approval under 46 C.F.R. § 162.060-10. This matter and your appeal are quite technical and detailed, and the issues raised will be discussed in more detail in this letter.⁴ To briefly summarize, during the development of the ballast water regulations, the Coast Guard explicitly rejected the use of BWMS that "may act to make organisms unviable or unable to reproduce rather than killing or removing them." This policy decision to reject "viability" was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations, and MSC lacked the discretion to accept your proposed

¹ Type testing and approval are used in equipment and manufacturing to determine that a specific "type" of equipment or process meets a minimum set of requirements. In this context, "type approval" is a vessel equipment approval process.

² Neither the hard nor electronic copy of DESMI's appendix included Exhibit 17.

³ I note that DESMI, by incorporation by reference, has reserved the right to "supplement" its appeal to address my response. That is unnecessary. This response constitutes final agency action on the issues raised in DESMI's appeal.

⁴ I have substantively responded to your appeal in the interest of transparency. This response does not waive any defenses the Coast Guard may have as to the timeliness of your assertions or failure to exhaust your administrative remedies.

testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). As your type approval application was based on testing that does not meet the regulatory standards and was not approved as an alternative, MSC was correct in denying your application for type approval of the DESMI RayClean BWMS. Your appeal is denied, and this decision constitutes final agency action.

Background

Marine environmental protection is one of the Coast Guard's core statutory and operational missions.⁵ As stewards of the marine environment, the Coast Guard maintains a robust environmental protection regulatory program and also assists other federal agencies in enforcing laws to protect, preserve, and remediate waters subject to the jurisdiction of the United States. The Coast Guard also leads and participates in initiatives at the International Maritime Organization (IMO), the intergovernmental organization specializing in commercial shipping safety, security, and environmental protection standards, to raise and standardize global shipping practices. These activities all have one desired end state: to help ensure the health and vitality of waters of the United States and its living marine natural resources.

The Coast Guard manages its marine environmental protection obligations through a well established network of Headquarters, regional and field offices. Within Coast Guard Headquarters, located in Washington, D.C., there are several organizations, or "programs," responsible for developing, promulgating, and enforcing marine environmental protection standards. The Office of Operating and Environmental Standards (CG-OES) and the Office of Design & Engineering Standards (CG-ENG) have the lead roles in promulgating and implementing (but not enforcing) Coast Guard environmental regulations, including requirements for approval of equipment installed on vessels. MSC's role focuses on regulatory compliance and policy development, generally related to plan reviews for domestic vessels and type approvals for vessel equipment. While MSC can be involved in the clearance process for rulemakings, MSC is not the lead office for environmental standards development. In other words, MSC applies environmental regulations but does not create them. All three programs are within the Directorate of Commercial Regulations and Standards (CG-5PS).

The Coast Guard's ballast water program is one of the Coast Guard's long-standing marine environmental protection programs. It is established under the authority of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, as amended by the National Invasive Species Act of 1996 (NANPCA/NISA).⁶ As mandated by NANPCA, the Coast Guard's program began in 1991 with voluntary ballast water management guidelines for the Great

⁵ See Section 888, Homeland Security Act of 2002, Pub. L. No. 107-296 (H.R. 5005), 116 Stat. 2135 (2002), as amended, *classified to* 6 U.S.C. § 468.

⁶ Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (NANPCA/NISA), Pub. L. No. 101-646 (H.R. 5390), 104 Stat. 4761 (1990), *as amended; codified in* 16 U.S.C. §§ 4701, 4702, 4711, 4712-4714, 4721-4728, 4741, 4751. Congress enacted NANPCA in response to the disruption and damage caused by the introduction of nonindigenous zebra mussels into the Great Lakes, likely released via discharges of ships' ballast water. NANPCA originally focused on ballast water operations in the Great Lakes but was later amended by NISA to cover waters nationwide.

Lakes.⁷ Mandatory requirements for the Great Lakes followed these voluntary guidelines in 1993.⁸ In 1996, NISA amended NANPCA to, among other things, cover all navigable waters of the United States.⁹ In the 1990s, the best method available for preventing the discharge of aquatic nuisance species from ballast water was an operational practice known as ballast water exchange. For this reason, NANPCA/NISA contained a specific requirement for certain vessels to conduct ballast water exchange.¹⁰ The Coast Guard updated its regulations to include nationwide, mandatory ballast water exchange requirements in 2004.¹¹ However, ballast water exchange was an interim measure until more effective ballast water technology could be developed and verified. Under the NANPCA/NISA mandate to “ensure to the maximum extent practicable that aquatic nuisance species are not discharged into waters of the United States from vessels,” the Coast Guard collaborated with domestic and international partners to identify better methods and technology to prevent the introduction and spread of aquatic nuisance species. These efforts ultimately resulted in the Coast Guard’s 2012 Final Rule, Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters (2012 Final Rule).¹²

NANPCA/NISA is a domestic legal authority and does not explicitly implement the international standard for ballast water discharges, which is found in the IMO International Convention for the Control and Management of Ships’ Ballast Water and Sediments, 2004 (BWM Convention).¹³ The disconnect between U.S. domestic law and the international standard is partly timing: NANPCA was enacted in 1990, many years before the international community adopted the BWM Convention in 2004. Additionally, at this time, the United States is not a contracting government to the BWM Convention¹⁴ and the BWM Convention has not received enough ratifications to enter into force. The Coast Guard attempted to harmonize the 2012 Final Rule with the BWM Convention to the extent possible within its statutory authority.¹⁵ Ultimately, the

⁷ NANPCA/NISA, *supra*, n. 6, § 1101(a); *codified in* 16 U.S.C. § 4711(a). *See also* Ballast Water Management for Vessels Entering the Great Lakes, Final Rule, 58 Federal Register (Fed. Reg.) 18330 (April 8, 1993).

⁸ *Id.*

⁹ *See* NANPCA/NISA, *supra*, n. 6, § 1101(a) – (c); *codified in* 16 U.S.C. § 4711(a) – (c). This statutory bifurcation is the historical reason why Coast Guard ballast water regulations are promulgated in two subparts of 33 C.F.R. Part 151, despite there now being few differences between the ballast water management standards for the Great Lakes and those for the rest of the United States.

¹⁰ Ballast water exchange is a process by which a vessel replaces water, generally coastal water, in its ballast tanks. *See* definition of “exchange,” 33 C.F.R. § 151.2005(b).

¹¹ Mandatory Ballast Water Management Program for U.S. Waters, 69 Fed. Reg. 44952 (July 28, 2004).

¹² 77 Fed. Reg. 17254, (March 23, 2012).

¹³ IMO Doc. BWM/CONF/36.

¹⁴ If the United States became party to the BWM Convention, the Coast Guard could implement the convention through NANPCA/NISA even though there is no explicit reference. *See* NANPCA/NISA, *supra*, n. 6, § 1101(f)(3); *codified in* 16 U.S.C. § 4711(f)(3).

¹⁵ *See, e.g.*, 77 Fed. Reg. 17260. I note that the Coast Guard has made some statements which could confuse the issue of whether the Coast Guard ballast water discharge standard is “identical” to the BWM Convention standard. From the Coast Guard’s perspective, as discussed further *infra*, the BWM Convention’s use of “viable” is synonymous with the Coast Guard’s use of “living,” and in that sense the standards are the same. However, there are some minor differences between the two regimes. That is why the Coast Guard has used certain language, such as “align with” and “equivalent to,” to show that the Coast Guard regime is *not* identical to the BWM Convention regime.

BWM Convention is not a treaty of the United States, and the Coast Guard has a mandate to implement NANPCA/NISA as written.¹⁶

The Coast Guard's ballast water regulations are codified into Titles 33 and 46 of the C.F.R. The regulations in 33 C.F.R. Part 151 Subparts C (Great Lakes) and D (Nationwide) are operational or performance standards, and the regulations in 46 C.F.R. Subpart 162.060 are equipment standards.¹⁷ In this case, the Title 33 operational requirements apply to vessels, vessel owners or operators, or other persons associated with the vessel, and the Title 46 equipment requirements apply to BWMS manufacturers seeking equipment approvals. However, as discussed *infra*, the two are still interrelated and should be read together.

The 2012 changes to 33 C.F.R. Part 151 introduced a scheduled phase-out of ballast water exchange as an accepted operational measure to reduce the introduction and spread of aquatic nuisance species and provided the new option of using a BWMS to treat ballast water prior to discharging it.¹⁸ The 2012 changes also added other ballast water management options, including the use of water from U.S. public water systems and discharge to a reception facility. 33 C.F.R. Part 151 now contains a numeric discharge standard for the maximum number of living organisms in ballast water, a standard which all Coast Guard-approved BWMS must meet.¹⁹ For a vessel meeting the discharge standard by using a BWMS to be in compliance with 33 C.F.R. Part 151, its BWMS must have received type approval under the standards located in 46 C.F.R. Subpart 162.060.²⁰

I now address the issues raised in your appeal, as well as the principal arguments raised by the other manufacturers in their respective appeals, which have been incorporated by reference, as follows:

1. MSC did not have the discretion to approve DESMI's request

¹⁶ As discussed further, *infra*, the Coast Guard's regulatory standard is "to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels..." NANPCA/NISA, *supra*, n. 6, § 1101; *codified in* 16 U.S.C. § 4711. The Coast Guard believes the "practicability" element of this standard requires the U.S. ballast water discharge standard to take into account the relevant IMO standard, as most vessels engaged on international voyages will likely need to comply with the BWM Convention once it comes into force. If the U.S. domestic standard conflicts with or frustrates an international standard, the result could range from increased vessel costs to preventing the vessel from calling on certain ports (implementing whichever standard the vessel cannot meet).

¹⁷ Generally, for the C.F.R., the reason vessel operational standards appear in Title 33 and vessel equipment standards appear in Title 46 is because many Coast Guard statutory authorities are contained either in Title 33 of the United States Code (Navigation and Navigable Waters) or Title 46 (Shipping). Title 33 authorities are generally more "operational," such as the Ports and Waterways Safety Act (Pub. L. No. 92-340 (H.R. 8140), Tit.I, 86 Stat. 424 (1972), *as amended*; *codified in* 33 U.S.C. §§ 1221 – 1232) and the Act to Prevent Pollution from Ships (Pub. L. No. 96-478, 94 Stat. 2297 (1980), *as amended*; *codified in* 33 U.S.C. §§ 1901 – 1911). On the technical side, the Coast Guard maintains broad authority to regulate inspected vessel equipment under 46 U.S.C. § 3306, and its vessel equipment approval processes are correspondingly located in Title 46 of the C.F.R. This is true even when the underlying statutory authority is contained in a different title. In this case, the Coast Guard has overlapping statutory authority to set BWMS equipment standards, under both NANPCA/NISA and 46 U.S.C. § 3306.

¹⁸ 33 C.F.R. § 151.1510(a); 33 C.F.R. § 151.2025(a).

¹⁹ 33 C.F.R. § 151.1511; 33 C.F.R. § 151.2030.

²⁰ *See, e.g.*, 33 C.F.R. § 151.2025(a)(1).

I agree with MSC that it lacked the discretion to approve DESMI's 46 C.F.R. § 162.060-10(b)(1) request, which sought approval of a testing method that measured "viable" rather than "living" organisms in ballast water. The Coast Guard's type approval regulations exclude "viability" as an option. This was an environmentally conservative policy decision, based on best scientific information available, which went through the public notice and comment process. Therefore, MSC could not grant an alternative request that circumvented the text and policy position of the Coast Guard's ballast water regulations. Since DESMI's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying DESMI's type approval application.

Issue:

DESMI is a BWMS manufacturer who requested that the Coast Guard approve an alternative to the Coast Guard's BWMS type approval requirements. DESMI manufactures the RayClean BWMS, which uses ultra-violet radiation (UV) to treat ballast water. DESMI submitted its application for a BWMS testing alternative under 46 C.F.R. § 162.060-10(b)(1), and the crux of this appeal revolves around the type of tests that can be used, under the Coast Guard regulations, to validate the efficacy of UV BWMS. Specifically, DESMI requested to use a "Most Probable Number assay" (MPN) in lieu of 5-chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) direct staining methods to test for certain organisms to meet type approval requirements.²¹ The simplified distinction between these measurement methods is that DESMI's preferred measurement method measures "viability" of an organism, while the regulatory requirement method measures whether an organism is "living." This appeal concerns the testing method being used and not whether the Coast Guard can or will grant type approval to UV BWMS as a class of system.²² While one appellant (Hyde) has characterized the question as "whether the measurement method – MPN – is reliable and accurate," the main question before me is whether the ballast water regulations allowed MSC to approve MPN as a measurement tool.²³

Applicable regulatory standards and the meaning of "living":

46 C.F.R. § 162.060-10(b)(1) provides:

If an evaluation, inspection, or test required by this section is not practicable or applicable, a manufacturer or independent laboratory may submit a written request to the Commanding Officer (MSC), Attn: Marine Safety Center, U.S. Coast Guard Stop 7410, 4200 Wilson Boulevard Suite 400, Arlington, VA 20598-7410, or by email to *msc@uscg.mil*, for approval of alternatives as equivalent to the requirements in this section. The request must include the manufacturer's justification for any proposed changes and contain full descriptions of any proposed alternative tests.

²¹ DESMI appeal, Exhibit 22.

²² Not all UV BWMS are designed like DESMI's: some UV BWMS are designed to kill organisms rather than to render them unviable. Thus, my decision should not be interpreted to mean that the Coast Guard will not grant type approval to *any* UV BWMS under the current regulatory standards.

²³ A follow-on question, answered in Section 3 of this response, is whether DESMI met all elements of 46 C.F.R. § 162.060-10(b)(1).

The Coast Guard applies 46 C.F.R. § 162.060-10(b)(1) by considering the following four elements:

1. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?
2. Is that evaluation, inspection or test not practicable or applicable?
3. Is the proposed alternative equivalent to the regulatory requirement?
4. Does the request include a full description of the proposed alternative?

MSC's decision denying DESMI's application specifically addressed items 2 and 3 and found them both in the negative.²⁴ MSC found that DESMI's proposed alternative was not equivalent because

...it does not measure the efficacy of the ballast water treatment system to the performance standard required by the regulations. The regulations specifically require ballast water treatment systems to be evaluated based on their ability to kill certain organisms. Since the proposed MPN method assesses the viability of an organism to colonize after treatment, it measures to a different standard than that required by the regulations.²⁵

You contend for several reasons that this reasoning was unsound.

However, I believe MSC was correct that the Coast Guard's ballast water management regulations do not allow MSC to approve a method that measures viability in lieu of the regulatory standards.²⁶ I believe the reference, above, to the "performance standard required by the regulations" means the ballast water discharge standard contained in 33 C.F.R. Part 151.²⁷ As a general principle, a technical equipment standard in Title 46 of the C.F.R. would not be able to override a performance or operational standard in Title 33 of the C.F.R. In this case, the technical equipment standard and the operational standard are inextricably linked, and the meaning of "living" cannot be resolved by viewing a stark dichotomy between Titles 33 and 46 of the C.F.R.

To understand why, it is helpful to begin with the text of 46 C.F.R. § 162.060-10(b)(1). DESMI's request must meet the first prong of 46 C.F.R. § 162.060-10(b)(1), requesting an alternative to an "evaluation, inspection, or test required by this section." This is a reference to the requirements contained in 46 C.F.R. § 162.060-10(f) (emphasis below added):

²⁴ Having found at least one of the elements in the negative, there was no need for MSC to opine on all of these elements.

²⁵ Letter dated December 14, 2015, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to DESMI Ocean Guard A/S, "Request for Approval of the Use of the Most Probable Number (MPN) Method to Determine Biological Efficacy of the DESMI RayClean™ Ballast Water Management System (BWMS)".

²⁶ This is not a matter of literal "equivalency" of testing methods, which is discussed *infra*, Section 3.

²⁷ See Letter dated February 2, 2016, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Hyde Marine Inc., "Request for Review and Reconsideration of December 14, 2105 Decision of Marine Safety Center Denying Application for Approval of Equivalent Test Method Under 46 C.F.R. § 162.060-10(b)(1); DESMI Exhibit 6, ("Therefore, in order to demonstrate compliance with the BWDS as set forth at 33 CFR §§ 151.1511 and 151.2030, a BWMS must be evaluated on the basis of counting living/dead vice viable/unviable" organisms.").

A BWMS is eligible for approval if –

(2) It is evaluated, inspected, and tested under land-based and shipboard conditions in accordance with §162.060-26 and 162.060-28 of this subpart, respectively, and thereby **demonstrates that it consistently meets the ballast water discharge standard in 33 CFR part 151, subparts C and D;**

(3) All applicable components of the BWMS meet the component testing requirements of §162.060-30;

(4) The BWMS meets the requirements of §162.060-32 of this subpart if the BWMS uses an active substance or preparation...

This provision clearly delineates the operational standard contained in 33 C.F.R. Part 151 from the equipment standard in 46 C.F.R. Subpart 162.060 and supports MSC's reasoning that a Title 46 alternative or equivalency for vessel equipment testing cannot be used to override a Title 33 performance standard. However, the actual text in 33 C.F.R. Part 151 shows that the two standards are inextricably linked (emphasis below added):

(a) Vessels employing a Coast Guard-approved ballast water management system (BWMS) must meet the following BWDS by the date in §151.1512(b) of this subpart:

(2) For organisms less than 50 micrometers and greater than or equal to 10 micrometers: discharge must include fewer than 10 **living** organisms per milliliter (mL) of ballast water.²⁸

There is no definition of “living” or any other regulatory text in 33 C.F.R. Part 151 that explains this important detail of the discharge standard. The only way a ballast water manufacturer can understand the “living” organism standard in 33 C.F.R. Part 151 is by referring to 46 C.F.R. Subpart 162.060 and its technical requirements and reading the preamble of the final rule. Thus, while the discharge standard in 33 C.F.R. Part 151 is an operational standard which applies to vessels, it is inextricably intertwined with the technical equipment standards contained in 46 C.F.R. Subpart 162.060 which apply to ballast water manufacturers.²⁹ The substance of “living” remains within 46 C.F.R. Subpart 162.060, and MSC was not solely bound by the indeterminate “living” language contained in 33 C.F.R. Part 151.

46 C.F.R. Subpart 162.060 also has no definition of “living,”³⁰ but this subpart contains extensive efficacy requirements which constructively define the term. Specifically, the Coast

²⁸ 33 C.F.R. § 151.1511, emphasis added.

²⁹ For vessels engaged on international voyages, this interlinkage between performance standards and technical standards is common, as typically a vessel's operation of a type approved or certificated piece of equipment satisfies the operational requirement unless a Coast Guard inspector or investigator has reason to believe that the equipment is not operating or being operated properly.

³⁰ DESMI's focus on the definition of BWMS (46 C.F.R. § 162.060-3) is misplaced. The BWMS definition alone does not set a performance or technical standard for BWMS. It merely identifies a category of equipment that the Coast Guard is regulating. The performance and technical standards in Titles 33 and 46, respectively, set the requirements for BWMS, and meeting the broad definition of BWMS does not necessarily mean that the BWMS meets all of the technical and performance standards in the regulations. Specific requirements control general terms.

Guard's testing regulations incorporate the Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV Protocol)³¹ by reference.³² The ETV Protocol contains staining test requirements³³ that evaluate the functioning of enzyme systems and cell membrane integrity of organisms, thereby defining "living" by virtue of these critical functions necessary for organisms to persist.³⁴ The ETV Protocol uses the term "viable," but defines it as "organisms and any life stages thereof that are living."³⁵ The ETV Protocol also explains why the ETV Technical Panel³⁶ decided to limit "viability":

Note that it is understood that many of the proposed regulatory discharge standards, and in fact the desired effect of BWTSs,³⁷ is that these technologies should render organisms unviable or incapable of reproduction. In other words, to "kill, remove or inactivate" is technically unnecessary when the objective is to eliminate the organism's capability for reproduction. However, as the introduction of "viability" as a measure of efficacy significantly complicates the Protocol and test methods, and since "kill, remove or inactivate" is a conservative approach, the latter has been adopted as the measure of biological efficacy in this Protocol.³⁸

For an analogy, see the definition of "tank vessel" contained in 46 U.S.C. § 2101. The fact that a vessel may meet this broad definition does not mean that it can be certificated as a tank vessel under the inspection requirements in C.F.R. Title 46. The definition identifies a broad category of thing that the Coast Guard is regulating. The vessel would still need to meet the specific tank vessel inspection requirements to receive a tank vessel Certificate of Inspection.

³¹ "Generic Protocol for the Verification of Ballast Water Treatment Technology," EPA/600/R-10/146, September 2010, available at https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=230926. The ETV Protocol is a document of the Environmental Technology Verification Program (ETV Program), funded in whole or in part by the U.S. Environmental Protection Agency.

³² See, e.g., 46 C.F.R. § 162.060-26(a), which refers to the Subpart's "Incorporation by Reference" section, § 162.060-5.

³³ The ETV Protocol "requires" the determination of organism concentration, but it is true that the offered testing method – the dual stain method – is stated in voluntary terms. For example, the ETV Protocol states, "[t]his protocol recommends use a [sic] combination of two vital stains..." (paragraph 5.4.6.5). This is because the ETV Protocol recognizes that there are other stains and measurement methods that may be acceptable, as long as they measure the same thing: whether something is alive. If a manufacturer does not want to use the dual stain method to meet the ETV Protocol requirements, then a manufacturer uses the USCG approval process in 46 C.F.R. § 162.060-10(b)(1) to request an alternative for the purposes of complying with the USCG regulations.

³⁴ DESMI raises divergent arguments, sometimes claiming that the ETV Protocol trumps the regulatory text and other times claiming that the Coast Guard cannot rely on the final ETV Protocol at all because it was not properly incorporated into the ballast water NPRM. As to the latter argument, the Coast Guard fully explained in its response to comments the logical outgrowth basis of its decision to adopt the final ETV Protocol into the 2012 Final Rule. 77 Fed. Reg. at 17258. Additionally, FACA does not apply to collaborations or partnerships that gather and share information, provide individual or unsolicited advice, or that do not seek consensus. See Federal Advisory Committee Act of 1972, Pub. L. No. 92-463 (H.R. 4383), 86 Stat. 770 (1972), as amended, *classified to 5 U.S.C. App. §§ 1 – 16*.

³⁵ ETV Protocol, page xii.

³⁶ The ETV Technical Panel is a group "comprised of a subset of stakeholders and other individuals with a technical expertise in ballast water and environmental technology issues." Members include fresh water and marine biologists, environmental scientists, engineers, and ship architects. See ETV Protocol, page 4.

³⁷ The ETV Protocol uses the term 'Ballast Water Treatment System(s)' (BWTS); which the Coast Guard views as consistent with the term 'Ballast Water Management System(s)' (BWMS) in Coast Guard regulations. See, e.g., 33 C.F.R. § 151.1504.

³⁸ ETV Protocol, page 5.

The ETV Protocol is unequivocal on the viability issue.³⁹ “Living” does not mean “viable.”⁴⁰ The language of 46 C.F.R. § 162.060-10(b)(1) provides flexibility for the Coast Guard to accept a test method other than what is expressly enumerated in the ETV Protocol, but the alternative must be demonstrated to measure what the discharge standard requires.⁴¹ A 46 C.F.R. § 162.060-10(b)(1) approval can be used to accept a different measurement tool (e.g., a 12 inch ruler versus a yard stick) but not to substitute the underlying measurement requirement (e.g., length versus weight).⁴² Thus, the efficacy standards in 46 C.F.R. Subpart 162.060 are a bar to MSC’s ability to accept the proposed viability measurement as a testing alternative.

Coast Guard policy and intent:

The preamble to the 2012 Final Rule is consistent with this ETV Protocol language. The Coast Guard responded to a comment directly on point regarding whether “living” meant or included “viable”:

One commenter stated that because some types of treatment processes, such as UV, may act to make organisms unviable or unable to reproduce rather than killing them outright, the Coast Guard should include viability as a criterion for determination of BWMS efficacy. The Coast Guard disagrees. This issue has been the point of much discussion both in the United States and internationally in association with the IMO BWM Convention. The Coast Guard has decided to use live/dead rather than viable/unviable, because the latter designations would require culturing potentially large numbers of different kinds of organisms to determine whether they were capable of reproduction. This would be made even more problematic by the fact that scientists are not able to culture many of the organisms in question. Finally, it is more conservative, and thus more protective, to base efficacy decision [sic] on the basis of live/dead, rather than viable/unviable.⁴³

³⁹ The ETV Protocol was developed independently from the USCG ballast water regulations. Its first two chapters describe an organizational process that is superseded by, or otherwise irrelevant to, the specific USCG equipment approval process contained in 46 C.F.R. § 162.060.

⁴⁰ This ETV Protocol language is a counterpoint to Hyde’s argument that viability was only addressed in the 2012 Final Rule preamble, but not in the regulation text. The ETV Protocol was incorporated by reference into the regulation text of 46 C.F.R. Subpart 162.060. *See, e.g.*, 46 C.F.R. § 162.060-26(a).

⁴¹ I note the existence of ETV Protocol paragraph 5.4.8, which provides for the use of “alternative and emerging methods.” This section is expressly limited to methods relating to “living” organisms, not organisms capable of reproduction, and even if it were not, the Coast Guard’s process set forth in 46 C.F.R. § 162.060-10 provides the relevant process for seeking alternatives. The specific alternative procedures created by the Coast Guard supersede the ETV Protocol’s generalized procedures. Additionally, if there is a conflict between the ETV Protocol and the regulation text, the two must be read such that the ETV Protocol does not render 46 C.F.R. § 162.060-10 meaningless. Finally, the Coast Guard disagrees with any interpretation of paragraph 5.4.8 which would effectively eliminate agency oversight of a federal regulatory program.

⁴² I disagree that MSC’s denial required MPN to be “identical” to the staining methods identified in the ETV Protocol. Hyde Appeal, page 26. MSC has approved other 46 C.F.R. § 162.060-10(b)(1) requests.

⁴³ 77 Fed. Reg. at 17274. I note the incongruity of Hyde’s argument that the Coast Guard cannot rely on this language to deny Hyde’s application while Hyde then quotes multiple preambular passages from multiple Coast Guard ballast water rulemakings in its favor.

This response to comment is unequivocal, and I find Hyde's arguments to discredit it, incorporated by reference, unpersuasive. First, the commenter who submitted this UV-oriented, "viable/nonviable" comment was Hyde, a manufacturer of UV-BWMS and also an appellant of the MSC decision under review here.⁴⁴ If Hyde believed that the standard clearly meant to include viable organisms, it would not have submitted this comment. In fact, it appears Hyde believed the opposite:

The characteristic "unviable" should be used in place of "dead" in determining the efficacy of BWMS... We wish to emphasize that the terms "kill" and "dead" should be replaced with "make unviable" and "unviable" throughout the proposed regulation.⁴⁵

At the time of this comment, the word "living" did not appear in the proposed discharge standard in either 33 C.F.R. § 151.1511 or 33 C.F.R. § 151.2030. The plain reading of the proposed discharge standard was that ballast water could contain only a maximum of *any* organism, living or dead. This is an extremely conservative and still practicably unachievable standard.⁴⁶ With such a conservative standard, it is understandable why a comment would be made to change the discharge standard. The upshot of the exchange is a clearly articulated statement of Coast Guard policy in the preamble to the Final Rule.

Additionally, this Coast Guard response is consistent with another section of the 2012 Final Rule preamble, which provided:

One commenter requested that the proposed BWDS include language necessary for differentiation between living and nonliving organisms. Another said that the standard should allow for the presence of nonliving organisms since some treatment technologies act to kill living organisms without necessarily removing them from the ballast water.

The Coast Guard acknowledges that the proposed BWDS is slightly different in this respect from the IMO discharge standard, which uses the term "viable" instead of "living." It is important to note that, while the text of the IMO BWM Convention refers to "viable" organisms, the G8 guidelines define "viable" as "living." Therefore, the Coast Guard has decided that this issue is best addressed in the BWMS approval process, and will not alter the standard as suggested by these commenters. We note that the standard and approval process do allow for the presence of nonliving organisms. Additionally, we corrected a technical error present in the NPRM, which mistakenly omitted the term "living" from the proposed 33 CFR 151.1511(a). This final rule corrects that omission.⁴⁷

⁴⁴ Letter dated December 3, 2009, Hyde Marine, Inc. to U.S. Department of Transportation, "Reference: Docket # USCG-2001-10486."

⁴⁵ *Id.* at page 5.

⁴⁶ In response to comments, the Coast Guard corrected the intended discharge standard by adding the word "living."

⁴⁷ 77 Fed. Reg. 17266. DESMI cannot read the adjective "viable" into regulatory text where it does not exist. The record shows that the Coast Guard intended both subparts (33 C.F.R. Subparts C and D) to have the same discharge standard, which was meant to align with the BWM Convention discharge standard. See 77 Fed. Reg. 17260 ("We corrected the BWDS in both subparts C and D to align with the IMO BWM Convention."). If the nationwide

Like the ETV Protocol, this response explains that “viable” means “living” and not vice versa. As discussed, *supra*, the IMO BWM Convention provides the international standard for organisms discharged from vessels’ ballast water. The Coast Guard’s ballast water discharge standard is nearly identical to the BWM Convention discharge standard. One difference is that the IMO standard contains the adjective “viable” rather than “living” to modify “organisms.” This Coast Guard response shows that its deviation from the IMO standard text was deliberate. This response also tries to articulate that, from the Coast Guard’s perspective, the two standards are substantively the same.⁴⁸ The BWM Convention does not define “viable.” However, one BWM Convention guidance document, the Guidelines for approval of ballast water management systems (G8 Guidelines),⁴⁹ defines “viable” to mean “living.”⁵⁰ While DESMI flips the definition around, so that “living” means “viable,” that is not reflected in the text of the G8 Guidelines. Instead, the G8 Guidelines provide a more narrowed interpretation of the BWM Convention text, consistent with the Coast Guard’s discharge standard.⁵¹ That is why there was no need to alter the proposed discharge standard in 33 C.F.R. Part 151 to harmonize with the IMO standard.

The Coast Guard’s response goes on to fully respond to the comments by stating that “this issue” is “best addressed in the BWMS approval process...” This means the Coast Guard’s intent was to leave “living” in 33 C.F.R. Part 151 undefined while relying on the technical efficacy requirements in 46 C.F.R. Subpart 162.060 to set the standard by defining how the standard would be measured. Subpart 162.060 contains a myriad of equipment requirements, but its efficacy requirements incorporate the ETV Protocol, which does not include viability as a measurement of efficacy.⁵² “[B]est addressed in the BWMS approval process...” does not mean

discharge standard is read without the “living” modifier, the standard becomes *stricter* than the Great Lakes discharge standard. Such a result is nonsensical.

⁴⁸ I note DESMI’s argument claiming that the Coast Guard failed in its rulemaking to consider the extra cost to foreign flag vessels which would be barred from using foreign-approved UV BWMS using an MPN-based test method. This argument confuses an environmental analysis with a Regulatory Analysis. The Coast Guard properly considered and calculated the costs to foreign flagged vessels in its Regulatory Analysis as a sensitivity analysis. Coast Guard developed a range of cost for a ballast water treatment systems based on potential technologies, calculating total costs based on the low end of the system cost estimates. Since UV BWMS are not the lowest cost option, the fact that some are not available for use in U.S. waters does not impact the Coast Guard’s analysis. Additionally, the reference to “more stringent measures” is to the “phase-two” ballast water discharge standard proposed in the 2009 NPRM but not promulgated as part of the 2012 Final Rule.

⁴⁹ Guidelines for approval of ballast water management systems, Resolution MEPC.174(58), adopted October 10, 2008. (Titles of IMO guidance documents are typically not capitalized except for the first word.)

⁵⁰ *Id.* at paragraph 3.12 (“Viable Organisms are organisms and any life stages thereof that are living.”). This language should be read as it is written and not reversed, so that living means viable. At the most recent meeting of the IMO Marine Environment Protection Committee (MEPC), this specific issue – whether the G8 Guidelines should be amended to remove or change the definition of “viable” – was raised and considered. MEPC did not reach a conclusion either way (i.e., contrary to Hyde’s assertion, the international community is not agreed that the G8 Guidelines allow BWMS to be type approved for viability rather than live/dead). See IMO Report of the Marine Environment Protection Committee on its Sixty-Ninth Session, MEPC 69/21, May 13, 2016, paragraph 4.39.

⁵¹ Because “living” is broader than “viable,” the term covers more organisms, and thus a discharge standard incorporating the broader term into a maximum allowable concentration means that fewer organisms can remain in the discharged ballast water. That is why it is a more protective standard, and a “more narrowed” interpretation, despite “living” being a broader term than “viable.”

⁵² See, e.g., 46 C.F.R. § 162.060-26(a) and 46 C.F.R. § 162.060-28(j). I note the Coast Guard’s use of “viability” in § 162.060-28(j). While it is true that the Coast Guard often uses “viable” and “living” synonymously, it is not in a

that 46 C.F.R. § 162.060-10(b)(1) can be used as a back door to insert viability into the discharge standard after it was deliberately excluded via rulemaking. It means that the ETV Protocol, or the regulation text, can be amended or updated to include viability as a measurement option once better scientific and technical capabilities are discovered. A new version of the ETV Protocol would still need to be incorporated by reference into the Coast Guard's rulemaking, via the public notice and comment process.⁵³ At a minimum, the Coast Guard's policy decision on viability was established through public notice and comment and would need to go back through public notice and comment to change.

Clarification of the administrative record:

I acknowledge some confusion in the administrative record regarding the interactions between DESMI and the Coast Guard on accepting viability as part of the discharge standard. I think more blame for this confusion falls on the appellants than it does on the Coast Guard. After the Coast Guard rejected the request to include viability in the 2012 rulemaking, the appellants then voluntarily launched a campaign to change the Coast Guard's position on the matter.⁵⁴ This campaign was not initially for an alternative to the new, existing standards but for the Coast Guard to reopen the rulemaking it had just completed and *change the discharge standard*. The Coast Guard was clear from the beginning that it was very unlikely to open up the rulemaking any time soon.⁵⁵ The appellants persisted in its campaign, however, and the drawn out process that followed is merely the result of a government agency trying its best to hear and try to accommodate a member of the regulated public, within the bounds of law and policy. Many of MSC's procedural recommendations were in direct response to their insistence to be heard, by any means possible.⁵⁶ There were no guarantees that the process would work in the appellants'

favorable way for DESMI. Both the ETV Protocol and the G8 Guidelines define "viable" as "living" and not the other way around. It should also be noted that the Coast Guard's use of the term "viable" or "viability" has changed over time. In the 1990s and early 2000s, the Coast Guard used the term viability because of the state of available science. As technology and knowledge advanced, the Coast Guard developed a more narrow view of "viability" given the unknowns involved with trying to verify it. An agency is allowed to refine its policy position over time and is not bound to archaic or outdated ideas, particularly if the agency has a statutory mandate to base decisions on best scientific information available. I agree that the Coast Guard has considered UV BWMS as part of a suite of BWMS options for a long time, but under the current regulations, a UV BWMS must meet the prescribed technical requirements.

⁵³ 46 C.F.R. § 162.060-5(d)(1) incorporates a specific version of the ETV Protocol, and so this reference would need to be updated.

⁵⁴ I disagree with Hyde's characterization of its interactions with the Coast Guard in late 2012, which paint a picture of dealing with an agency with no corporate knowledge of its own regulations. The Coast Guard had just completed what was a very labor intensive, very high profile rulemaking in which the viability issue was directly considered and addressed. In bureaucratic terms, "the ink wasn't even dry yet." The fact that there may have been internal Coast Guard uncertainty over how to procedurally address Hyde's persistent requests is understandable considering the rulemaking – specifically the type approval program – was in the process of initial rollout and implementation.

⁵⁵ See, e.g., HYDE APPX-000002. Additionally, I find Hyde's criticism, in footnote 3, of the Coast Guard's "delay" to be misleading and without merit. The Coast Guard was waiting on the final decision of the ETV Technical Panel on the MPN issue.

⁵⁶ See, e.g., MSC email, dated 12 February 2015, HYDE APPX 000417. This email begins, "Please submit your request for alternates as equivalent as stated. We'll review your request and determine if a meeting is warranted." This is in direct response to an email from Hyde, dated 10 February 2015, which ended "Alternately, please let us know of your continued refusal of this meeting and we will submit our official 162.060.10(A) and 162.060.10(B) letters by the end of the week." HYDE APPX-000416. MSC's remarks were not an endorsement but an attempt to be responsive to a direct Hyde demand.

favor. Additionally, the Coast Guard does not prohibit the submission of applications even when a Coast Guard employee anticipates it will be denied on the merits.⁵⁷ How can the Coast Guard precisely know what the applicant is requesting if it does not see the actual request? In fact, if the Coast Guard refused to allow the appellants to submit an alternative request, that would have exposed the agency to claims of arbitrary and capricious behavior. The record shows that the appellants was persistent in trying to change the Coast Guard's decision on viability, and the efforts Coast Guard employees went through to provide an answer to them, at their insistence, should not be held against them now.

I will also clear up some confusion over the "ETV process," as Hyde refers to it. The "ETV process" can mean different things. One "ETV process" is the process by which Independent Laboratories (ILs) use the ETV Protocol to conduct testing pursuant to the ballast water regulation requirements.⁵⁸ A different "ETV process" is the process by which the ETV Technical Panel considers new developments in ballast water treatment technology and whether to update or amend the ETV Protocol. In this case, the ETV Technical Panel convened to consider general issues relating to the ETV Protocol, including whether the use of viability as a measurement and the MPN assay as a measurement tool was acceptable, independently of Hyde's November 2012 MPN campaign.⁵⁹ Since MSC knew that this work was underway, it is reasonable that MSC (or CG-OES) would mention it to Hyde and suggest that Hyde wait for the ETV Technical Panel to conclude its work. If the ETV Technical Panel found in Hyde's favor, that decision would provide the basis for future Coast Guard action, including updating the regulations to include viability. It would also be very difficult for the Coast Guard to come to an independent conclusion on viability, as the Coast Guard does not have the same scientific and technical resources as the ETV Panel. Additionally, the Coast Guard never suggested that the ILs or the ETV Technical Panel could decide or approve a 46 C.F.R. § 162.060-10(b)(1) request. However, information from either "ETV process" can certainly be submitted to support a 46 C.F.R. § 162.060-10(b)(1) request.

Finally, I reject DESMI's characterization of the Coast Guard's *Shipboard Technology Evaluation Program* (STEP) program and development of its current ballast water requirements as implicitly accepting the MPN method. As I mentioned earlier, the Coast Guard has regulated ballast water for decades. The Coast Guard ballast water requirements began with a Congressionally-mandated ballast water exchange requirement, discussed *supra*, certain best management practices, and reporting and record keeping requirements. During this time, and in

⁵⁷ See HYDE APPX-000417. "If manufacturers do not want to wait until the ETV technical panel process plays out, they can submit a -10(b)(1) proposal for acceptance of an alternative method...however, that request would have to meet the -10(b)(1) requirements..." This is an objective, likely palliative, instruction that does not take into account specific facts or presuppose that the request will be granted. MSC's following comments provide the specific warning that a "-10(b)(1)" request for MPN would not be simple. Even after receiving a favorable opinion from the ETV Technical Panel (which has not occurred), Hyde would still need to come back to the Coast Guard for further consideration.

⁵⁸ See, e.g., 46 C.F.R. § 162.060-42.

⁵⁹ "Currently, the MPN remains an unapproved method for determining the biological efficacy of the BWMS. The method remains under review by the EPA tech panel and we have no outlook on when an answer may be reached or any indication as to what that answer may be. I would be cautious of conducting testing prior to approval of this method if your system will rely solely on this method to meet the discharge standards...MPN data may be accepted as existing data following testing provided that...the MPN method is accepted as an approved method." MSC email, dated 6 February 2015, HYDE APPX-000413. This warning from MSC is categorical.

accordance with its statutory mandate,⁶⁰ the Coast Guard considered and reviewed various technological alternatives to ballast water exchange. These alternatives included BWMS based on UV technology. The STEP program is “intended to facilitate the development of effective [BWMS] technologies, to create more options for vessel owners/operators seeking alternatives to ballast water exchange...vessel owners/operators have expressed a reluctance to invest the resources to install and operate an experimental treatment system that might not meet discharge standards mandated by future regulations.”⁶¹ In other words, the point of the STEP program is that those BWMS are experimental. The fact that the Coast Guard, in 2008,⁶² accepted a UV BWMS for use on a STEP-enrolled vessel does not mean that the particular UV BWMS, regardless of its efficacy, *meets the regulatory discharge standard*.⁶³ At the end of the day, MSC’s actions were to uphold a regulatory discharge standard in light of a proposed alternative test method and were not an opinion on UV BWMS efficacy.⁶⁴

In sum, I find that MSC did not have the discretion to approve viability as an alternative measurement to the regulatory standards under 46 C.F.R. § 162.060-10(b)(1). The ballast water discharge standard in 33 C.F.R. Part 151 must be read together with the BWMS type approval requirements found in 46 C.F.R. Subpart 162.060 to understand the meaning of the word “living” in the Coast Guard’s ballast water regulations. The type approval requirements do not allow “living” to be substituted with “viable,” and therefore MSC did not have the discretion to approve a testing alternative that would insert viability into the discharge standard. While the appellants argue that certain Coast Guard employees agreed that 46 C.F.R. § 162.060-10(b)(1) could be used to insert viability into the discharge standard, I do not believe the administrative record definitively or specifically proves this assertion. Additionally, the Coast Guard’s regulations and viability policy decision went through the public notice and comment process, and those decisions cannot be changed without returning to the public notice and comment process.

2. The Coast Guard has the statutory and regulatory discretion to reject alternative proposals

⁶⁰ NANPCA/NISA, *supra*, n. 6, § 1101(e); *codified in* 16 U.S.C. § 4711(e).

⁶¹ Navigation and Vessel Inspection Circular (NVIC) 01-04. The STEP program predates the 2012 Final Rule.

⁶² Letter dated October 31, 2008, from M. L. Blair, Captain, U.S. Coast Guard, Office of Operating and Environmental Standards, to Princess Cruise Lines, no. 33.151.2035.0040. This acceptance pre-dated the 2009 NPRM’s publication.

⁶³ A similar reasoning dismisses DESMI’s assertions that the Coast Guard violated the National Environmental Policy Act, Pub. L. No. 91-190, §2, 83 Stat. 852 (1969), as amended, *codified in* 42 U.S.C. §§ 4321, 4331-4335, 4341-4346, 4346a, 4346b, 4347. The purpose and need of the Coast Guard’s 2012 Final Rule Final Environmental Impact Statement (FEIS) is to provide “an assessment of the potential environmental impacts associated with the proposed establishment of a ballast water discharge standard. The standard would be used to approve alternative ballast water management methods that are effective in preventing or reducing the introduction of nonindigenous species via discharged ballast water into the waters of the United States.” FEIS Appendix F, which lists BWMS enrolled in the STEP program, is meant to provide a “rational basis” that BWMS exist that could achieve the discharge standard. The FEIS was not intended to prove that any particular system met the type approval standards established in 46 C.F.R. Subpart 162.060. Additionally, it is clear from the description of each of the alternative concentration levels considered as being “living organisms (per volume)” and in the description of UV BWMS assessed, that the Coast Guard examined the impacts of the regulatory discharge standard in the context of killing organisms (*see, e.g.*, Pages 2-5 and 2-6 and Appendix F).

⁶⁴ In fact, the Coast Guard still considers UV BWMS a valid ballast water treatment technology and believes that UV BWMS can be type approved under the existing regulatory requirements.

DESMI and the other appellants contend that MSC's rejection of their applications for regulatory alternatives was tantamount to failing to consider them and that such failure was arbitrary, capricious, and inconsistent with law. As an initial matter, I believe the appellants confuse "to consider" with "to approve." It is clear from the record, including MSC's rejection letter of December 14, 2015, that MSC considered DESMI's application. As discussed *supra*, MSC found that DESMI's application failed to meet the second and third prongs of 46 C.F.R. § 162.060-10(b)(1). The fact that MSC ultimately denied DESMI's application does not mean MSC did not consider it. If there were any defect in MSC's consideration,⁶⁵ I cure it now by independently finding that DESMI's application fails to meet the requirements of 46 C.F.R. § 162.060(b)(1). That reasoning is provided in Section 3, *infra*.

DESMI and the other appellants make various legal arguments asserting various levels of Coast Guard discretion or mandate to accept alternatives to the Coast Guard regulatory requirements. The Coast Guard does not dispute that it has the discretion, in theory, to accept viability as a BWMS efficacy measurement.⁶⁶ The Coast Guard also has not permanently rejected MPN as a BWMS measurement tool by a regulatory change. The 2012 Final Rule and its 2009 Notice of Public Rulemaking (2009 NPRM)⁶⁷ were very clear that the 2012 Final Rule is an interim phase of ballast water treatment and management. At that time, the Coast Guard did not have sufficient information to include viability as an approval criterion. However, NANPCA/NISA contains a mandate which requires the Coast Guard to periodically review and revise its ballast water regulations based on the best scientific information available.⁶⁸ If and when the Coast Guard has such information, it can reconsider whether to include viability. As this criterion would differ from the existing regulatory text and policy established through public notice and comment, this change would need to go through public notice and comment rulemaking.

While I agree, in principle, about the Coast Guard's discretion to accept viability, there are some very important statutory and regulatory limitations that these arguments raise that must be addressed within this response.

⁶⁵ MSC should have waited for the final results of the independent analysis before denying the application. We now have the final results, and my decision is based on those results.

⁶⁶ As a counterpoint to the appellants' arguments that NANPCA/NISA mandate that the Coast Guard accept viability by virtue of the NANPCA/NISA definition of "nonindigenous species" including the word "viable," Congress enacted the assumption that vessel water treatment systems should "kill" aquatic nuisance species: "provide an exemption from ballast water exchange requirements to passenger vessels with...treatment systems designed to kill aquatic organisms in ballast water..." NANPCA/NISA § 1101(c)(2)(K), 16 U.S.C. § 4711(c)(2)(K) (emphasis added). "Viable" is used in this definition to cover things like viruses, which are not universally considered to be "living." In any event, NANPCA/NISA is based on a precautionary rather than prescriptive framework. That means that the Coast Guard has the authority, under "maximum extent practicable," to regulate the concentration of both indigenous and nonindigenous, as well as invasive and noninvasive, species in ballast water in order to prevent the introduction and spread of aquatic nuisance species. For example, ballast water exchange does not discriminate among "viable" or "nonviable" organisms.

⁶⁷ See, e.g., Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters, Notice of Proposed Rulemaking, 74 Fed. Reg. 44632 at 44634 – 44635 (Aug. 28, 2009).

⁶⁸ NANPCA/NISA, *supra*, n. 6, § 1101(e)(1)(D); *codified in* 16 U.S.C. § 4711(e)(1)(D). The Coast Guard also committed to a regulatory obligation to conduct a practicability review for a more stringent standard, initiating a rulemaking by 2017 if appropriate. See, e.g., 33 C.F.R. § 151.1511.

The Coast Guard interprets NANPCA/NISA Section 1101 (16 U.S.C. § 4711) differently than DESMI and the other appellants. In particular, the Coast Guard disagrees with the applicability and interpretation of Section 1101(c)(2)(D) and believes Section 1101(e)(1) expressly or constructively “trumps” Section 1101(c)(2)(D).

Section 1101 can be difficult to understand if it is not read in the context in which it was originally enacted and subsequently amended.⁶⁹ When NANPCA was amended to include voluntary guidelines for the entire United States (Section 1101(c)(2)), the specific mandates in Section 1101(c)(2)(D) were for those initial voluntary guidelines:

The voluntary guidelines issued under this subsection shall-

- (D) direct a vessel that is carrying ballast water into waters of the United States after operating beyond the exclusive economic zone to-
- (i) carry out the exchange of ballast water of the vessel in waters beyond the exclusive economic zone;
 - (ii) exchange the ballast water of the vessel in other waters where the exchange does not pose a threat of infestation or spread of nonindigenous species in waters of the United States, as recommended by the Task Force under section 4712(a)(1) of this title; or
 - (iii) use environmentally sound alternative ballast water management methods, including modification of the vessel ballast water tanks and intake systems, if the Secretary determines that such alternative methods are at least as effective as ballast water exchange in preventing and controlling infestations of aquatic nuisance species...

In other words, there was no existing nationwide standard, and Congress provided an initial *minimum* or *floor* for the Coast Guard⁷⁰ to meet. The Coast Guard’s initial guidelines were based on the framework contained in Section 1101(c)(2)(D) and included guidance on conducting ballast water exchange for vessels carrying ballast water into waters of the United States after operating beyond the U.S. exclusive economic zone.⁷¹ Eventually, the Coast Guard converted its voluntary guidelines to mandatory, regulatory requirements under the mandate contained in Section 1101(f)(1).⁷² These regulations were also based on the framework contained in Section 1101(c)(2)(D), as ballast water exchange remained the best available ballast water management option.

However, Section 1101(e)(1) requires the Coast Guard to consider revising its ballast water regulations no less than every three years. After conducting this periodic review, the Coast Guard is required under Section 1101(e)(1) to amend its ballast water regulations if, based on the

⁶⁹ Please refer to the background section of this response for this discussion.

⁷⁰ The Act refers to the “Secretary,” defined as the Secretary of the department in which the Coast Guard is operating. For simplicity, I will instead refer to the Coast Guard, as the properly delegated entity of the Department of Homeland Security.

⁷¹ See Implementation of the National Invasive Species Act of 1996 (NISA), Interim Rule, 64 Fed. Reg. 26672 (May 17, 1999).

⁷² See Mandatory Ballast Water Management Program for U.S. Waters, Final Rule, 69 Fed. Reg. 44952 (July 28, 2004).

best scientific information available, the existing guidelines and regulations implementing Section 1101(c) do not effectively reduce the introduction and spread of aquatic nuisance species by vessels. The Coast Guard's 2012 Final Rule was the result of an on-going review that began almost immediately after promulgation of the initial voluntary guidelines for ballast water exchange, and continued through participation in the development of the IMO ballast water management convention and development of test protocols for BWMS, and the best scientific information available showed that some BWMS were more effective than ballast water exchange in reducing the introduction and spread of aquatic nuisance species. While the Coast Guard initially characterized its new requirement of BWMS as an approval of an "environmentally sound alternative ballast water management method" under Section 1101(c)(2)(D)(iii),⁷³ the Coast Guard later explained its reasoning that subparagraph (c)(2)(D) merely set forth initial ballast water requirements for certain vessels and it was acting under the broader mandates found in paragraphs (a) and (e).⁷⁴ To read Section 1101 as permanently binding the Coast Guard to the initial floor set by Section 1101(c)(2)(D) would render Sections 1101(c)(2)(A) and 1101(e)(1) meaningless.⁷⁵ The BWMS manufacturers' argument that the Coast Guard is bound to implement all of Section 1101(c)(2)(D) is even more perplexing considering it would mean the Coast Guard has no discretion to phase out ballast water exchange in favor of BWMS.⁷⁶ The Coast Guard has moved beyond the initial mandate contained in Section 1101(c)(2)(D) to the more stringent mandate contained in Section 1101(c)(2)(A), which requires the Coast Guard to ensure, to the maximum extent practicable, that aquatic nuisance species are not discharged into the waters of the United States from vessels.⁷⁷

⁷³ See, e.g., 2009 NPRM, 74 Fed. Reg. 44633.

⁷⁴ 77 Fed. Reg. 17282, 17286. This analysis also explains why the Coast Guard believes it has the authority to require all vessels equipped with ballast tanks – and not just those that have operated beyond the exclusive economic zone – to comply with its ballast water management requirements. The Coast Guard has previously, and publicly, rejected the legal argument that Section 1101(c)(2)(D) contains specific requirements which control "broader" requirements in Section 1101.

⁷⁵ I note Trojan's argument that NANPCA/NISA mandates the Coast Guard use "best science available," which Trojan evidently believes requires the Coast Guard to perpetually amend its equipment standards without going through a public notice and comment rulemaking. The Coast Guard properly incorporated the ETV Protocol into its regulations, as it does for many other vessel equipment standards such as those from the International Organization for Standardization. To suggest that the Coast Guard can "undo" a proper regulatory incorporation by reference without a notice and comment rulemaking is untenable. Additionally, the Coast Guard used "best science available" at the time it promulgated the 2012 Final Rule, four years ago. The Coast Guard is now completing its NANPCA/NISA-required periodic review, in which it is considering any new, properly validated scientific information. That information will inform whether to amend the Coast Guard regulations. Vessels must be able to keep up with changing equipment standards or the Coast Guard would not be maintaining NANPCA/NISA's "maximum extent practicable" mandate.

⁷⁶ Even reading Section 1101(c)(2)(D) alone, without reference to any other parts of NANPCA/NISA, cannot support this conclusion. Section 1101(c)(2)(D) is formed in the disjunctive, allowing the Coast Guard to choose all or only one of the options under it, while it was still operative. DESMI and Alfa Laval may have used a Coast Guard preambular statement out of context on page 12 of their respective appeals. The quoted statement, from the ballast water NPRM, meant that any alternatives to ballast water exchange must be approved by the Coast Guard, not that the Coast Guard was required to approve all alternatives to ballast water exchange.

⁷⁷ For this reason, Trojan's argument about accepting a BWMS as an alternate management system (AMS) fails. There is a difference between the initial standard of "at least as effective as ballast water exchange," which is consistent with the purpose of AMS – a bridging strategy between ballast water exchange and BWMS – and an evolved "maximum extent practicable" standard. Type approved BWMS must meet the *discharge standard*, not meet the floor of "at least as effective as ballast water exchange."

Thus, while I generally agree with the appellants' arguments that NANPCA/NISA gives the Coast Guard the discretion to consider and accept viability, I do not agree with all of their interpretations and analysis of NANPCA/NISA § 1101.⁷⁸ NANPCA/NISA does not "mandate" that the Coast Guard accept viability unless and until viability falls within the Coast Guard's responsibility to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels. Given the then-current state of science and technology, discussed *infra*, live/dead was the most environmentally conservative and practicably achievable standard, and viability does not yet fall within this mandate.

3. DESMI's application fails to meet all elements of 46 C.F.R. § 162.060-10(b)(1)

I find that DESMI's application did not meet the minimum requirements in 46 C.F.R. § 162.060-10(b)(1) and confirm MSC's denial of DESMI's alternative request.

This decision is based, in part, on the technical review and conclusions of the Naval Research Laboratory (NRL),⁷⁹ whom the Coast Guard contracted to review the technical aspects of the alternative request submitted by Trojan.⁸⁰ The NRL review resulted in a comprehensive evaluation and the report includes more detailed descriptions and comments, not all of which were considered pertinent to the narrow issue of the 46 C.F.R. § 162.060-10(b)(1) requirement. I find the NRL report persuasive, and summarize my agreement with it as follows:

The applicants request that Coast Guard approve as equivalent an alternative to the required test specified in the ETV Protocol for determining the concentration of living organisms in the 10-50 micrometers (μm)⁸¹ size range in samples of water during type approval testing.⁸² The proposed alternative method is composed of two separate procedures, one (based on "viable") for autotrophic (photosynthetic) organisms, and one (based on "living") for heterotrophic organisms.

⁷⁸ I also reject Trojan's argument that NANPCA/NISA does not apply to non-reproductive organisms, and therefore the Coast Guard lacks the authority to promulgate the existing discharge standard. (Trojan appeal, page 45 – 46). NANPCA/NISA's reference to aquatic nuisance species, of course, concerns the species prior to treatment in a BWMS.

⁷⁹ NRL, "Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(B)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{M}$ and $<50 \mu\text{M}$," Feb. 10, 2016, 3900 Ser 6130/1622.

⁸⁰ The NRL reviewed only one 46 C.F.R. § 162.060-10(b)(1) application: Trojan. Nonetheless, the NRL report on Trojan is relevant to all four applicants. Three of the four applicants (Trojan, DESMI, and Alfa Laval) submitted a base set of the same six documents containing a description of the proposed test, an overview of the use of the most probable number approach for evaluating BWMS, and results of various experiments conducted during development of the method, including several in which the alternative and required methods were compared. Hyde submitted only three of the six documents. Trojan also submitted additional documentation. Thus, Trojan's application was the most comprehensive of the four and covered all of the evidence provided by all four applicants. If Trojan's application fails to meet 46 C.F.R. § 162.060-10(b)(1), then all four fail. I have also included a discussion of arguments (vice documentation) of the individual applicants.

⁸¹ A micrometer equals 0.001 millimeter.

⁸² I also note that all four applicants relied on the recent work of the ETV Technical Panel in their respective 46 C.F.R. § 162.060-10(b)(1) requests. While the appellants characterize their MPN-based method as being singular (i.e., "the MPN method") and in use for decades, the record shows that the "MPN" method they submitted for alternative approval is the preliminary draft MPN-based method developed through the work of the ETV Technical Panel, which has yet to be validated (i.e., the specific submitted method was developed after the testing was conducted for their foreign type approvals).

The autotroph procedure is based on using a “grow-out” approach, wherein samples are serially diluted, and replicate tubes at each dilution are cultured for a period of time, and then assayed for population growth of phytoplankton by detecting changes in the concentration of chlorophyll. The pattern of tubes with and without positive population growth is then used to estimate the probable original concentration, through a calculation termed MPN. The MPN statistical approach has been used for over a century to quantify monocultures (single species) of bacteria and phytoplankton, and several automated “calculators” are available for use. Significantly, the applicants propose to use MPN as the basis for quantifying concentrations of mixed assemblages of phytoplankton, composed of numerous different species in varying relative abundance.⁸³ This is a “new” use for MPN as a statistical approach under a regulatory context and has not been adequately validated for such purpose.

The heterotroph procedure, for heterotrophic organisms, uses microscopy to count numbers of non-photosynthetic organisms that are motile, where movement is the criterion for determining an organism is alive. This method is conceptually similar to the required method, in that direct counts of “living” (not “viable”) organisms are made using a microscope. However, the specific procedures for determining whether an organism is “living” and the type of microscope are different than specified in the required method.

Compliance with 46 C.F.R. § 162.060-10(b)(1):

A. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?

I find that the applicant meets the first prong of 46 C.F.R. § 162.060-10(b)(1). DESMI’s request to use the MPN assay is a request for an alternative to the testing requirements of the ETV Protocol, incorporated by reference into 46 C.F.R. § 162.060-26 and 46 C.F.R § 162.060-28, which are referred to in 46 C.F.R. § 162.060-10(f)(2).

B. Is that evaluation, inspection or test not practicable or applicable?

I find that DESMI has not provided sufficient justification that the testing requirement is not practicable or applicable.⁸⁴ Three applicants argued, in various ways, that the required method was not practicable and/or not applicable for evaluating the efficacy of UV-based BWMS. Some of the arguments were not always clear whether they were being made on the basis of practicability or applicability, and so for this review I consider the two issues to have been combined, and I review the appellants’ arguments together. The applicants essentially make a two-part argument: first, that the phrasing of the discharge standard should be in terms of, or include, “viable” (or reproductive) organisms instead of, or in addition to, “living” organisms as currently phrased in the discharge standard; and second, that the required method for

⁸³ I will refer to the applicants’ approach as the “alternative method.” I note that there is no globally accepted “MPN method” for BWTS type approval. While the applicants used the same MPN-based method, it appears that MPN approaches vary across flag administrations.

⁸⁴ I agree that MSC’s conclusion on practicability was not sufficiently justified. For that reason, I vacate the practicability decision of MSC and reach my own decision.

enumerating “living” organisms in the type approval requirements is not applicable to BWMSs intended to render organisms non-viable.

Only one applicant (Trojan) offered any objective basis to argue that the required method was not practicable (Miller 2015d). Trojan essentially argued that it is too expensive to use UV to kill organisms rather than render them non-reproductive, and so UV should be used to render organisms non-viable. This, according to Trojan, would provide an equivalent level of protection for the environment as killing them, because non-viable and dead organisms represented the same level of risk reduction with respect to biological invasions. Rather than supporting an argument that the required method is not practicable for evaluating numbers of living organisms, I view this as an argument that UV is not practicable for killing organisms in ballast water. Under the current type approval requirements, BWMS are required to be tested to demonstrate efficacy in reducing numbers of “living” organisms below concentrations in the discharge standard, and to do so using specific test methods that measure numbers of “living” organisms. As explained, *supra*, the Coast Guard cannot grant a waiver to the “living” requirement under the current regulations.

Furthermore, the proposed alternative method includes an assessment of “living” organisms in the case of heterotrophic organisms, which undermines the contention that the required method, which assesses “living” organisms is impracticable or inapplicable. In the proposed alternative method, a culture-based viability assay is used to assess viable autotrophic organisms (organisms capable of synthesizing their own food from inorganic substances using light or chemical energy; e.g., plants and algae) while a “living” assay is used for heterotrophic organisms (organisms that cannot manufacture their own food and instead obtain food and energy by taking in organic substances, usually plant or animal matter; e.g., animals and fungi).⁸⁵ DESMI does not explain why it is practicable and applicable to evaluate “living” organisms when assessing the efficacy of UV systems in treating heterotrophic organisms, but it is not when the focus is autotrophic organisms. This inconsistency must be explained.⁸⁶

Specific to Hyde, I disagree that the required method for assessing the concentration of living organisms in treated ballast water is not applicable to UV-based BWMS. To make this assertion, Hyde is forced to define the term “living” in the discharge standard to mean viable or reproductive. As I explained, *supra*, I disagree that this is an appropriate definition of “living.” The Coast Guard intentionally used the broader term “living” because Coast Guard considered it less difficult and problematic to distinguish living organisms from dead organisms than to distinguish between reproductive and non-reproductive living organisms. While Hyde argues

⁸⁵ The required method is used to enumerate both of these two components of the size group.

⁸⁶ DESMI makes a similar argument against the Coast Guard, stating that for organisms in the <10 μ size class, tests for only 3 indicator species are allowed. This argument conflates the ETV Protocol with the overarching Coast Guard regulatory standard, found in 33 C.F.R. §§ 151.1511 and 151.2030. There is no “<10 μ size class.” The Coast Guard regulations set forth specific requirements for those three indicator microorganisms, and they are not ‘indicators’ for a size class. DESMI also points to the Coast Guard’s incorporation of the option to use water from a U.S. public water system for ballast water management as evidence that MPN is acceptable. However, this point proves the opposite: the Coast Guard *limited* the option to *only* U.S. sources because of the difficulties in determining water quality from foreign sources. In the case of the United States, the regulatory requirements for these facilities are known, uniform, and verifiable. Additionally, water treatment facilities use MPN-based methods to estimate abundances of *specific* well known species, not the thousands in ballast water.

that conceptually, a non-reproductive organism represents the same risk of a biological invasion as does a non-living organism, and I do not disagree with that conceptual perspective, Hyde does not successfully argue that a reliable method exists for determining the reproductive ability of the thousands of species occurring in ballast water (this is discussed further below regarding the technical aspects of the proposed method).

For these general reasons, I find that the applicant's assertion that the required method is not applicable to evaluating UV-based BWMS is unsubstantiated.

I address the following specific points raised by DESMI:

- i. *UV treatment of ballast water does not outright kill organisms in the water, but destroys their DNA and RNA making them incapable of reproduction.* This is a decision made by treatment system manufacturers on the basis of economic considerations, and reflects the specific, and differentiated from shipboard, circumstances of drinking water, food safety and waste water concerns. UV can have a biocidal (i.e., killing) effect. Currently the type approval testing requirements specify measurement of numbers of living organisms as determined by the specific method described in the ETV protocol. This method is as appropriate for enumerating living organisms in UV-treated water as it is for the same purpose in water treated by other means.
- ii. *Reproductive capability is central to the definition of invasive species laid out by the US Federal Aquatic Nuisance Species Task Force in 2012. It follows that organisms incapable of reproduction cannot become invasive species.* A 46 C.F.R. § 162.060-10(b)(1) analysis does not consider the definition of "invasive species" or whether organisms that are incapable of reproduction cannot become invasive species. Rather, this section is concerned with the method(s) accepted by the Coast Guard under these equipment type approval regulations.
- iii. *Destruction of organisms' ability to reproduce therefore grants the same protection against aquatic invasive species as is obtained by killing the organisms.* The crux of the matter is the degree to which "ability to reproduce" can be determined for all of the different kinds of organisms in ballast water. I do not believe that there is currently an acceptably validated method that can be used to consistently assess whether organisms in treated ballast water are capable of reproduction.
- iv. *The FDA/CMFDA staining method prescribed in the ETV protocol for testing of ballast water treatment does not show whether an organism is capable of reproducing or not.* I do not dispute this assertion. However, if it is not practicable to adequately determine whether living organisms in treated water are able to reproduce, then limiting the number of "live" organisms includes both viable and non-viable individuals, and so provides a conservative, more protective limit.⁸⁷

⁸⁷ 77 F.R. 17274 ("Finally, it is more conservative, and thus more protective, to base efficacy decision on the basis of live/dead, rather than viable/unviable.").

- v. *Therefore, the FDA/CMFDA method is not well suited for assessment of the effectiveness of a UV based ballast water treatment system.* The relevant question is whether the required FDA/CMFDA method in the ETV Protocol is suitable for assessing effectiveness of a BWMS in meeting the “live” criterion in the USCG discharge standard, not whether it is well suited for a BWMS meeting some other discharge standard.

- vi. *On this basis it is concluded that:*
 - a) *The method contained in the ETV protocol for organisms in the 10-50 micron size category is not practicable or applicable to evaluate the performance of UV based ballast water treatment systems.* I disagree. DESMI frames practicability in terms of measuring something (“viable”) that is not pertinent to the actual standard, which is framed in terms of “live” organisms. Again, the required method is practical for enumerating the number of living organisms in a sample of ballast water. The applicant desires to be held to a different end-point – not “living” but instead “able to reproduce”.

 - b) *The proposed alternative test method provides same level of protection against invasive species, and is equivalent to existing requirements in terms of accuracy.* This is irrelevant to the issue of whether the required method is practical or applicable in the case of assessing the effectiveness of a BWMS in meeting the discharge standard. This point is addressed, *infra*, in regard to whether the proposed alternative is equivalent to the requirement.

I also address the following specific points raised by Hyde (HMI 2015):

- i. *“The inherent nature of the proposed staining technique measures the capability of organisms to hold fluorescence rather than to provide a measurement of “living/dead”...”* The fluorescent markers in the required method, as well as many other fluorescent markers specific for other biochemical characteristics of living organisms, are widely used in biology to differentiate living from dead.

- ii. *“...all scientific definitions of the word “living” include the ability to reproduce”.* This assertion is a gross mischaracterization of the definition of “living” in the context under discussion, which is how one can differentiate between a living organism and a dead organism. The context for the assertion is the higher-level issue of differentiating between “living” things (i.e., organisms) such as animals, plants, fungi, bacteria, etc and “non-living” things (e.g., rocks, water, fire, etc), wherein living things are defined in part as those things that exhibit reproduction at some time. Such an overarching definition is not pertinent to the issue at hand, as the required method is used to differentiate “living” organisms from “dead” organisms, not organisms from rocks (although the fluorescent “signal” from the marker does assist in seeing living organisms within the jumble of nonliving material such as bits of rock, shell fragments, and plant detritus.) Many “living” organisms are not capable of reproduction (adult humans who have undergone sterilization procedures; sterile

castes in social insects, mules, female mammals that have reached menopause, etc). Viable organisms are by definition included in the larger sub-set of 'living' organisms (because an organism must be alive to reproduce). If a reliable procedure can be identified by which viable individuals of thousands of different species can be consistently discriminated from non-viable individuals, then such methods could be included in the approved methods for determining numbers of living organisms through amendment of the regulations. To avoid reducing the protectiveness of the discharge limits, any such viability assessment must also have levels of accuracy and precision equivalent to that of the method used to determine the number of living organisms.

- iii. *"UV as a disinfection product sterilizes organisms by disrupting their DNA and interrupting their ability to reproduce"*. The use of UV as a disinfection process is not the issue. The issue is whether UV has been, or can be, demonstrated to be an effective disinfection process for the vast number of different species in the 10-50 μm size group found in ships' ballast water. UV is generally used to render organisms non-reproductive because it is cheaper than using UV to "kill." Using UV to render organisms non-reproductive, rather than to kill, depends on a good understanding of the specific UV dose necessary to render specific organisms incapable of reproduction. This has been done for a relatively small subset of organisms that are either human pathogens (e.g. *Vibrio cholera*, several species of the genus *Cryptosporidium*, *Hepatitis A*, etc) or indicators of poor water quality due to unsanitary conditions (e.g., *Escherichia coli* and several species of *Enterococcus*) that can be associated with the presence of such pathogens). By contrast, there are many thousands of species carried in ships' ballast water, and little or nothing is known about the specific UV doses required to render these permanently incapable of reproduction, nor is there a good understanding of how these many species could be consistently cultured in the laboratory to detect viability.
- iv. *In order not to eliminate UV disinfection in ballast water, a reproductive measurement assay must be used.* This is an argument based on economics, not the question of whether the required method is applicable to testing whether UV-based technologies are effective in achieving the limits on concentrations of living organisms.
- v. *"...in any disinfection enumeration, looking at the reproductive capability of the organisms studied is a more accurate representation of effectiveness than looking simply at their ability to hold a stain"*. This statement is only true for those disinfection processes intended to render organisms non-reproductive. For a process intended and designed to kill organisms, the appropriate approach in measuring effectiveness would be to identify the numbers of living organisms. The assertion that the required method "simply" looks at the ability of organisms to "hold stain" is a gross over-simplification and ignores completely the broad use of vital markers to identify "living" cells.

- vi. *“The international testing community and US EPA have historically used reproductive assays in lieu of staining to demonstrate the effectiveness of UV systems in ballast and other applications”.* The “international testing community,” meaning the test facilities conducting tests of BWMS for foreign administrations, may have used, collectively, a range of methods that involved reproductive assays, but these have not been made generally available to the public, including in particular the documentation demonstrating the careful validation of culture-based methods. Several different “methods” have been used by different facilities, and the validations that these methods were able to measure what they were intended to measure, or the comparisons among methods, have never been made public; indeed, at least one test facility has long asserted that the details of its methods are “proprietary”. When the ETV panel first began to consider the issue of whether an acceptable viability assessment could be identified for use in testing BWMS, the various test facilities and manufacturers were unable to provide or point to any specific validations, and had to subsequently conduct much of the validation work submitted (after much testing of BWMS had been completed) in support of the method being developed within the ETV Program, a draft of which was submitted to the Coast Guard for consideration under the -10(b)(1) provision.

I address the following specific points raised by Trojan (Miller 2015d):

- i. *The existing method is not practicable for evaluation of UV-based BWTS. The current requirement for analysis of the organisms in the 10-50 μ m size class in the ETV protocol requires the use of FDA/CMFDA stains to categorize organisms as being alive or dead. The stains evaluate the functioning of an organism’s esterase system as a proxy for cell death. Treatment with UV irradiation, causes damage that prevents cell replication (and thus precludes invaders from colonizing), but esterases are not directly affected at UV doses typically employed for disinfection, and thus effective treatment by UV is not evaluated by the existing method.* Trojan asserts that the required method is not practicable for evaluation of UV-based BWMS that are intended to render organisms non-reproductive rather than dead. I do not dispute that the staining methods evaluate the functioning of an organism’s esterase system rather than the ability of an organism to reproduce, but the issue is that the discharge standard is not phrased in terms of “non-reproductive” or “non-viable” organisms, but instead is phrased in terms of “living” organisms. At the time of the 2012 Final Rule, the Coast Guard believed that there were significant difficulties associated with determining the reproductive ability of the thousands of species of organisms found in ships’ ballast water. Because of those difficulties, the Coast Guard determined that it was more practical and protective of the environment to phrase the standard in terms of living organisms. Concurrently with establishing the discharge standard, the Coast Guard established the required methods by which numbers of living organisms would be determined during type approval testing. The required method for organisms in the 10-50 μ m size class is the FDA/CMFDA fluorescent marking method specified in the ETV Protocol. The applicant has not provided an adequate argument that the required method is not practicable for evaluating the number of living organisms in ballast water.

- ii. *A BWTS designed for UV doses equivalent to ten (10) times the current UV doses employed in the industry would require 10X the number of UV lamps, and would therefore be approximately 10X the footprint and require 10X the electrical power for operation. This increase would render UV systems impractical for implementation on board vessels.* Rather than making an argument that the required method is not practicable for enumerating living organisms after UV treatment, Trojan essentially argues that the required method is not applicable to UV treatment when such treatment is intended to render organisms non-reproductive. In essence, Trojan does not present an argument that the required method is not practicable, but rather that it is not practicable to use UV treatment to achieve the required discharge standard. Trojan argues that the UV doses commonly used in BWMS that are intended to render organisms non-reproductive are not great enough to induce mortality during type approval testing. Increased UV doses would not only prevent reproduction or cellular division, but if sufficient, also induce mortality that is detected following treatment using the required method. Thus, the required method would indicate treatment efficacy in meeting the “live” discharge standard because the dead cells would not fluoresce green. An alternative method would not be needed.
- iii. The applicant asserts that it is not practicable to increase the UV dose so treated cells are dead (as determined by the required method) rather than not viable (as determined by the alternative method). The applicant provides no data on the actual UV dosages necessary to kill the many species of organisms found in ballast water. Using derived ratios of UV doses needed to kill to doses needed to render organisms non-reproductive (no actual doses or dose-response curves were provided) from a study using 12 species of algae, the applicants argued that a UV dose sufficient to damage cells’ non-specific esterases—the foundation of the required method—and reduce concentrations of algae by 100-fold (from 1000 cells mL⁻¹ to 10 cells mL⁻¹) would be “extremely high”. The study found that for the 12 species, on average, a 10-fold increase in UV dose was needed to kill cells using the required method compared to the dose needed to show cells were non-viable using a culture-based viability assay. However, no data on actual dosages required to kill organisms or the power required to achieve those dosages were provided, so it is not possible to objectively evaluate this claim.

Consequently, I find that none of the applicants provided an adequate justification that the required method was not practicable and/or not applicable for its intended purpose when used to evaluate the efficacy of BWMS that used UV to treat organisms in the 10-50 µm size range in ballast water to meet the discharge limits set in the Coast Guard’s March 2012 Final Rule.

C. Is the proposed alternative equivalent to the regulatory standard?

I find that DESMI has not justified the proposed alternative’s equivalency to the requirement. “Equivalence” among testing methods, in a literal sense, entails different methods of achieving the same measurement. A simple example would be measuring the length of an object by using a ruler in a specific manner or taking a photo of the object and analyzing length using an image

analysis software program (in which case the program converts pixels along the delineation into length, based on a user-supplied calibration between pixels and length). In such a case, the methods result in measurement of the same thing (in the example, length). In a more technical sense, “equivalence” among testing methods⁸⁸ means that the methods must have the same or very similar accuracy (closeness to true value) and precision (degree of repeatability under unchanged conditions).⁸⁹

I have already found that the proposed alternative is not “equivalent” in the literal sense, and MSC did not have the discretion to use 46 C.F.R. § 162.060-10(b)(1) to rewrite the discharge standard. Even if the Coast Guard could accept viability without going through public notice and comment, which it cannot, the UV applicants’ alternative would still fail for technical reasons.

To determine if the alternative “viable” and required “live” methods are equivalent, a proposal for the alternative must demonstrate equivalent accuracy and precision of measurement – even if the actual parameters being measured are different. Precision can be assessed within a method, but accuracy is usually evaluated through comparison with a “true” value.

Accuracy or Agreement

“Accuracy” means that the two methods should return the same result in terms of closeness to a known or “true” value. In this case, we have no way of knowing the “true” concentration that is independent of the methods being evaluated. In other words, how do we count the organisms without using the methods under consideration? In the absence of a way to evaluate accuracy, several approaches were taken to evaluate the degree of agreement between the proposed alternative and the required method in determining the number of living and viable organisms.⁹⁰

The applicants analyzed three subsamples of each of three concentrations of the cultured phytoplankter *Tetraselmis suecica*, using both the required and the autotroph (MPN) methods (Miller and Petri 2015).⁹¹ In this experiment, the cultures of the alga were healthy and robust,

⁸⁸ I will assume for the sake of argument that “viable” and “living” relate equivalently to the risk of biological invasion. However, I do not reach a conclusion on that issue.

⁸⁹ Neither Hyde nor Alfa Laval included any additional arguments for accuracy or precision in their respective alternative requests, outside of the included documentation. DESMI did not provide direct examination of either accuracy (agreement) or precision, outside of its included documentation. DESMI presented information on the relative potential for “false negative” results in the required and proposed alternative approaches. While false negatives in the required method (living organisms that are not motile and do not stain) and proposed alternative (viable autotrophic organisms that do not reproduce under the provided conditions and living heterotrophic organisms that are not motile) are contributors to potential differences in agreement and precision, they are not the sole sources of error.

⁹⁰ “Agreement” means that the proposed alternative method provides at least the same result when circumstances are such that the results should be the same. In the supporting material, the applicants provided results from an experiment in which the two methods were used to measure the concentration of samples in which all cells were likely both living and viable. In such case, the two methods should result in the same numbers. If, as in this case, accuracy cannot be evaluated because there is no independently derived “true” value against which to assess the methods, then agreement can be used to evaluate equivalency.

⁹¹ *MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 µm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology*. Only autotrophs were used; no heterotrophs were used in this case. Since all organisms were phytoplankton and presumably the phytoplankton were capable of growth (since they were in culture, under

and it could be expected that all or most of the living cells were likely also viable. To evaluate agreement between the two methods, the applicants calculated the mean of the three subsamples using the autotroph method divided by the mean concentration determined using the required method, multiplied by 100 (the required method was considered the benchmark for this comparison). The value (converted to a percent) would be 100% if both methods yielded the exact same concentration. Because the “true” concentration is unknown, this metric actually quantifies the agreement between the required and alternative methods (rather than accuracy, which is the term used in the proposal).

In this test, where most if not all the cells were both living and viable, the percent difference in measured concentration between the required and alternative methods ranged from 38% difference at a concentration of 1000 cells/mL to 412% difference at the lowest concentration of 10 cell/mL. In other words, in a circumstance where one would expect the results to be the same, the result using the alternative was at best 38% different, and at worst 412% different than the result using the required method. It is particularly concerning that the percent difference was so great at the lowest concentration, which is essentially at the level of the discharge standard. However, due to the high degree of variability in results (6 of 9 means for the required test had standard deviations less than 10, while 6 of the 9 means for the alternative test had standard deviations greater than 46), it is difficult to draw any further conclusions from this test regarding how agreement between methods might vary as a function of concentration.

The applicants also evaluated the agreement between the alternative method and the required method when all of the organisms in the samples were heterotrophic. Cultures of the rotifer *Brachionus plicatilis* were evaluated using the heterotrophic component of the alternative method and direct counts using a stereomicroscope; in both of which, movement of organisms was the parameter used to assess whether the organisms were living. There was no difference between the two methods in the concentration of rotifers. However, in this experiment, the alternative heterotrophic method was not compared to the actual required method, which uses epifluorescence microscopy to detect positive reactions of two vital stains and cell movement. For a legitimate comparison, both the heterotroph and required methods must be used.

When using the two methods to assess samples of ambient communities (Miller *et al.*, 2015b), the applicant examined the agreement between methods using a calculated “factor of agreement” (FOA, calculated as the average alternative concentration/average required concentration). The FOA varied widely among different experiments, from 0.47 to 33.8. Half of the 16 comparisons had an FOA of less than one, and half had values greater than one; six of the latter values were greater than 5.0. It is difficult to draw any conclusions from this experiment, given the possible sources of confounding effects: the MPN counts could have been reduced due to the presence of viable but non-culturable species, or increased due to the presence of organisms smaller than 10 μm , while the stain counts could have been depressed by the presence of non-motile, non-staining organisms that were nonetheless living (false negatives). These potentially confounding effects would have to be carefully partitioned in experiments using ambient organisms, and that would entail a significant amount of work. Alternatively, consistently culturable species known to take up the fluorescent stains of the required method and larger than 10 μm in size could be

favorable light and nutrient conditions), there was no need to perform the heterotroph method in addition to the autotroph method to determine the total number of organisms.

used, in laboratory tests using mono- and mixed cultures, to evaluate the degree of agreement between the two methods. The need for statistical adjustments for the presence of viable but non-culturable species would need to be evaluated and possibly developed and incorporated into the method, and the effect of contamination by organisms less than 10 µm in size would also need to be addressed. Even with pre-filtration to remove organisms less than 10 µm, some contamination is unavoidable in samples of natural assemblages, and controls for this source of error will need to be developed.

Given the findings discussed above, I cannot agree that the proposed alternative has been reasonably shown to be equivalent to the required method in terms of agreement.

Precision

A measure of equal precision means that the two methods should demonstrate the same result in terms of the variability in the measurement (e.g., variance around the mean). Unlike accuracy, the variance in result can be examined without having to know the “true” concentration. Repeatability, even if “wrong”, is the key. Precision can be examined systematically for known cultures, both singly and mixed, as well as for a variety of ambient assemblages, although the assumption that all of the living organisms are also viable gets more tenuous when samples of organisms from the environment are used, rather than organisms from known cultures in a healthy phase of population growth.

Problems arise when the ability to get organisms to consistently reproduce sufficient for detection by a method is unknown. When organisms are obtained from culture collections, these are, to a degree, “predisposed” to growing under culture during an MPN-based method. In those cases, organisms are likely to be detected by serial dilution and culturing. For ambient organisms collected from the environment, one would have to run repeated culture experiments: either by selecting organisms and culturing from an initial “seed” individual, or by somehow demonstrating that every time organisms of that species are present in an ambient sample, they demonstrate increased concentration under the provided culture conditions.⁹² In the experiments conducted by the applicants, this is at least partly examined when taxonomic analyses are conducted for the post-incubation dilution tubes. However, the focus in the analysis was on species shown to increase in population size at least some of the time, rather than on species that were shown to be culturable in all tests. For a repeatable standard method, consistent culturability would be critical.

Laboratory experiments with monocultures of the autotrophic organism *Tetraselmis* were conducted (Miller and Petri 2015) to compare the precision of the autotrophic component of the alternative method to that of the required method.⁹³ The results from this test showed the precision was better in the required method than in the alternative method: the coefficients of variation (CVs, a measure of precision, with larger CVs indicating lower precision; calculated from the data in Miller and Petri 2015) were 1-26% (average = 13%) for the required method and

⁹² This would potentially be confounded by inhibition or encouragement of reproduction by other organisms that would not be in effect if the target species was present as one individual in a MPN dilution tube.

⁹³ In this case, all of the organisms were autotrophic, so the heterotrophic component of the alternative method was not pertinent.

20-135% (average = 50%) for the proposed alternative. The means of the CVs resulting from the two methods were significantly different ($p < 0.05$), when compared using a non-parametric Mann-Whitney U-test. In all but one case, the CV of the required method was lower than the CV of the alternative method. Thus, by this comparison, the required method has greater precision than the proposed alternative.

DESMI also compared precision between the heterotrophic component of the alternative method and a similar microscopical method in experiments using monocultures of the rotifer *Brachionus* (Miller and Petri, 2015). In this comparison, there was no difference between the results produced by the two methods. However, while the two methods were shown to have similar precision (CVs of 23% and 30% respectively), the method against which the alternative was compared was not the required method. Thus, the comparison is invalid and irrelevant for proving equivalent precision with the required method.

Precision was not specifically examined in the experiments conducted with ambient assemblages at two test facilities (Miller *et al.*, 2015b).⁹⁴ However the experiments did report the results of comparisons between concentrations measured with the required method and with the proposed alternative method at the two locations and on two separate dates, providing 4 measurements each for five methods.⁹⁵ The applicant did not provide raw data for the results from the experiment for the required method. However, a summary figure was produced showing average concentrations of organisms obtained using the five methods, and also showing some measure of the variance around the averages as “error bars.”⁹⁶ The degree of variance as indicated by the error bars is clearly greater for the alternative method results than for the required method. Additionally, the CVs for the measurements using the alternative method are provided in a table. Interestingly, the range of CVs was 19-145 for the 14 tests where “0” variance due to method problems was not an issue, and the average CV was 58. This is very similar to the range and average observed for the alternative method when used to measure concentrations of the single autotroph *Tetraselmis* (range: 20-135 and average: 50), as discussed above.

On the basis of these comparisons, the Coast Guard cannot agree that required and alternative methods are equivalent with respect to precision.

D. Does the request include a full description of the proposed alternative?

The request does not contain a full description of the proposed alternative. Overall, the proposed alternative method is clearly described and understandable. However, in several specific sections there are ambiguities or potential errors that must be addressed:⁹⁷

⁹⁴ MPN Method Development Report No. 5; Miller, A. F. Norlin and B. Petri; 2015. These experiments were focused on evaluating the best culture conditions and in examining the Factor of Agreement (FOA) between the two methods.

⁹⁵ The 5 methods were the required method and four combinations of the proposed alternative method using two media and two temperatures.

⁹⁶ It is unclear what the “error bars” represent. They could be standard deviations, standard errors, or CVs.

⁹⁷ These issues are separate from the problems related to lack of equivalence identified *supra*.

While the MPN statistical approach is a common microbiological assay for calculating concentrations based on observations of population growth in replicate serial dilution cultures, DESMI has failed to justify why it is appropriate to use for BWMS.

Many microorganisms, such as bacteria, fungi, and algae, can reproduce via asexual cell division, and under the appropriate conditions for growth, a single cell can reproduce to concentrations that are easily detectable. For autotrophic organisms such as phytoplankton, a suspension containing a single organism, given ideal conditions (e.g., nutrients, substrate, temperature, and light), will undergo exponential population growth. After sufficient time for such population increase, bulk metrics can be used to indicate the presence of that population, indicating that at least one reproductive organism must have been present in the original suspension. Changes in common bulk metrics, including chl-a fluorescence for algae and turbidity for bacteria, between initial measurements and measurements after a period of culturing, denote the presence of at least one organism in the initial diluted sample capable of undergoing reproduction.

MPN's success under these circumstances does not mean that it is appropriate for all circumstances, particularly for use with ambient, mixed assemblages of phytoplankton rather than a monoculture. In a given water body, the number of species varies geographically and seasonally, but it is on the order of dozens, if not hundreds, of species.⁹⁸ Applying MPN to such diverse communities may violate the assumptions of the method.

The following assumptions, which were written in reference to bacteria but are also applicable for phytoplankton, are "necessary to support the MPN method" and are stated up front in all specific versions of the MPN statistical approach:⁹⁹

- a) The organisms are distributed randomly within the sample.
- b) The organisms are separate, neither clustered together nor repelling each other.
- c) Every replicate (tube, plate, etc.) whose inoculum contains even one viable organism will produce detectable population growth or change.
- d) The individual tubes of the sample are independent.

A full description of the method must explain why apparent violations of these assumptions are either not critical, or are otherwise accounted for.

The most critical assumption of MPN as a statistical approach is that each viable cell is capable of reproducing, so its original presence in the diluted subsample is detectable through population growth and an increase in the measured parameter over the course of the MPN incubation. The ability of an organism to reproduce must be independent of other organisms, inhibitory factors, such as toxicity due to the presence of trace metals and viruses in the cultures. If every cell may not be capable of being cultured in MPN tubes, and in turn, may not be detected, then the mathematical underpinnings of the MPN calculations are not applicable and procedures that incorporate the MPN methodology will not return accurate estimates. One of the most important

⁹⁸ For example, over a thousand phytoplankton species have been identified in the Chesapeake Bay estuary.

⁹⁹ See, e.g., US Food and Drug Administration, *BAM Appendix 2: Most probable number from serial dilutions*, Washington, DC, (2010).

aspects of test procedures that incorporate MPN as a statistical approach is that all of the necessary conditions for reproduction and population increase be present during the incubation period. If this is not the case, the method will not detect the presence of organisms that do not reproduce sufficiently to result in a detectable population increase, and the procedure will not result in an accurate measurement of original concentration.

With the above fundamental requirement in mind, it is important to recognize that the information submitted in support of the proposed method includes data showing not all species of phytoplankton are capable of being consistently cultured, at least under the conditions that were used, during all tests. This point is not addressed in the application, other than to assert, without substantiation, that it will present a small bias. The applicants argue that the important issue is whether all species have been observed to reproduce under the provided conditions at least once, rather than consistently (i.e., every time culturing is attempted), and the species that are not known to have reproduced at least once approaches 0% when a long-term historical record is considered. However, the key issue is not whether a viable organism of a species has been observed to reproduce at least once in the past, but instead whether it is known to reproduce consistently whenever present during a test. Thus the percentage of organisms that may be viable but non-culturable is greater when the ability to grow in each test is considered.

The data submitted with the proposed alternative indicate that the proportion of species observed to consistently demonstrate reproduction and population increase varied between the two locations where the issue was examined. At one site, 20-44% of species present in the samples had consistently demonstrated population growth in culture, while at the other the range was 56-89%. Hence, the proportion of species present that had not been observed to reproduce consistently in tests intended to detect organisms capable of reproduction was 56-80% and 11-44% at the other. The conclusion is that the test is not capable of detecting whether members of these species are capable of reproduction.

The assumption that organisms do not aggregate is clearly called into question for mixed assemblages of phytoplankton because of the existence of many species that naturally occur as multi-celled colonies. It may be that this problem could be ameliorated by gentle agitation of the sample to break up the colonies into individual cells, but many of the colonial types adhere quite strongly, and the ability to disaggregate the colonies without causing mortality would need to be established for all the colonial species that might occur in test waters.

The ability of every cell to be cultured is a fundamental tenet of MPN, and a significant shortfall in meeting that criterion calls into question the use of the proposed alternative method. This critical shortfall needs to be resolved.¹⁰⁰

Additionally, there is further uncertainty regarding the estimated MPN in the proposal. Typically, the upper and lower values defining the confidence interval (CI) are reported with MPN estimates. Standard methods for MPN analyses, including methods published by the EPA

¹⁰⁰ This is and remains a central issue of discussion within EPA's ETV Technical Panel formed to consider, among other things, the acceptability of an MPN-based viability assay. The data presented in support of the proposed alternative method have been the basis for many of the panel's discussions, and a generally accepted resolution has not yet been identified.

(U.S. EPA 1978), the U.S. Food and Drug Administration (U.S. FDA 2010), and other scientific authorities (APHA et al. 1999), include tables that list the corresponding 95% CIs. These values must be included when reporting the outcome of an MPN analysis to assure data quality.¹⁰¹ Likewise, the calculators evaluated in the submitted documents all report CIs. However, the proposed method does not require reporting the CIs, nor explain why. As for all calculations of standard errors (SE), of which the CI is an example, larger sample sizes (n) generally result in smaller SE. Take for example, a single dilution MPN with a sample volume of 0.1 mL and an original undiluted concentration of 2.23 viable organisms per mL. Varying the numbers of tubes in an MPN, while holding the percentage of sample tubes positive for growth in each test at 20%, results in a wide range of confidence interval sizes around the MPN. Using 5, 10 or 20 tubes per test results in confidence intervals of 0.31 – 15.9; 0.56 – 8.9; and 0.84 – 5.96, respectively. The precision of the estimate of a 5-tube MPN was limited: the range of CI spanned two orders of magnitude (from 0.31-15.9 organisms mL⁻¹). Higher numbers of sample tubes yield narrower CI ranges, and thus greater confidence in the calculated MPN value.

The proposed method stipulates an MPN matrix should consist of 3 dilutions x 5 tubes per dilution. As shown from the above example, a larger number of tubes must be used to ensure that an MPN value generated from an MPN table does not have a large CI. If the 5 tube case described above were observed in an approval test, even though the MPN is 2.23 viable organisms per mL, a value below the discharge standard, the upper confidence limit is 15.9 viable organisms, a value above the discharge limit.

Of further concern is that the proposed alternative method does not account for the CIs generated by MPN tables. The CI can be relatively large (as noted above), and excluding the CIs can potentially result in a BWMS being considered to meet the discharge standard on the basis of the MPN, whereas the BWMS may not meet the discharge standard if the upper CI was taken into consideration. The lack of consideration of the CI in the alternative method is not explained, other than by a statement that they are not used. The CI, and its meaning with respect to the calculated MPN, must be explained in the alternative method and it should be reported with all results.

Additionally, several sections of the method description require clarification, as discussed below:

- i. Sampling: The sampling scheme described in the method allows for two options: "...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken." Having two options allows for unnecessary and potentially disruptive differences among practitioners. Furthermore, the latter option (one sample with 3 subsamples) could greatly affect the outcome of the sampling and is inappropriate for use in this circumstance. This option results in "pseudoreplication,"¹⁰² in which the "replicates" are actually "subsamples" that violate the assumption of independence among samples. If the latter option were used, the statistical analyses would be flawed, potentially yielding

¹⁰¹ See, e.g., EPA, *Soil sampling quality assurance user's guide*, Report number EPA/600/S4-84-043, Washington, DC.

¹⁰² Hurlbert SH, *Pseudoreplication and the design of ecological field experiments*, *Ecol Monogr* 54:187–211 (1984).

- results that were wrong. Thus, there must be only one recommended sampling scheme, it should be the first option (three replicate samples).¹⁰³
- ii. Autotrophs – Filtering: The proposed alternative method directs that samples for the autotroph method be filtered onto 10- μ m filters. However, if organisms <10 μ m are retained on the filter, as could be expected, estimates of organism numbers from the MPN analysis will be artificially inflated, because the final MPN number would include organisms that are regulated by the Coast Guard (≥ 10 μ m and <50 μ m) as well as those that are not regulated by Coast Guard (<10 μ m). Similarly, organisms ≥ 50 μ m could also be retained on the filter, again, artificially inflating the MPN estimate. The applicant asserts this bias would be small, but in any case, it would be prudent for each test facility using this approach to examine the potential for these circumstances to bias the estimates from the MPN analysis. According to the method description, the filter may or may not be left in the MPN tube during the grow-out period. It is unclear if the practice of leaving a filter in the tube affects the potential for population growth due to smaller organisms entrained on the filter being included in the MPN tubes, or if the presence of the filter itself affects the fluorescence reading. The alternative method must provide unambiguous direction, and if the direction is to leave the filter in the tube, data showing that the practice does not affect the results must be presented.
 - iii. Autotrophs – Measurements: To determine if reproduction and population growth has occurred in an MPN tube, the proposed method specified a minimum threshold fluorescence of four times the standard deviation (SD) of fluorescence measurements from method blanks (tubes containing no chlorophyll). In order for this threshold to be uniformly applied across laboratories, the fluorometers would need to be calibrated following the same, standardized procedure, and demonstrate consistency in measuring the threshold value. This specification must be included in the proposed method.
 - iv. Heterotrophs – Detection: The heterotroph component of the proposed alternative method relies partly on the ability to detect the red autofluorescence of chlorophyll-a (chl-a) containing organisms using epifluorescence microscopy. However, the optical filter set specified in the method is optimized to detect the fluorescence from fluorescein, not chl-a; according to the wavelengths of the filter set specified in the heterotroph method (B-2E/C), the red autofluorescence of chl-a would not be visible (i.e., the optical filter would not serve to identify organisms with chl-a). Potentially, all organisms detected would not fluoresce, and would appear to lack chl-a and, therefore, if they were motile, they would be scored as heterotrophs. Filter sets are available that allow chl-a fluorescence to be detected and must be used. Additionally, the procedure used to quantify living heterotrophic organisms scores cells that are moving and do not show red autofluorescence (i.e., they do not contain chl a) as living heterotrophs. However, if organisms do not exhibit fluorescence and are not viewed with another light source (one is not stipulated in the proposal), they will, whether moving or stationary, be, at best, dimly illuminated by light and difficult to see. This potential problem should be addressed.

¹⁰³ This does not apply to sample tubes used in the MPN approach; in such case, water for all dilution tubes (i.e., an array consisting of 3 dilutions, each with 5 replicates) should be drawn from the same population. Tubes are inoculated with water from a single, original sample.

- v. Data Analysis: It is unclear how the uncertainties around the counts resulting from the autotroph and heterotroph components of the proposed alternative method are applied. Each set of replicate measurements will result in an estimate of the variance around the mean. If the variance estimates are to be added together, as are the means to arrive at a total number of organisms, there is no statistical justification provided for doing so. Instructions, and justifications for such, should be provided.

Conclusion

In sum, I deny your appeal and affirm MSC's decision denying DESMI's request for a testing equivalency and type approval under 46 C.F.R. § 162.060-10. When promulgating its ballast water regulations, the Coast Guard explicitly rejected "viability" as part of its ballast water discharge standard. This policy decision was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying DESMI's proposal because DESMI's proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations. MSC lacked the discretion to accept DESMI's proposed testing method. Even if MSC did have the discretion to accept viability, DESMI's application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). Because DESMI's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying DESMI's type approval application.

This decision constitutes final agency action on the issues raised in your appeal.

Sincerely,



Linda L. Fagan

Rear Admiral, U. S. Coast Guard

Deputy for Operations Policy and Capabilities

Technical References

1. (Miller 2015a) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Supplemental Information 02 MAR 2015
2. (Miller 2015b) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Updated Documentation 06 MAY 2015
3. (Trojan Marinex 2015a) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples 31 JAN 2015
4. (Trojan Marinex 2015b) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples, 05 MAY 2015
5. (Petri 2015a) Evaluating the MPN Dilution-Culture Method for the Enumeration of Viable Phytoplankton Cells
6. (Maurer and Welschmeyer 2015a) Flow Cytometric Analysis of the Relative Abundance of Heterotrophs and Autotrophs in the Regulated 10-50 μm Size Class
7. (DHI 2014) MPN Assay – Analyses of Algal Regrowth for Performance Evaluation of Ballast Water Management Systems Primary Validation
8. (Petri 2015b) MPN Method Development Experiments 1 to 3 Inter-Lab Comparison of the MPN Dilution-Culture Method and Fluorescein-Based Staining Methods for the Enumeration of Viable or Living Phytoplankton Cells
9. (Miller et al. 2015a) MPN Method Development Report Experiment 4
10. (Miller et al. 2015b) MPN Method Development Report Experiment 5
11. (Miller and Petri 2015) MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 μm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology
12. (Cullen and MacIntyre 2015) On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment
13. (Maurer and Welschmeyer 2015b) Rationale for the Use of Most Probable Number (MPN) Technique in the Evaluation of UV-based Ballast Water Management Systems
14. (MacIntyre et al. 2015) Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water

15. (Miller 2015c) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 03 JUN 2015
16. (Miller 2015d) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 30 JUN 2015
17. (Miller 2015e) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 23 JUL 2015
18. (Miller 2015f) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 26 JUL

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JUL 12 2016

The Boutique Firm PLC
Attn: Matthew D. Melewski
3928 Xerxes Ave S
Minneapolis, MN 55410

Dear Mr. Melewski,

I refer to your appeal dated February 11, 2016, on behalf of Alfa Laval Tumba AB (Alfa Laval). You requested a formal administrative appeal of the decision of the Marine Safety Center (MSC) denying Alfa Laval's request for a testing alternative to be used in the Coast Guard's ballast water management system (BWMS) type approval process and Alfa Laval's application for USCG type approval of the Alfa Laval PureBallast BWMS.¹

This is an administrative appeal of an MSC decision or action taken pursuant to 46 Code of Federal Regulations (C.F.R.) § 162.060-10(b) and is reviewed by my office under 46 C.F.R. § 159.001-2 and as provided in 46 C.F.R. § 1.03-15. In considering your request, I reviewed your appeal (including its appendix and the administrative appeals of Hyde Marine, Inc., Trojan Technologies, and DESMI Ocean Guard A/S, incorporated by reference by your appeal, footnote 30), the administrative record, and applicable laws, regulations, and policy.²

Based on this review, I hereby deny your appeal, affirming MSC's decision to deny Alfa Laval's request for testing equivalency and type approval under 46 C.F.R. § 162.060-10. This matter and your appeal are quite technical and detailed, and the issues raised will be discussed in more detail in this letter.³ To briefly summarize, during the development of the ballast water regulations, the Coast Guard explicitly rejected the use of BWMS that "may act to make organisms unviable or unable to reproduce rather than killing or removing them." This policy decision to reject "viability" was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations, and MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed

¹ Type testing and approval are used in equipment and manufacturing to determine that a specific "type" of equipment or process meets a minimum set of requirements. In this context, "type approval" is a vessel equipment approval process.

² I note that Trojan, and, by incorporation by reference, Alfa Laval, has reserved the right to "supplement" its appeal to address my response. That is unnecessary. This response constitutes final agency action on the issues raised in Alfa Laval's appeal.

³ I have substantively responded to your appeal in the interest of transparency. This response does not waive any defenses the Coast Guard may have as to the timeliness of your assertions or failure to exhaust your administrative remedies.

to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). As your type approval application was based on testing that does not meet the regulatory standards and was not approved as an alternative, MSC was correct in denying your application for type approval of the Alfa Laval PureBallast BWMS. Your appeal is denied, and this decision constitutes final agency action.

Background

Marine environmental protection is one of the Coast Guard's core statutory and operational missions.⁴ As stewards of the marine environment, the Coast Guard maintains a robust environmental protection regulatory program and also assists other federal agencies in enforcing laws to protect, preserve, and remediate waters subject to the jurisdiction of the United States. The Coast Guard also leads and participates in initiatives at the International Maritime Organization (IMO), the intergovernmental organization specializing in commercial shipping safety, security, and environmental protection standards, to raise and standardize global shipping practices. These activities all have one desired end state: to help ensure the health and vitality of waters of the United States and its living marine natural resources.

The Coast Guard manages its marine environmental protection obligations through a well established network of Headquarters, regional and field offices. Within Coast Guard Headquarters, located in Washington, D.C., there are several organizations, or "programs," responsible for developing, promulgating, and enforcing marine environmental protection standards. The Office of Operating and Environmental Standards (CG-OES) and the Office of Design & Engineering Standards (CG-ENG) have the lead roles in promulgating and implementing (but not enforcing) Coast Guard environmental regulations, including requirements for approval of equipment installed on vessels. MSC's role focuses on regulatory compliance and policy development, generally related to plan reviews for domestic vessels and type approvals for vessel equipment. While MSC can be involved in the clearance process for rulemakings, MSC is not the lead office for environmental standards development. In other words, MSC applies environmental regulations but does not create them. All three programs are within the Directorate of Commercial Regulations and Standards (CG-5PS).

The Coast Guard's ballast water program is one of the Coast Guard's long-standing marine environmental protection programs. It is established under the authority of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, as amended by the National Invasive Species Act of 1996 (NANPCA/NISA).⁵ As mandated by NANPCA, the Coast Guard's program began in 1991 with voluntary ballast water management guidelines for the Great Lakes.⁶ Mandatory requirements for the Great Lakes followed these voluntary guidelines in

⁴ See Section 888, Homeland Security Act of 2002, Pub. L. No. 107-296 (H.R. 5005), 116 Stat. 2135 (2002), as amended, *classified to* 6 U.S.C. § 468.

⁵ Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (NANPCA/NISA), Pub. L. No. 101-646 (H.R. 5390), 104 Stat. 4761 (1990), *as amended; codified in* 16 U.S.C. §§ 4701, 4702, 4711, 4712-4714, 4721-4728, 4741, 4751. Congress enacted NANPCA in response to the disruption and damage caused by the introduction of nonindigenous zebra mussels into the Great Lakes, likely released via discharges of ships' ballast water. NANPCA originally focused on ballast water operations in the Great Lakes but was later amended by NISA to cover waters nationwide.

⁶ NANPCA/NISA, *supra*, n. 5, § 1101(a); *codified in* 16 U.S.C. § 4711(a). *See also* Ballast Water Management for Vessels Entering the Great Lakes, Final Rule, 58 Federal Register (Fed. Reg.) 18330 (April 8, 1993).

1993.⁷ In 1996, NISA amended NANPCA to, among other things, cover all navigable waters of the United States.⁸ In the 1990s, the best method available for preventing the discharge of aquatic nuisance species from ballast water was an operational practice known as ballast water exchange. For this reason, NANPCA/NISA contained a specific requirement for certain vessels to conduct ballast water exchange.⁹ The Coast Guard updated its regulations to include nationwide, mandatory ballast water exchange requirements in 2004.¹⁰ However, ballast water exchange was an interim measure until more effective ballast water technology could be developed and verified. Under the NANPCA/NISA mandate to “ensure to the maximum extent practicable that aquatic nuisance species are not discharged into waters of the United States from vessels,” the Coast Guard collaborated with domestic and international partners to identify better methods and technology to prevent the introduction and spread of aquatic nuisance species. These efforts ultimately resulted in the Coast Guard’s 2012 Final Rule, Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters (2012 Final Rule).¹¹

NANPCA/NISA is a domestic legal authority and does not explicitly implement the international standard for ballast water discharges, which is found in the IMO International Convention for the Control and Management of Ships’ Ballast Water and Sediments, 2004 (BWM Convention).¹² The disconnect between U.S. domestic law and the international standard is partly timing: NANPCA was enacted in 1990, many years before the international community adopted the BWM Convention in 2004. Additionally, at this time, the United States is not a contracting government to the BWM Convention¹³ and the BWM Convention has not received enough ratifications to enter into force. The Coast Guard attempted to harmonize the 2012 Final Rule with the BWM Convention to the extent possible within its statutory authority.¹⁴ Ultimately, the

⁷ *Id.*

⁸ See NANPCA/NISA, *supra*, n. 5, § 1101(a) – (c); *codified in* 16 U.S.C. § 4711(a) – (c). This statutory bifurcation is the historical reason why Coast Guard ballast water regulations are promulgated in two subparts of 33 C.F.R. Part 151, despite there now being few differences between the ballast water management standards for the Great Lakes and those for the rest of the United States.

⁹ Ballast water exchange is a process by which a vessel replaces water, generally coastal water, in its ballast tanks. See definition of “exchange,” 33 C.F.R. § 151.2005(b).

¹⁰ Mandatory Ballast Water Management Program for U.S. Waters, 69 Fed. Reg. 44952 (July 28, 2004).

¹¹ 77 Fed. Reg. 17254 (March 23, 2012).

¹² IMO Doc. BWM/CONF/36.

¹³ If the United States became party to the BWM Convention, the Coast Guard could implement the convention through NANPCA/NISA even though there is no explicit reference. See NANPCA/NISA, *supra*, n. 5, § 1101(f)(3); *codified in* 16 U.S.C. § 4711(f)(3).

¹⁴ See, e.g., 77 Fed. Reg. 17260. I note that the Coast Guard has made some statements which could confuse the issue of whether the Coast Guard ballast water discharge standard is “identical” to the BWM Convention standard. From the Coast Guard’s perspective, as discussed further *infra*, the BWM Convention’s use of “viable” is synonymous with the Coast Guard’s use of “living,” and in that sense the standards are the same. However, there are some minor differences between the two regimes. That is why the Coast Guard has used certain language, such as “align with” and “equivalent to,” to show that the Coast Guard regime is *not* identical to the BWM Convention regime.

BWM Convention is not a treaty of the United States, and the Coast Guard has a mandate to implement NANPCA/NISA as written.¹⁵

The Coast Guard's ballast water regulations are codified into Titles 33 and 46 of the C.F.R. The regulations in 33 C.F.R. Part 151 Subparts C (Great Lakes) and D (Nationwide) are operational or performance standards, and the regulations in 46 C.F.R. Subpart 162.060 are equipment standards.¹⁶ In this case, the Title 33 operational requirements apply to vessels, vessel owners or operators, or other persons associated with the vessel, and the Title 46 equipment requirements apply to BWMS manufacturers seeking equipment approvals. However, as discussed *infra*, the two are still interrelated and should be read together.

The 2012 changes to 33 C.F.R. Part 151 introduced a scheduled phase-out of ballast water exchange as an accepted operational measure to reduce the introduction and spread of aquatic nuisance species and provided the new option of using a BWMS to treat ballast water prior to discharging it.¹⁷ 33 C.F.R. Part 151 now contains a numeric discharge standard for the maximum number of living organisms in ballast water, a standard which all Coast Guard-approved BWMS must meet.¹⁸ The 2012 changes also added other ballast water management options, including the use of water from U.S. public water systems and discharge to a reception facility. For a vessel meeting the discharge standard by using a BWMS to be in compliance with 33 C.F.R. Part 151, its BWMS must have received type approval under the standards located in 46 C.F.R. Subpart 162.060.¹⁹

I now address the issues raised in your appeal, as well as the principal arguments raised by the other manufacturers in their respective appeals, which have been incorporated by reference, as follows:

1. MSC did not have the discretion to approve Alfa Laval's request

¹⁵ As discussed further, *infra*, the Coast Guard's regulatory standard is "to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels..." NANPCA/NISA, *supra*, n. 5, § 1101; *codified in* 16 U.S.C. § 4711. The Coast Guard believes the "practicability" element of this standard requires the U.S. ballast water discharge standard to take into account the relevant IMO standard, as most vessels engaged on international voyages will likely need to comply with the BWM Convention once it comes into force. If the U.S. domestic standard conflicts with or frustrates an international standard, the result could range from increased vessel costs to preventing the vessel from calling on certain ports (implementing whichever standard the vessel cannot meet).

¹⁶ Generally, for the C.F.R., the reason vessel operational standards appear in Title 33 and vessel equipment standards appear in Title 46 is because many Coast Guard statutory authorities are contained either in Title 33 of the United States Code (Navigation and Navigable Waters) or Title 46 (Shipping). Title 33 authorities are generally more "operational," such as the Ports and Waterways Safety Act (Pub. L. No. 92-340 (H.R. 8140), Tit.I, 86 Stat. 424 (1972), *as amended*; *codified in* 33 U.S.C. §§ 1221 – 1232) and the Act to Prevent Pollution from Ships (Pub. L. No. 96-478, 94 Stat. 2297 (1980), *as amended*; *codified in* 33 U.S.C. §§ 1901 – 1911). On the technical side, the Coast Guard maintains broad authority to regulate inspected vessel equipment under 46 U.S.C. § 3306, and its vessel equipment approval processes are correspondingly located in Title 46 of the C.F.R. This is true even when the underlying statutory authority is contained in a different title. In this case, the Coast Guard has overlapping statutory authority to set BWMS equipment standards, under both NANPCA/NISA and 46 U.S.C. § 3306.

¹⁷ 33 C.F.R. § 151.1510(a); 33 C.F.R. § 151.2025(a).

¹⁸ 33 C.F.R. § 151.1511; 33 C.F.R. § 151.2030.

¹⁹ *See, e.g.*, 33 C.F.R. § 151.2025(a)(1).

I agree with MSC that it lacked the discretion to approve Alfa Laval's 46 C.F.R. § 162.060-10(b)(1) request, which sought approval of a testing method that measured "viable" rather than "living" organisms in ballast water. The Coast Guard's type approval regulations exclude "viability" as an option. This was an environmentally conservative policy decision, based on best scientific information available, which went through the public notice and comment process. Therefore, MSC could not grant an alternative request that circumvented the text and policy position of the Coast Guard's ballast water regulations. Since Alfa Laval's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying Alfa Laval's type approval application.

Issue:

Alfa Laval is a BWMS manufacturer who requested that the Coast Guard approve an alternative to the Coast Guard's BWMS type approval requirements. Alfa Laval manufactures the PureBallast BWMS, which uses ultra-violet radiation (UV) to treat ballast water. Alfa Laval submitted its application for a BWMS testing alternative under 46 C.F.R. § 162.060-10(b)(1), and the crux of this appeal revolves around the type of tests that can be used, under the Coast Guard regulations, to validate the efficacy of UV BWMS. Specifically, Alfa Laval requested to use a "Most Probable Number assay" (MPN) in lieu of 5-chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) direct staining methods to test for certain organisms to meet type approval requirements.²⁰ The simplified distinction between these measurement methods is that Alfa Laval's preferred measurement method measures "viability" of an organism, while the regulatory requirement method measures whether an organism is "living." This appeal concerns the testing method being used and not whether the Coast Guard can or will grant type approval to UV BWMS as a class of system.²¹ While one appellant (Hyde) has characterized the question as "whether the measurement method – MPN – is reliable and accurate," the main question before me is whether the ballast water regulations allowed MSC to approve MPN as a measurement tool.²²

Applicable regulatory standards and the meaning of "living":

46 C.F.R. § 162.060-10(b)(1) provides:

If an evaluation, inspection, or test required by this section is not practicable or applicable, a manufacturer or independent laboratory may submit a written request to the Commanding Officer (MSC), Attn: Marine Safety Center, U.S. Coast Guard Stop 7410, 4200 Wilson Boulevard Suite 400, Arlington, VA 20598-7410, or by email to msc@uscg.mil, for approval of alternatives as equivalent to the requirements in this section. The request must include the manufacturer's justification for any proposed changes and contain full descriptions of any proposed alternative tests.

²⁰ Alfa Laval appeal, Exhibit 21.

²¹ Not all UV BWMS are designed like Alfa Laval's: some UV BWMS are designed to kill organisms rather than to render them unviable. Thus, my decision should not be interpreted to mean that the Coast Guard will not grant type approval to *any* UV BWMS under the current regulatory standards.

²² A follow-on question, answered in Section 3 of this response, is whether Alfa Laval met all elements of 46 C.F.R. § 162.060-10(b)(1).

The Coast Guard applies 46 C.F.R. § 162.060-10(b)(1) by considering the following four elements:

1. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?
2. Is that evaluation, inspection or test not practicable or applicable?
3. Is the proposed alternative equivalent to the regulatory requirement?
4. Does the request include a full description of the proposed alternative?

MSC's decision denying Alfa Laval's application specifically addressed items 2 and 3 and found them both in the negative.²³ MSC found that Alfa Laval's proposed alternative was not equivalent because

...it does not measure the efficacy of the ballast water treatment system to the performance standard required by the regulations. The regulations specifically require ballast water treatment systems to be evaluated based on their ability to kill certain organisms. Since the proposed MPN method assesses the viability of an organism to colonize after treatment, it measures to a different standard than that required by the regulations.²⁴

You contend for several reasons that this reasoning was unsound.

However, I believe MSC was correct that the Coast Guard's ballast water management regulations do not allow MSC to approve a method that measures viability in lieu of the regulatory standards.²⁵ I believe the reference, above, to the "performance standard required by the regulations" means the ballast water discharge standard contained in 33 C.F.R. Part 151.²⁶ As a general principle, a technical equipment standard in Title 46 of the C.F.R. would not be able to override a performance or operational standard in Title 33 of the C.F.R. In this case, the technical equipment standard and the operational standard are inextricably linked, and the meaning of "living" cannot be resolved by viewing a stark dichotomy between Titles 33 and 46 of the C.F.R.

To understand why, it is helpful to begin with the text of 46 C.F.R. § 162.060-10(b)(1). Alfa Laval's request must meet the first prong of 46 C.F.R. § 162.060-10(b)(1), requesting an alternative to an "evaluation, inspection, or test required by this section." This is a reference to the requirements contained in 46 C.F.R. § 162.060-10(f) (emphasis below added):

²³ Having found at least one of the elements in the negative, there was no need for MSC to opine on all of these elements.

²⁴ Letter dated December 14, 2015, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Alfa Laval Tumba AB, "Request for Approval of the Use of the Most Probable Number (MPN) Method to Determine Biological Efficacy of the Alfa Laval PureBallast Ballast Water Management System (BWMS)".

²⁵ This is not a matter of literal "equivalency" of testing methods, which is discussed *infra*, Section 3.

²⁶ See Letter dated February 2, 2016, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Hyde Marine Inc., "Request for Review and Reconsideration of December 14, 2105 Decision of Marine Safety Center Denying Application for Approval of Equivalent Test Method Under 46 C.F.R. § 162.060-10(b)(1); (Alfa Laval Exhibit 6), ("Therefore, in order to demonstrate compliance with the BWDS as set forth at 33 CFR §§ 151.1511 and 151.2030, a BWMS must be evaluated on the basis of counting living/dead vice viable/unviable" organisms.").

A BWMS is eligible for approval if –

- (2) It is evaluated, inspected, and tested under land-based and shipboard conditions in accordance with §162.060-26 and 162.060-28 of this subpart, respectively, and thereby **demonstrates that it consistently meets the ballast water discharge standard in 33 CFR part 151, subparts C and D;**
- (3) All applicable components of the BWMS meet the component testing requirements of §162.060-30;
- (4) The BWMS meets the requirements of §162.060-32 of this subpart if the BWMS uses an active substance or preparation...

This provision clearly delineates the operational standard contained in 33 C.F.R. Part 151 from the equipment standard in 46 C.F.R. Subpart 162.060 and supports MSC's reasoning that a Title 46 alternative or equivalency for vessel equipment testing cannot be used to override a Title 33 performance standard. However, the actual text in 33 C.F.R. Part 151 shows that the two standards are inextricably linked (emphasis below added):

(a) Vessels employing a Coast Guard-approved ballast water management system (BWMS) must meet the following BWDS by the date in §151.1512(b) of this subpart:

- (2) For organisms less than 50 micrometers and greater than or equal to 10 micrometers: discharge must include fewer than 10 **living** organisms per milliliter (mL) of ballast water.²⁷

There is no definition of “living” or any other regulatory text in 33 C.F.R. Part 151 that explains this important detail of the discharge standard. The only way a ballast water manufacturer can understand the “living” organism standard in 33 C.F.R. Part 151 is by referring to 46 C.F.R. Subpart 162.060 and its technical requirements and reading the preamble of the final rule. Thus, while the discharge standard in 33 C.F.R. Part 151 is an operational standard which applies to vessels, it is inextricably intertwined with the technical equipment standards contained in 46 C.F.R. Subpart 162.060 which apply to ballast water manufacturers.²⁸ The substance of “living” remains within 46 C.F.R. Subpart 162.060, and MSC was not solely bound by the indeterminate “living” language contained in 33 C.F.R. Part 151.

46 C.F.R. Subpart 162.060 also has no definition of “living,”²⁹ but this subpart contains extensive efficacy requirements which constructively define the term. Specifically, the Coast

²⁷ 33 C.F.R. § 151.1511, emphasis added.

²⁸ For vessels engaged on international voyages, this interlinkage between performance standards and technical standards is common, as typically a vessel's operation of a type approved or certificated piece of equipment satisfies the operational requirement unless a Coast Guard inspector or investigator has reason to believe that the equipment is not operating or being operated properly.

²⁹ Alfa Laval's focus on the definition of BWMS (46 C.F.R. § 162.060-3) is misplaced. The BWMS definition alone does not set a performance or technical standard for BWMS. It merely identifies a category of equipment that the Coast Guard is regulating. The performance and technical standards in Titles 33 and 46, respectively, set the requirements for BWMS, and meeting the broad definition of BWMS does not necessarily mean that the BWMS

Guard's testing regulations incorporate the Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV Protocol)³⁰ by reference.³¹ The ETV Protocol contains staining test requirements³² that evaluate the functioning of certain enzyme systems and cell membrane integrity of organisms, thereby defining "living" by virtue of these critical functions necessary for organisms to persist.³³ The ETV Protocol uses the term "viable," but defines it as "organisms and any life stages thereof that are living."³⁴ The ETV Protocol also explains why the ETV Technical Panel³⁵ decided to limit "viability":

Note that it is understood that many of the proposed regulatory discharge standards, and in fact the desired effect of BWTs,³⁶ is that these technologies should render organisms unviable or incapable of reproduction. In other words, to "kill, remove or inactivate" is technically unnecessary when the objective is to eliminate the organism's capability for reproduction. However, as the introduction of "viability" as a measure of efficacy significantly complicates the Protocol and test methods, and since "kill, remove or inactivate" is a conservative

meets all of the technical and performance standards in the regulations. Specific requirements control general terms. For an analogy, see the definition of "tank vessel" contained in 46 U.S.C. § 2101. The fact that a vessel may meet this broad definition does not mean that it can be certificated as a tank vessel under the inspection requirements in C.F.R. Title 46. The definition identifies a broad category of thing that the Coast Guard is regulating. The vessel would still need to meet the specific tank vessel inspection requirements to receive a tank vessel Certificate of Inspection.

³⁰ "Generic Protocol for the Verification of Ballast Water Treatment Technology," EPA/600/R-10/146, September 2010, available at https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=230926. The ETV Protocol is a document of the Environmental Technology Verification Program (ETV Program), funded in whole or in part by the U.S. Environmental Protection Agency.

³¹ See, e.g., 46 C.F.R. § 162.060-26(a), which refers to the Subpart's "Incorporation by Reference" section, § 162.060-5.

³² The ETV Protocol "requires" the determination of organism concentration, but it is true that the offered testing method – the dual stain method – is stated in voluntary terms. For example, the ETV Protocol states, "[t]his protocol recommends use a [sic] combination of two vital stains..." (paragraph 5.4.6.5). This is because the ETV Protocol recognizes that there are other stains and measurement methods that may be acceptable, as long as they measure the same thing: whether something is alive. If a manufacturer does not want to use the dual stain method to meet the ETV Protocol requirements, then a manufacturer uses the USCG approval process in 46 C.F.R. § 162.060-10(b)(1) to request an alternative for the purposes of complying with the USCG regulations.

³³ Alfa Laval raises divergent arguments, sometimes claiming that the ETV Protocol trumps the regulatory text and other times claiming that the Coast Guard cannot rely on the final ETV Protocol at all because it was not properly incorporated into the ballast water NPRM. As to the latter argument, the Coast Guard fully explained in its response to comments the logical outgrowth basis of its decision to adopt the final ETV Protocol into the 2012 Final Rule. 77 Fed. Reg. at 17258. Additionally, FACA does not apply to collaborations or partnerships that gather and share information, provide individual or unsolicited advice, or that do not seek consensus. See Federal Advisory Committee Act of 1972, Pub. L. No. 92-463 (H.R. 4383), 86 Stat. 770 (1972), as amended, *classified to 5 U.S.C. App. §§ 1 – 16*.

³⁴ ETV Protocol, page xii.

³⁵ The ETV Technical Panel is a group "comprised of a subset of stakeholders and other individuals with a technical expertise in ballast water and environmental technology issues." Members include fresh water and marine biologists, environmental scientists, engineers, and ship architects. See ETV Protocol, page 4.

³⁶ The ETV Protocol uses the term 'Ballast Water Treatment System(s)' (BWTs); which the Coast Guard views as consistent with the term 'Ballast Water Management System(s)' (BWMS) in Coast Guard regulations. See, e.g., 33 C.F.R. § 151.1504.

approach, the latter has been adopted as the measure of biological efficacy in this Protocol.³⁷

The ETV Protocol is unequivocal on the viability issue.³⁸ “Living” does not mean “viable.”³⁹ The language of 46 C.F.R. § 162.060-10(b)(1) provides flexibility for the Coast Guard to accept a test method other than what is expressly enumerated in the ETV Protocol, but the alternative must be demonstrated to measure what the discharge standard requires.⁴⁰ A 46 C.F.R. § 162.060-10(b)(1) approval can be used to accept a different measurement tool (e.g., a 12 inch ruler versus a yard stick) but not to substitute the underlying measurement requirement (e.g., length versus weight).⁴¹ Thus, the efficacy standards in 46 C.F.R. Subpart 162.060 are a bar to MSC’s ability to accept the proposed viability measurement as a testing alternative.

Coast Guard policy and intent:

The preamble to the 2012 Final Rule is consistent with this ETV Protocol language. The Coast Guard responded to a comment directly on point regarding whether “living” meant or included “viable”:

One commenter stated that because some types of treatment processes, such as UV, may act to make organisms unviable or unable to reproduce rather than killing them outright, the Coast Guard should include viability as a criterion for determination of BWMS efficacy. The Coast Guard disagrees. This issue has been the point of much discussion both in the United States and internationally in association with the IMO BWM Convention. The Coast Guard has decided to use live/dead rather than viable/unviable, because the latter designations would require culturing potentially large numbers of different kinds of organisms to determine whether they were capable of reproduction. This would be made even more problematic by the fact that scientists are not able to culture many of the organisms in question. Finally, it is more conservative, and thus more protective,

³⁷ ETV Protocol, page 5.

³⁸ The ETV Protocol was developed independently from the USCG ballast water regulations. Its first two chapters describe an organizational process that is superseded by, or otherwise irrelevant to, the specific USCG equipment approval process contained in 46 C.F.R. § 162.060.

³⁹ This ETV Protocol language is a counterpoint to Hyde’s argument that viability was only addressed in the 2012 Final Rule preamble, but not in the regulation text. The ETV Protocol was incorporated by reference into the regulation text of 46 C.F.R. Subpart 162.060. *See, e.g.*, 46 C.F.R. § 162.060-26(a).

⁴⁰ I note the existence of ETV Protocol paragraph 5.4.8, which provides for the use of “alternative and emerging methods.” This section is expressly limited to methods relating to “living” organisms, not organisms capable of reproduction, and even if it were not, the Coast Guard’s process set forth in 46 C.F.R. § 162.060-10 provides the relevant process for seeking alternatives. The specific alternative procedures created by the Coast Guard supersede the ETV Protocol’s generalized procedures. Additionally, if there is a conflict between the ETV Protocol and the regulation text, the two must be read such that the ETV Protocol does not render 46 C.F.R. § 162.060-10 meaningless. Finally, the Coast Guard disagrees with any interpretation of paragraph 5.4.8 which would effectively eliminate agency oversight of a federal regulatory program.

⁴¹ I disagree that MSC’s denial required MPN to be “identical” to the staining methods identified in the ETV Protocol. Hyde Appeal, page 26. MSC has approved other 46 C.F.R. § 162.060-10(b)(1) requests.

to base efficacy decision [sic] on the basis of live/dead, rather than viable/unviable.⁴²

This response to comment is unequivocal, and I find Hyde's arguments, incorporated by reference, to discredit it unpersuasive. First, the commenter who submitted this UV-oriented, "viable/nonviable" comment was Hyde, a manufacturer of UV-BWMS and also an appellant of the MSC decision under review here.⁴³ If Hyde believed that the standard clearly meant to include viable organisms, it would not have submitted this comment. In fact, it appears Hyde believed the opposite:

The characteristic "unviable" should be used in place of "dead" in determining the efficacy of BWMS... We wish to emphasize that the terms "kill" and "dead" should be replaced with "make unviable" and "unviable" throughout the proposed regulation.⁴⁴

At the time of this comment, the word "living" did not appear in the proposed discharge standard in either 33 C.F.R. § 151.1511 or 33 C.F.R. § 151.2030. The plain reading of the proposed discharge standard was that ballast water could contain only a maximum of *any* organism, living or dead. This is an extremely conservative and still practicably unachievable standard.⁴⁵ With such a conservative standard, it is understandable why a comment would be made to change the discharge standard. The upshot of the exchange is a clearly articulated statement of Coast Guard policy in the preamble to the Final Rule.

Additionally, this Coast Guard response is consistent with another section of the 2012 Final Rule preamble, which provided:

One commenter requested that the proposed BWDS include language necessary for differentiation between living and nonliving organisms. Another said that the standard should allow for the presence of nonliving organisms since some treatment technologies act to kill living organisms without necessarily removing them from the ballast water.

The Coast Guard acknowledges that the proposed BWDS is slightly different in this respect from the IMO discharge standard, which uses the term "viable" instead of "living." It is important to note that, while the text of the IMO BWM Convention refers to "viable" organisms, the G8 guidelines define "viable" as "living." Therefore, the Coast Guard has decided that this issue is best addressed in the BWMS approval process, and will not alter the standard as suggested by these commenters. We note that the standard and approval process do allow for the presence of nonliving organisms. Additionally, we corrected a technical error

⁴² 77 Fed. Reg. at 17274. I note the incongruity of Hyde's argument that the Coast Guard cannot rely on this language to deny Hyde's application while Hyde then quotes multiple preambular passages from multiple Coast Guard ballast water rulemakings in its favor.

⁴³ Letter dated December 3, 2009, Hyde Marine, Inc. to U.S. Department of Transportation, "Reference: Docket # USCG-2001-10486."

⁴⁴ *Id.* at page 5.

⁴⁵ In response to comments, the Coast Guard corrected the intended discharge standard by adding the word "living."

present in the NPRM, which mistakenly omitted the term “living” from the proposed 33 CFR 151.1511(a). This final rule corrects that omission.⁴⁶

Like the ETV Protocol, this response explains that “viable” means “living” and not vice versa. As discussed, *supra*, the IMO BWM Convention provides the international standard for organisms discharged from vessels’ ballast water. The Coast Guard’s ballast water discharge standard is nearly identical to the BWM Convention discharge standard. One difference is that the IMO standard contains the adjective “viable” rather than “living” to modify “organisms.” This Coast Guard response shows that its deviation from the IMO standard text was deliberate. This response also tries to articulate that, from the Coast Guard’s perspective, the two standards are substantively the same.⁴⁷ The BWM Convention does not define “viable.” However, one BWM Convention guidance document, the Guidelines for approval of ballast water management systems (G8 Guidelines),⁴⁸ defines “viable” to mean “living.”⁴⁹ While Alfa Laval flips the definition around, so that “living” means “viable,” that is not reflected in the text of the G8 Guidelines. Instead, the G8 Guidelines provide a more narrowed interpretation of the BWM Convention text, consistent with the Coast Guard’s discharge standard.⁵⁰ That is why there was no need to alter the proposed discharge standard in 33 C.F.R. Part 151 to harmonize with the IMO standard.

The Coast Guard’s response goes on to fully respond to the comments by stating that “this issue” is “best addressed in the BWMS approval process...” This means the Coast Guard’s intent was to leave “living” in 33 C.F.R. Part 151 undefined while relying on the technical efficacy

⁴⁶ 77 Fed. Reg. 17266. Alfa Laval cannot read the adjective “viable” into regulatory text where it does not exist. The record shows that the Coast Guard intended both subparts (33 C.F.R. Subparts C and D) to have the same discharge standard, which was meant to align with the BWM Convention discharge standard. *See* 77 Fed. Reg. 17260 (“We corrected the BWDS in both subparts C and D to align with the IMO BWM Convention.”). If the nationwide discharge standard is read without the “living” modifier, the standard becomes *stricter* than the Great Lakes discharge standard. Such a result is nonsensical.

⁴⁷ I note Alfa Laval’s argument claiming that the Coast Guard failed in its rulemaking to consider the extra cost to foreign flag vessels which would be barred from using foreign-approved UV BWMS using an MPN-based test method. This argument confuses an environmental analysis with a Regulatory Analysis. The Coast Guard properly considered and calculated the costs to foreign flagged vessels in its Regulatory Analysis as a sensitivity analysis. Coast Guard developed a range of cost for a ballast water treatment systems based on potential technologies, calculating total costs based on the low end of the system cost estimates. Since UV BWMS are not the lowest cost option, the fact that some are not available for use in U.S. waters does not impact the Coast Guard’s analysis. Additionally, the reference to “more stringent measures” is to the “phase-two” ballast water discharge standard proposed in the 2009 NPRM but not promulgated as part of the 2012 Final Rule.

⁴⁸ Guidelines for approval of ballast water management systems, Resolution MEPC.174(58), adopted October 10, 2008. (Titles of IMO guidance documents are typically not capitalized except for the first word.)

⁴⁹ *Id.* at paragraph 3.12 (“Viable Organisms are organism and any life stages thereof that are living.”). This language should be read as it is written and not reversed, so that living means viable. At the most recent meeting of the IMO Marine Environment Protection Committee (MEPC), this specific issue – whether the G8 Guidelines should be amended to remove or change the definition of “viable” – was raised and considered. MEPC did not reach a conclusion either way (i.e., contrary to Hyde’s assertion, the international community is not agreed that the G8 Guidelines allow BWMS to be type approved for viability rather than live/dead). *See* IMO Report of the Marine Environment Protection Committee on its Sixty-Ninth Session, MEPC 69/21, May 13, 2016, paragraph 4.39.

⁵⁰ Because “living” is broader than “viable,” the term covers more organisms, and thus a discharge standard incorporating the broader term into a maximum allowable concentration means that fewer organisms can remain in the discharged ballast water. That is why it is a more protective standard, and a “more narrowed” interpretation, despite “living” being a broader term than “viable.”

requirements in 46 C.F.R. Subpart 162.060 to set the standard by defining how the standard would be measured. Subpart 162.060 contains a myriad of equipment requirements, but its efficacy requirements incorporate the ETV Protocol, which does not include viability as a measurement of efficacy.⁵¹ “[B]est addressed in the BWMS approval process...” does not mean that 46 C.F.R. § 162.060-10(b)(1) can be used as a back door to insert viability into the discharge standard after it was deliberately excluded via rulemaking. It means that the ETV Protocol, or the regulation text, can be amended or updated to include viability as a measurement option once better scientific and technical capabilities are discovered. A new version of the ETV Protocol would still need to be incorporated by reference into the Coast Guard’s rulemaking, via the public notice and comment process.⁵² At a minimum, the Coast Guard’s policy decision on viability was established through public notice and comment and would need to go back through public notice and comment to change.

Clarification of the administrative record:

I acknowledge some confusion in the administrative record regarding the interactions between Alfa Laval and the Coast Guard on accepting viability as part of the discharge standard. I think more blame for this confusion falls on the appellants than it does on the Coast Guard. After the Coast Guard rejected therequest to include viability in the 2012 rulemaking, the appellants then voluntarily launched a campaign to change the Coast Guard’s position on the matter.⁵³ This campaign was not initially for an alternative to the new, existing standards but for the Coast Guard to reopen the rulemaking it had just completed and *change the discharge standard*. The Coast Guard was clear from the beginning that it was very unlikely to open up the rulemaking any time soon.⁵⁴ The appellants persisted in its campaign, however, and the drawn out process that followed is merely the result of a government agency trying its best to hear and try to accommodate a member of the regulated public, within the bounds of law and policy. Many of MSC’s procedural recommendations were in direct response to their insistence to be heard, by

⁵¹ See, e.g., 46 C.F.R. § 162.060-26(a) and 46 C.F.R. § 162.060-28(j). I note the Coast Guard’s use of “viability” in § 162.060-28(j). While it is true that the Coast Guard often uses “viable” and “living” synonymously, it is not in a favorable way for Alfa Laval. Both the ETV Protocol and the G8 Guidelines define “viable” as “living” and not the other way around. It should also be noted that the Coast Guard’s use of the term “viable” or “viability” has changed over time. In the 1990s and early 2000s, the Coast Guard used the term viability because of the state of available science. As technology and knowledge advanced, the Coast Guard developed a more narrow view of “viability” given the unknowns involved with trying to verify it. An agency is allowed to refine its policy position over time and is not bound to archaic or outdated ideas, particularly if the agency has a statutory mandate to base decisions on best scientific information available. I agree that the Coast Guard has considered UV BWMS as part of a suite of BWMS options for a long time, but under the current regulations, a UV BWMS must meet the prescribed technical requirements.

⁵² 46 C.F.R. § 162.060-5(d)(1) incorporates a specific version of the ETV Protocol, and so this reference would need to be updated.

⁵³ I disagree with Hyde’s characterization of its interactions with the Coast Guard in late 2012, which paint a picture of dealing with an agency with no corporate knowledge of its own regulations. The Coast Guard had just completed what was a very labor intensive, very high profile rulemaking in which the viability issue was directly considered and addressed. In bureaucratic terms, “the ink wasn’t even dry yet.” The fact that there may have been internal Coast Guard uncertainty over how to procedurally address Hyde’s persistent requests is understandable considering the rulemaking – specifically the type approval program – was in the process of initial rollout and implementation.

⁵⁴ See, e.g., HYDE APPX-000002. Additionally, I find Hyde’s criticism, in footnote 3, of the Coast Guard’s “delay” to be misleading and without merit. The Coast Guard was waiting on the final decision of the ETV Technical Panel on the MPN issue.

any means possible.⁵⁵ There were no guarantees that the process would work in the appellants' favor. Additionally, the Coast Guard does not prohibit the submission of applications even when a Coast Guard employee anticipates it will be denied on the merits.⁵⁶ How can the Coast Guard precisely know what the applicant is requesting if it does not see the actual request? In fact, if the Coast Guard refused to allow the appellants to submit an alternative request, that would have exposed the agency to claims of arbitrary and capricious behavior. The record shows that the appellants were persistent in trying to change the Coast Guard's decision on viability, and the efforts Coast Guard employees went through to provide an answer to them, at their insistence, should not be held against them now.

I will also clear up some confusion over the "ETV process," as Hyde refers to it. The "ETV process" can mean different things. One "ETV process" is the process by which Independent Laboratories (ILs) use the ETV Protocol to conduct testing pursuant to the ballast water regulation requirements.⁵⁷ A different "ETV process" is the process by which the ETV Technical Panel considers new developments in ballast water treatment technology and whether to update or amend the ETV Protocol. In this case, the ETV Technical Panel convened to consider general issues relating to the ETV Protocol, including whether the use of viability as a measurement and the MPN assay as a measurement tool was acceptable, independently of Hyde's November 2012 MPN campaign.⁵⁸ Since MSC knew that this work was underway, it is reasonable that MSC (or CG-OES) would mention it to Hyde and suggest that Hyde wait for the ETV Technical Panel to conclude its work. If the ETV Technical Panel found in Hyde's favor, that decision would provide the basis for future Coast Guard action, including updating the regulations to include viability. It would also be very difficult for the Coast Guard to come to an independent conclusion on viability, as the Coast Guard does not have the same scientific and technical resources as the ETV Panel. Additionally, the Coast Guard never suggested that the ILs or the ETV Technical Panel could decide or approve a 46 C.F.R. § 162.060-10(b)(1) request. However, information from either "ETV process" can certainly be submitted to support a 46 C.F.R. § 162.060-10(b)(1) request.

⁵⁵ See, e.g., MSC email, dated 12 February 2015, HYDE APPX 000417. This email begins, "Please submit your request for alternates as equivalent as stated. We'll review your request and determine if a meeting is warranted." This is in direct response to an email from Hyde, dated 10 February 2015, which ended "Alternately, please let us know of your continued refusal of this meeting and we will submit our official 162.060.10(A) and 162.060.10(B) letters by the end of the week." HYDE APPX-000416. MSC's remarks were not an endorsement but an attempt to be responsive to a direct Hyde demand.

⁵⁶ See HYDE APPX-000417. "If manufacturers do not want to wait until the ETV technical panel process plays out, they can submit a -10(b)(1) proposal for acceptance of an alternative method...however, that request would have to meet the -10(b)(1) requirements..." This is an objective, likely palliative, instruction that does not take into account specific facts or presuppose that the request will be granted. MSC's following comments provide the specific warning that a "-10(b)(1)" request for MPN would not be simple. Even after receiving a favorable opinion from the ETV Technical Panel (which has not occurred), Hyde would still need to come back to the Coast Guard for further consideration.

⁵⁷ See, e.g., 46 C.F.R. § 162.060-42.

⁵⁸ "Currently, the MPN remains an unapproved method for determining the biological efficacy of the BWMS. The method remains under review by the EPA tech panel and we have no outlook on when an answer may be reached or any indication as to what that answer may be. I would be cautious of conducting testing prior to approval of this method if your system will rely solely on this method to meet the discharge standards...MPN data may be accepted as existing data following testing provided that...the MPN method is accepted as an approved method." MSC email, dated 6 February 2015, HYDE APPX-000413. This warning from MSC is categorical.

Finally, I reject Alfa Laval's characterization of the Coast Guard's *Shipboard Technology Evaluation Program* (STEP) program and development of its current ballast water requirements as implicitly accepting the MPN method. As I mentioned earlier, the Coast Guard has regulated ballast water for decades. The Coast Guard ballast water requirements began with a Congressionally-mandated ballast water exchange requirement, discussed *supra*, certain best management practices, and reporting and record keeping requirements. During this time, and in accordance with its statutory mandate,⁵⁹ the Coast Guard considered and reviewed various technological alternatives to ballast water exchange. These alternatives included BWMS based on UV technology. The STEP program is "intended to facilitate the development of effective [BWMS] technologies, to create more options for vessel owners/operators seeking alternatives to ballast water exchange...vessel owners/operators have expressed a reluctance to invest the resources to install and operate an experimental treatment system that might not meet discharge standards mandated by future regulations."⁶⁰ In other words, the point of the STEP program is that those BWMS are experimental. The fact that the Coast Guard, in 2008,⁶¹ accepted a UV BWMS for use on a STEP-enrolled vessel does not mean that the particular UV BWMS, regardless of its efficacy, *meets the regulatory discharge standard*.⁶² At the end of the day, MSC's actions were to uphold a regulatory discharge standard in light of a proposed alternative test method and were not an opinion on UV BWMS efficacy.⁶³

In sum, I find that MSC did not have the discretion to approve viability as an alternative measurement to the regulatory standards under 46 C.F.R. § 162.060-10(b)(1). The ballast water discharge standard in 33 C.F.R. Part 151 must be read together with the BWMS type approval requirements found in 46 C.F.R. Subpart 162.060 to understand the meaning of the word "living" in the Coast Guard's ballast water regulations. The type approval requirements do not allow "living" to be substituted with "viable," and therefore MSC did not have the discretion to approve a testing alternative that would insert viability into the discharge standard. While the appellants argue that certain Coast Guard employees agreed that 46 C.F.R. § 162.060-10(b)(1) could be used to insert viability into the discharge standard, I do not believe the administrative record definitively or specifically proves this assertion. Additionally, the Coast Guard's

⁵⁹ NANPCA/NISA, *supra*, n. 5, § 1101(e); *codified in* 16 U.S.C. § 4711(e).

⁶⁰ Navigation and Vessel Inspection Circular (NVIC) 01-04. The STEP program predates the 2012 Final Rule.

⁶¹ Letter dated October 31, 2008, from M. L. Blair, Captain, U.S. Coast Guard, Office of Operating and Environmental Standards, to Princess Cruise Lines, no. 33.151.2035.0040. This acceptance pre-dated the 2009 NPRM's publication.

⁶² A similar reasoning dismisses Alfa Laval's assertions that the Coast Guard violated the National Environmental Policy Act, Pub. L. No. 91-190, §2, 83 Stat. 852 (1969), as amended, *codified in* 42 U.S.C. §§ 4321, 4331-4335, 4341-4346, 4346a, 4346b, 4347. The purpose and need of the Coast Guard's 2012 Final Rule Final Environmental Impact Statement (FEIS) is to provide "an assessment of the potential environmental impacts associated with the proposed establishment of a ballast water discharge standard. The standard would be used to approve alternative ballast water management methods that are effective in preventing or reducing the introduction of nonindigenous species via discharged ballast water into the waters of the United States." FEIS Appendix F, which lists BWMS enrolled in the STEP program, is meant to provide a "rational basis" that BWMS exist that could achieve the discharge standard. The FEIS was not intended to prove that any particular system met the type approval standards established in 46 C.F.R. Subpart 162.060. Additionally, it is clear from the description of each of the alternative concentration levels considered as being "living organisms (per volume)" and in the description of UV BWMS assessed, that the Coast Guard examined the impacts of the regulatory discharge standard in the context of killing organisms (*see, e.g.*, Pages 2-5 and 2-6 and Appendix F).

⁶³ In fact, the Coast Guard still considers UV BWMS a valid ballast water treatment technology and believes that UV BWMS can be type approved under the existing regulatory requirements.

regulations and viability policy decision went through the public notice and comment process, and those decisions cannot be changed without returning to the public notice and comment process.

2. The Coast Guard has the statutory and regulatory discretion to reject alternative proposals

Alfa Laval and the other appellants contend that MSC's rejection of their applications for regulatory alternatives was tantamount to failing to consider them and that such failure was arbitrary, capricious, and inconsistent with law. As an initial matter, I believe the appellants confuse "to consider" with "to approve." It is clear from the record, including MSC's rejection letter of December 14, 2015, that MSC considered Alfa Laval's application. As discussed *supra*, MSC found that Alfa Laval's application failed to meet the second and third prongs of 46 C.F.R. § 162.060-10(b)(1). The fact that MSC ultimately denied Alfa Laval's application does not mean MSC did not consider it. If there were any defect in MSC's consideration,⁶⁴ I cure it now by independently finding that Alfa Laval's application fails to meet the requirements of 46 C.F.R. § 162.060(b)(1). That reasoning is provided in Section 3, *infra*.

Alfa Laval and the other appellants make various legal arguments asserting various levels of Coast Guard discretion or mandate to accept alternatives to the Coast Guard regulatory requirements. The Coast Guard does not dispute that it has the discretion, in theory, to accept viability as a BWMS efficacy measurement.⁶⁵ The Coast Guard also has not permanently rejected MPN as a BWMS measurement tool by a regulatory change. The 2012 Final Rule and its 2009 Notice of Public Rulemaking (2009 NPRM)⁶⁶ were very clear that the 2012 Final Rule is an interim phase of ballast water treatment and management. At that time, the Coast Guard did not have sufficient information to include viability as an approval criterion. However, NANPCA/NISA contains a mandate which requires the Coast Guard to periodically review and revise its ballast water regulations based on the best scientific information available.⁶⁷ If and when the Coast Guard has such information, it can reconsider whether to include viability. As this criterion would differ from the existing regulatory text and policy established through public

⁶⁴ MSC should have waited for the final results of the independent analysis before denying the application. We now have the final results, and my decision is based on those results.

⁶⁵ As a counterpoint to the appellants' arguments that NANPCA/NISA mandate that the Coast Guard accept viability by virtue of the NANPCA/NISA definition of "nonindigenous species" including the word "viable," Congress enacted the assumption that vessel water treatment systems should "kill" aquatic nuisance species: "provide an exemption from ballast water exchange requirements to passenger vessels with...treatment systems designed to kill aquatic organisms in ballast water..." NANPCA/NISA § 1101(c)(2)(K), 16 U.S.C. § 4711(c)(2)(K) (emphasis added). "Viable" is used in this definition to cover things like viruses, which are not universally considered to be "living." In any event, NANPCA/NISA is based on a precautionary rather than prescriptive framework. That means that the Coast Guard has the authority, under "maximum extent practicable," to regulate the concentration of both indigenous and nonindigenous, as well as invasive and noninvasive, species in ballast water in order to prevent the introduction and spread of aquatic nuisance species. For example, ballast water exchange does not discriminate among "viable" or "nonviable" organisms.

⁶⁶ See, e.g., Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters, Notice of Proposed Rulemaking, 74 Fed. Reg. 44632 at 44634 – 44635 (Aug. 28, 2009).

⁶⁷ NANPCA/NISA, *supra*, n. 5, § 1101(e)(1)(D); codified in 16 U.S.C. § 4711(e)(1)(D). The Coast Guard also committed to a regulatory obligation to conduct a practicability review for a more stringent standard, initiating a rulemaking by 2017 if appropriate. See, e.g., 33 C.F.R. § 151.1511.

notice and comment, this change would need to go through public notice and comment rulemaking.

While I agree, in principle, about the Coast Guard's discretion to accept viability, there are some very important statutory and regulatory limitations that these arguments raise that must be addressed within this response.

The Coast Guard interprets NANPCA/NISA Section 1101 (16 U.S.C. § 4711) differently than Alfa Laval and the other appellants. In particular, the Coast Guard disagrees with the applicability and interpretation of Section 1101(c)(2)(D) and believes Section 1101(e)(1) expressly or constructively "trumps" Section 1101(c)(2)(D).

Section 1101 can be difficult to understand if it is not read in the context in which it was originally enacted and subsequently amended.⁶⁸ When NANPCA was amended to include voluntary guidelines for the entire United States (Section 1101(c)(2)), the specific mandates in Section 1101(c)(2)(D) were for those initial voluntary guidelines:

The voluntary guidelines issued under this subsection shall-

- (D) direct a vessel that is carrying ballast water into waters of the United States after operating beyond the exclusive economic zone to-
 - (i) carry out the exchange of ballast water of the vessel in waters beyond the exclusive economic zone;
 - (ii) exchange the ballast water of the vessel in other waters where the exchange does not pose a threat of infestation or spread of nonindigenous species in waters of the United States, as recommended by the Task Force under section 4712(a)(1) of this title; or
 - (iii) use environmentally sound alternative ballast water management methods, including modification of the vessel ballast water tanks and intake systems, if the Secretary determines that such alternative methods are at least as effective as ballast water exchange in preventing and controlling infestations of aquatic nuisance species...

In other words, there was no existing nationwide standard, and Congress provided an initial *minimum* or *floor* for the Coast Guard⁶⁹ to meet. The Coast Guard's initial guidelines were based on the framework contained in Section 1101(c)(2)(D) and included guidance on conducting ballast water exchange for vessels carrying ballast water into waters of the United States after operating beyond the U.S. exclusive economic zone.⁷⁰ Eventually, the Coast Guard converted its voluntary guidelines to mandatory, regulatory requirements under the mandate

⁶⁸ Please refer to the background section of this response for this discussion.

⁶⁹ The Act refers to the "Secretary," defined as the Secretary of the department in which the Coast Guard is operating. For simplicity, I will instead refer to the Coast Guard, as the properly delegated entity of the Department of Homeland Security.

⁷⁰ See Implementation of the National Invasive Species Act of 1996 (NISA), Interim Rule, 64 Fed. Reg. 26672 (May 17, 1999).

contained in Section 1101(f)(1).⁷¹ These regulations were also based on the framework contained in Section 1101(c)(2)(D), as ballast water exchange remained the best available ballast water management option.

However, Section 1101(e)(1) requires the Coast Guard to consider revising its ballast water regulations no less than every three years. After conducting this periodic review, the Coast Guard is required under Section 1101(e)(1) to amend its ballast water regulations if, based on the best scientific information available, the existing guidelines and regulations implementing Section 1101(c) do not effectively reduce the introduction and spread of aquatic nuisance species by vessels. The Coast Guard's 2012 Final Rule was the result of an on-going review that began almost immediately after promulgation of the initial voluntary guidelines for ballast water exchange, and continued through participation in the development of the IMO ballast water management convention and development of test protocols for BWMS, and the best scientific information available showed that some BWMS were more effective than ballast water exchange in reducing the introduction and spread of aquatic nuisance species. While the Coast Guard initially characterized its new requirement of BWMS as an approval of an "environmentally sound alternative ballast water management method" under Section 1101(c)(2)(iii),⁷² the Coast Guard later explained its reasoning that subparagraph (c)(2)(D) merely set forth initial ballast water requirements for certain vessels and it was acting under the broader mandates found in paragraphs (a) and (e).⁷³ To read Section 1101 as permanently binding the Coast Guard to the initial floor set by Section 1101(c)(2)(D) would render Sections 1101(c)(2)(A) and 1101(e)(1) meaningless.⁷⁴ The BWMS manufacturers' argument that the Coast Guard is bound to implement all of Section 1101(c)(2)(D) is even more perplexing considering it would mean the Coast Guard has no discretion to phase out ballast water exchange in favor of BWMS.⁷⁵ The Coast Guard has moved beyond the initial mandate contained in Section 1101(c)(2)(D) to the

⁷¹ See Mandatory Ballast Water Management Program for U.S. Waters, Final Rule, 69 Fed. Reg. 44952 (July 28, 2004).

⁷² See, e.g., 2009 NPRM, 74 Fed. Reg. 44633.

⁷³ 77 Fed. Reg. 17282, 17286. This analysis also explains why the Coast Guard believes it has the authority to require all vessels equipped with ballast tanks – and not just those that have operated beyond the exclusive economic zone – to comply with its ballast water management requirements. The Coast Guard has previously, and publicly, rejected the legal argument that Section 1101(c)(2)(D) contains specific requirements which control "broader" requirements in Section 1101.

⁷⁴ I note Trojan's argument that NANPCA/NISA mandates the Coast Guard use "best science available," which Trojan evidently believes requires the Coast Guard to perpetually amend its equipment standards without going through a public notice and comment rulemaking. The Coast Guard properly incorporated the ETV Protocol into its regulations, as it does for many other vessel equipment standards such as those from the International Organization for Standardization. To suggest that the Coast Guard can "undo" a proper regulatory incorporation by reference without a notice and comment rulemaking is untenable. Additionally, the Coast Guard used "best science available" at the time it promulgated the 2012 Final Rule, four years ago. The Coast Guard is now completing its NANPCA/NISA-required periodic review, in which it is considering any new, properly validated scientific information. That information will inform whether to amend the Coast Guard regulations. Vessels must be able to keep up with changing equipment standards or the Coast Guard would not be maintaining NANPCA/NISA's "maximum extent practicable" mandate.

⁷⁵ Even reading Section 1101(c)(2)(D) alone, without reference to any other parts of NANPCA/NISA, cannot support this conclusion. Section 1101(c)(2)(D) is formed in the disjunctive, allowing the Coast Guard to choose all or only one of the options under it, while it was still operative. DESMI and Alfa Laval may have used a Coast Guard preambular statement out of context on page 12 of their respective appeals. The quoted statement, from the ballast water NPRM, meant that any alternatives to ballast water exchange must be approved by the Coast Guard, not that the Coast Guard was required to approve all alternatives to ballast water exchange.

more stringent mandate contained in Section 1101(c)(2)(A), which requires the Coast Guard to ensure, to the maximum extent practicable, that aquatic nuisance species are not discharged into the waters of the United States from vessels.⁷⁶

Thus, while I generally agree with the Alfa Laval's arguments that NANPCA/NISA gives the Coast Guard the discretion to consider and accept viability, I do not agree with all of their interpretations and analysis of NANPCA/NISA § 1101.⁷⁷ NANPCA/NISA does not "mandate" that the Coast Guard accept viability unless and until viability falls within the Coast Guard's responsibility to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels. Given the then-current state of science and technology, discussed *infra*, live/dead was the most environmentally conservative and practicably achievable standard, and viability does not yet fall within this mandate.

3. Alfa Laval's application fails to meet all elements of 46 C.F.R. § 162.060-10(b)(1)

I find that Alfa Laval's application did not meet the minimum requirements in 46 C.F.R. § 162.060-10(b)(1) and confirm MSC's denial of Alfa Laval's alternative request.

This decision is based, in part, on the technical review and conclusions of the Naval Research Laboratory (NRL),⁷⁸ whom the Coast Guard contracted to review the technical aspects of the alternative request submitted by Trojan.⁷⁹ The NRL review resulted in a comprehensive evaluation and the report includes more detailed descriptions and comments, not all of which were considered pertinent to the narrow issue of the 46 C.F.R. § 162.060-10(b)(1) requirement. I find the NRL report persuasive, and summarize my agreement with it as follows:

The applicants request that Coast Guard approve as equivalent an alternative to the required test specified in the ETV Protocol for determining the concentration of living organisms in the 10-50

⁷⁶ For this reason, Trojan's argument about accepting a BWMS as an alternate management system (AMS) fails. There is a difference between the initial standard of "at least as effective as ballast water exchange," which is consistent with the purpose of AMS – a bridging strategy between ballast water exchange and BWMS – and an evolved "maximum extent practicable" standard. Type approved BWMS must meet the *discharge standard*, not meet the floor of "at least as effective as ballast water exchange."

⁷⁷ I also reject Trojan's argument that NANPCA/NISA does not apply to non-reproductive organisms, and therefore the Coast Guard lacks the authority to promulgate the existing discharge standard. (Trojan appeal, page 45 – 46). NANPCA/NISA's reference to aquatic nuisance species, of course, concerns the species prior to treatment in a BWMS.

⁷⁸ NRL, "Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(B)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{M}$ and $<50 \mu\text{M}$," Feb. 10, 2016, 3900 Ser 6130/1622.

⁷⁹ The NRL reviewed only one 46 C.F.R. § 162.060-10(b)(1) application: Trojan. Nonetheless, the NRL report on Trojan is relevant to all four applicants (Trojan, DESMI, and Alfa Laval) submitted a base set of the same six documents containing a description of the proposed test, an overview of the use of the most probable number approach for evaluating BWMS, and results of various experiments conducted during development of the method, including several in which the alternative and required methods were compared. Hyde submitted only three of the six documents. Trojan also submitted additional documentation. Thus, Trojan's application was the most comprehensive of the four and covered all of the evidence provided by all four applicants. If Trojan's application fails to meet 46 C.F.R. § 162.060-10(b)(1), then all four fail. I have also included a discussion of arguments (vice documentation) of the individual applicants.

micrometers (μm)⁸⁰ size range in samples of water during type approval testing.⁸¹ The proposed alternative method is composed of two separate procedures, one (based on “viable”) for autotrophic (photosynthetic) organisms, and one (based on “living”) for heterotrophic organisms.

The autotroph procedure is based on using a “grow-out” approach, wherein samples are serially diluted, and replicate tubes at each dilution are cultured for a period of time, and then assayed for population growth of phytoplankton by detecting changes in the concentration of chlorophyll. The pattern of tubes with and without positive population growth is then used to estimate the probable original concentration, through a calculation termed MPN. The MPN statistical approach has been used for over a century to quantify monocultures (single species) of bacteria and phytoplankton, and several automated “calculators” are available for use. Significantly, the applicants propose to use MPN as the basis for quantifying concentrations of mixed assemblages of phytoplankton, composed of numerous different species in varying relative abundance.⁸² This is a “new” use for MPN as a statistical approach under a regulatory context and has not been adequately validated for such purpose.

The heterotroph procedure, for heterotrophic organisms, uses microscopy to count numbers of non-photosynthetic organisms that are motile, where movement is the criterion for determining an organism is alive. This method is conceptually similar to the required method, in that direct counts of “living” (not “viable”) organisms are made using a microscope. However, the specific procedures for determining whether an organism is “living” and the type of microscope are different than specified in the required method.

Compliance with 46 C.F.R. § 162.060-10(b)(1):

A. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?

I find that the applicant meets the first prong of 46 C.F.R. § 162.060-10(b)(1). Alfa Laval’s request to use the MPN assay is a request for an alternative to the testing requirements of the ETV Protocol, incorporated by reference into 46 C.F.R. § 162.060-26 and 46 C.F.R § 162.060-28, which are referred to in 46 C.F.R. § 162.060-10(f)(2).

B. Is that evaluation, inspection or test not practicable or applicable?

⁸⁰ A micrometer equals 0.001 millimeter.

⁸¹ I also note that all four applicants relied on the recent work of the ETV Technical Panel in their respective 46 C.F.R. § 162.060-10(b)(1) requests. While the appellants characterize their MPN-based method as being singular (i.e., “the MPN method”) and in use for decades, the record shows that the “MPN” method they submitted for alternative approval is the preliminary draft MPN-based method developed through the work of the ETV Technical Panel, which has yet to be validated (i.e., the specific submitted method was developed after the testing was conducted for their foreign type approvals).

⁸² I will refer to the applicants’ approach as the “alternative method.” I note that there is no globally accepted “MPN method” for BWTS type approval. While the applicants used the same MPN-based method, it appears that MPN approaches vary across flag administrations.

I find that Alfa Laval has not provided sufficient justification that the testing requirement is not practicable or applicable.⁸³ Three applicants argued, in various ways, that the required method was not practicable and/or not applicable for evaluating the efficacy of UV-based BWMS. Some of the arguments were not always clear whether they were being made on the basis of practicability or applicability, and so for this review I consider the two issues to have been combined and I review the appellants' arguments together. The applicants essentially make a two-part argument: first, that the phrasing of the discharge standard should be in terms of, or include, "viable" (or reproductive) organisms instead of, or in addition to, "living" organisms as currently phrased in the discharge standard; and second, that the required method for enumerating "living" organisms in the type approval requirements is not applicable to BWMSs intended to render organisms non-viable.

Only one applicant (Trojan) offered any objective basis to argue that the required method was not practicable (Miller 2015d). Trojan essentially argued that it is too expensive to use UV to kill organisms rather than render them non-reproductive, and so UV should be used to render organisms non-viable. This, according to Trojan, would provide an equivalent level of protection for the environment as killing them, because non-viable and dead organisms represented the same level of risk reduction with respect to biological invasions. Rather than supporting an argument that the required method is not practicable for evaluating numbers of living organisms, I view this as an argument that UV is not practicable for killing organisms in ballast water. Under the current type approval requirements, BWMS are required to be tested to demonstrate efficacy in reducing numbers of "living" organisms below concentrations in the discharge standard, and to do so using specific test methods that measure numbers of "living" organisms. As explained, *supra*, the Coast Guard cannot grant a waiver to the "living" requirement under the current regulations.

Furthermore, the proposed alternative method includes an assessment of "living" organisms in the case of heterotrophic organisms, which undermines the contention that the required method, which assesses "living" organisms is impracticable or inapplicable. In the proposed alternative method, a culture-based viability assay is used to assess viable autotrophic organisms (organisms capable of synthesizing their own food from inorganic substances using light or chemical energy; e.g., plants and algae) while a "living" assay is used for heterotrophic organisms (organisms that cannot manufacture their own food and instead obtain food and energy by taking in organic substances, usually plant or animal matter; e.g., animals and fungi).⁸⁴ Alfa Laval did not specifically address the issue of whether the required method is practicable or applicable. Additionally, Alfa Laval did not explain why it is practicable and applicable to evaluate "living"

⁸³ I agree that MSC's conclusion on practicability was not sufficiently justified. For that reason, I vacate the practicability decision of MSC and reach my own decision.

⁸⁴ The required method is used to enumerate both of these two components of the size group.

organisms when assessing the efficacy of UV systems in treating heterotrophic organisms, but it is not when the focus is autotrophic organisms. This inconsistency must be explained.⁸⁵

Specific to Hyde, I disagree that the required method for assessing the concentration of living organisms in treated ballast water is not applicable to UV-based BWMS. To make this assertion, Hyde is forced to define the term “living” in the discharge standard to mean viable or reproductive. As I explained, *supra*, I disagree that this is an appropriate definition of “living.” The Coast Guard intentionally used the broader term “living” because Coast Guard considered it less difficult and problematic to distinguish living organisms from dead organisms than to distinguish between reproductive and non-reproductive living organisms. While Hyde argues that conceptually, a non-reproductive organism represents the same risk of a biological invasion as does a non-living organism, and I do not disagree with that conceptual perspective, Hyde does not successfully argue that a reliable method exists for determining the reproductive ability of the thousands of species occurring in ballast water (this is discussed further below regarding the technical aspects of the proposed method).

For these general reasons, I find that the applicant’s assertion that the required method is not applicable to evaluating UV-based BWMS is unsubstantiated.

I address the following specific points raised by DESMI:

- i. *UV treatment of ballast water does not outright kill organisms in the water, but destroys their DNA and RNA making them incapable of reproduction.* This is a decision made by treatment system manufacturers on the basis of economic considerations, and reflects the specific, and differentiated from shipboard, circumstances of drinking water, food safety and waste water concerns. UV can have a biocidal (i.e., killing) effect. Currently the type approval testing requirements specify measurement of numbers of living organisms as determined by the specific method described in the ETV protocol. This method is as appropriate for enumerating living organisms in UV-treated water as it is for the same purpose in water treated by other means.
- ii. *Reproductive capability is central to the definition of invasive species laid out by the US Federal Aquatic Nuisance Species Task Force in 2012. It follows that organisms incapable of reproduction cannot become invasive species.* A 46 C.F.R. § 162.060-10(b)(1) analysis does not consider the definition of “invasive species” or whether organisms that are incapable of reproduction cannot become invasive species.

⁸⁵ Alfa Laval makes a similar argument against the Coast Guard, stating that for organisms in the <10 µm size class, tests for only 3 indicator species are allowed. This argument conflates the ETV Protocol with the overarching Coast Guard regulatory standard, found in 33 C.F.R. §§ 151.1511 and 151.2030. There is no “<10 µm size class.” The Coast Guard regulations set forth specific requirements for those three indicator microorganisms, and they are not ‘indicators’ for a size class. Alfa Laval also points to the Coast Guard’s incorporation of the option to use water from a U.S. public water system for ballast water management as evidence that MPN is acceptable. However, this point proves the opposite: the Coast Guard *limited* the option to *only* U.S. sources because of the difficulties in determining water quality from foreign sources. In the case of the United States, the regulatory requirements for these facilities are known, uniform, and verifiable. Additionally, water treatment facilities use MPN-based methods to estimate abundances of *specific* well known species, not the thousands in ballast water.

Rather, this section is concerned with the method(s) accepted by the Coast Guard under these equipment type approval regulations.

- iii. *Destruction of organisms' ability to reproduce therefore grants the same protection against aquatic invasive species as is obtained by killing the organisms.* The crux of the matter is the degree to which "ability to reproduce" can be determined for all of the different kinds of organisms in ballast water. I do not believe that there is currently an acceptably validated method that can be used to consistently assess whether organisms in treated ballast water are capable of reproduction.
- iv. *The FDA/CMFDA staining method prescribed in the ETV protocol for testing of ballast water treatment does not show whether an organism is capable of reproducing or not.* I do not dispute this assertion. However, if it is not practicable to adequately determine whether living organisms in treated water are able to reproduce, then limiting the number of "live" organisms includes both viable and non-viable individuals, and so provides a conservative, more protective limit.⁸⁶
- v. *Therefore, the FDA/CMFDA method is not well suited for assessment of the effectiveness of a UV based ballast water treatment system.* The relevant question is whether the required FDA/CMFDA method in the ETV Protocol is suitable for assessing effectiveness of a BWMS in meeting the "live" criterion in the USCG discharge standard, not whether it is well suited for a BWMS meeting some other discharge standard.
- vi. *On this basis it is concluded that:*
 - a) *The method contained in the ETV protocol for organisms in the 10-50 micron size category is not practicable or applicable to evaluate the performance of UV based ballast water treatment systems.* I disagree. DESMI frames practicability in terms of measuring something ("viable") that is not pertinent to the actual standard, which is framed in terms of "live" organisms. Again, the required method is practical for enumerating the number of living organisms in a sample of ballast water. The applicant desires to be held to a different end-point – not "living" but instead "able to reproduce".
 - b) *The proposed alternative test method provides same level of protection against invasive species, and is equivalent to existing requirements in terms of accuracy.* This is irrelevant to the issue of whether the required method is practical or applicable in the case of assessing the effectiveness of a BWMS in meeting the discharge standard. This point is addressed, *infra*, in regard to whether the proposed alternative is equivalent to the requirement.

I also address the following specific points raised by Hyde (HMI 2015):

⁸⁶ 77 F.R. 17274 ("Finally, it is more conservative, and thus more protective, to base efficacy decision on the basis of live/dead, rather than viable/unviable.").

- i. *“The inherent nature of the proposed staining technique measures the capability of organisms to hold fluorescence rather than to provide a measurement of “living/dead”...”* The fluorescent markers in the required method, as well as many other fluorescent markers specific for other biochemical characteristics of living organisms, are widely used in biology to differentiate living from dead.
- ii. *“...all scientific definitions of the word “living” include the ability to reproduce”.* This assertion is a gross mischaracterization of the definition of “living” in the context under discussion, which is how one can differentiate between a living organism and a dead organism. The context for the assertion is the higher-level issue of differentiating between “living” things (i.e., organisms) such as animals, plants, fungi, bacteria, etc and “non-living” things (e.g., rocks, water, fire, etc), wherein living things are defined in part as those things that exhibit reproduction at some time. Such an overarching definition is not pertinent to the issue at hand, as the required method is used to differentiate “living” organisms from “dead” organisms, not organisms from rocks (although the fluorescent “signal” from the marker does assist in seeing living organisms within the jumble of nonliving material such as bits of rock, shell fragments, and plant detritus.) Many “living” organisms are not capable of reproduction (adult humans who have undergone sterilization procedures; sterile castes in social insects, mules, female mammals that have reached menopause, etc). Viable organisms are by definition included in the larger sub-set of “living” organisms (because an organism must be alive to reproduce). If a reliable procedure can be identified by which viable individuals of thousands of different species can be consistently discriminated from non-viable individuals, then such methods could be included in the approved methods for determining numbers of living organisms, through amendment of the regulations. To avoid reducing the protectiveness of the discharge limits, any such viability assessment must also have levels of accuracy and precision equivalent to that of the method used to determine the number of living organisms.
- iii. *“UV as a disinfection product sterilizes organisms by disrupting their DNA and interrupting their ability to reproduce”.* The use of UV as a disinfection process is not the issue. The issue is whether UV has been, or can be, demonstrated to be an effective disinfection process for the vast number of different species in the 10-50 μm size group found in ships’ ballast water. UV is generally used to render organisms non-reproductive because it is cheaper than using UV to “kill.” Using UV to render organisms non-reproductive, rather than to kill, depends on a good understanding of the specific UV dose necessary to render specific organisms incapable of reproduction. This has been done for a relatively small subset of organisms that are either human pathogens (e.g., *Vibrio cholera*, several species of the genus *Cryptosporidium*, *Hepatitis A*, etc.) or indicators of poor water quality due to unsanitary conditions (e.g., *Escherichia coli* and several species of *Enterococcus*) that can be associated with the presence of such pathogens. By contrast, there are many thousands of species carried in ships’ ballast water, and little or nothing is known about the specific UV doses required to render these permanently incapable of

reproduction, nor is there a good understanding of how these many species could be consistently cultured in the laboratory to detect viability.

- iv. *In order not to eliminate UV disinfection in ballast water, a reproductive measurement assay must be used.* This is an argument based on economics, not the question of whether the required method is applicable to testing whether UV-based technologies are effective in achieving the limits on concentrations of living organisms.
- v. *“...in any disinfection enumeration, looking at the reproductive capability of the organisms studied is a more accurate representation of effectiveness than looking simply at their ability to hold a stain.”* This statement is only true for those disinfection processes intended to render organisms non-reproductive. For a process intended and designed to kill organisms, the appropriate approach in measuring effectiveness would be to identify the numbers of living organisms. The assertion that the required method “simply” looks at the ability of organisms to “hold stain” is a gross over-simplification and ignores completely the broad use of vital markers to identify “living” cells.
- vi. *“The international testing community and US EPA have historically used reproductive assays in lieu of staining to demonstrate the effectiveness of UV systems in ballast and other applications.”* The “international testing community,” meaning the test facilities conducting tests of BWMS for foreign administrations, may have used, collectively, a range of methods that involved reproductive assays, but these have not been made generally available to the public, including in particular the documentation demonstrating the careful validation of culture-based methods. Several different “methods” have been used by different facilities, and the validations that these methods were able to measure what they were intended to measure, or the comparisons among methods, have never been made public; indeed, at least one test facility has long asserted that the details of its methods are “proprietary.” When the ETV panel first began to consider the issue of whether an acceptable viability assessment could be identified for use in testing BWMS, the various test facilities and manufacturers were unable to provide or point to any specific validations, and had to subsequently conduct much of the validation work submitted (after much testing of BWMS had been completed) in support of the method being developed within the ETV Program, a draft of which was submitted to the Coast Guard for consideration under the -10(b)(1) provision.

I address the following specific points raised by Trojan (Miller 2015d):

- i. *The existing method is not practicable for evaluation of UV-based BWTS. The current requirement for analysis of the organisms in the 10-50µm size class in the ETV protocol requires the use of FDA/CMFDA stains to categorize organisms as being alive or dead. The stains evaluate the functioning of an organism’s esterase system as a proxy for cell death. Treatment with UV irradiation, causes damage that prevents cell replication (and thus precludes invaders from colonizing), but esterases*

are not directly affected at UV doses typically employed for disinfection, and thus effective treatment by UV is not evaluated by the existing method. Trojan asserts that the required method is not practicable for evaluation of UV-based BWMS that are intended to render organisms non-reproductive rather than dead. I do not dispute that the staining methods evaluate the functioning of an organism's esterase system rather than the ability of an organism to reproduce, but the issue is that the discharge standard is not phrased in terms of "non-reproductive" or "non-viable" organisms, but instead is phrased in terms of "living" organisms. At the time of the 2012 Final Rule, the Coast Guard believed that there were significant difficulties associated with determining the reproductive ability of the thousands of species of organisms found in ships' ballast water. Because of those difficulties, the Coast Guard determined that it was more practical and protective of the environment to phrase the standard in terms of living organisms. Concurrently with establishing the discharge standard, the Coast Guard established the required methods by which numbers of living organisms would be determined during type approval testing. The required method for organisms in the 10-50 μm size class is the FDA/CMFDA fluorescent marking method specified in the ETV Protocol. The applicant has not provided an adequate argument that the required method is not practicable for evaluating the number of living organisms in ballast water.

- ii. *A BWTS designed for UV doses equivalent to ten (10) times the current UV doses employed in the industry would require 10X the number of UV lamps, and would therefore be approximately 10X the footprint and require 10X the electrical power for operation. This increase would render UV systems impractical for implementation on board vessels.* Rather than making an argument that the required method is not practicable for enumerating living organisms after UV treatment, Trojan essentially argues that the required method is not applicable to UV treatment when such treatment is intended to render organisms non-reproductive. In essence, Trojan does not present an argument that the required method is not practicable, but rather that it is not practicable to use UV treatment to achieve the required discharge standard. Trojan argues that the UV doses commonly used in BWMS that are intended to render organisms non-reproductive are not great enough to induce mortality during type approval testing. Increased UV doses would not only prevent reproduction or cellular division, but if sufficient, also induce mortality that is detected following treatment using the required method. Thus, the required method would indicate treatment efficacy in meeting the "live" discharge standard because the dead cells would not fluoresce green. An alternative method would not be needed.
- iii. The applicant asserts that it is not practicable to increase the UV dose so treated cells are dead (as determined by the required method) rather than not viable (as determined by the alternative method). The applicant provides no data on the actual UV dosages necessary to kill the many species of organisms found in ballast water. Using derived ratios of UV doses needed to kill to doses needed to render organisms non-reproductive (no actual doses or dose-response curves were provided) from a study using 12 species of algae, the applicants argued that a UV dose sufficient to damage cells' non-specific esterases—the foundation of the required method—and reduce

concentrations of algae by 100-fold (from 1000 cells mL⁻¹ to 10 cells mL⁻¹) would be “extremely high”. The study found that for the 12 species, on average, a 10-fold increase in UV dose was needed to kill cells using the required method compared to the dose needed to show cells were non-viable using a culture-based viability assay. However, no data on actual dosages required to kill organisms or the power required to achieve those dosages were provided, so it is not possible to objectively evaluate this claim.

Consequently, I find that none of the applicants provided an adequate justification that the required method was not practicable and/or not applicable for its intended purpose when used to evaluate the efficacy of BWMS that used UV to treat organisms in the 10-50 µm size range in ballast water to meet the discharge limits set in the Coast Guard’s March 2012 Final Rule.

C. Is the proposed alternative equivalent to the regulatory standard?

I find that Alfa Laval has not justified the proposed alternative’s equivalency to the requirement. “Equivalence” among testing methods, in a literal sense, entails different methods of achieving the same measurement. A simple example would be measuring the length of an object by using a ruler in a specific manner or taking a photo of the object and analyzing length using an image analysis software program (in which case the program converts pixels along the delineation into length, based on a user-supplied calibration between pixels and length). In such a case, the methods result in measurement of the same thing (in the example, length). In a more technical sense, “equivalence” among testing methods⁸⁷ means that the methods must have the same or very similar accuracy (closeness to true value) and precision (degree of repeatability under unchanged conditions).⁸⁸

I have already found that the proposed alternative is not “equivalent” in the literal sense, and MSC did not have the discretion to use 46 C.F.R. § 162.060-10(b)(1) to rewrite the discharge standard. Even if the Coast Guard could accept viability without going through public notice and comment, which it cannot, the UV applicants’ alternative would still fail for technical reasons.

To determine if the alternative “viable” and required “live” methods are equivalent, a proposal for the alternative must demonstrate equivalent accuracy and precision of measurement – even if the actual parameters being measured are different. Precision can be assessed within a method, but accuracy is usually evaluated through comparison with a “true” value.

⁸⁷ I will assume for the sake of argument that “viable” and “living” relate equivalently to the risk of biological invasion. However, I do not reach a conclusion on that issue.

⁸⁸ Neither Hyde nor Alfa Laval included any additional arguments for accuracy or precision in their respective alternative requests, outside of the included documentation. Alfa Laval did not provide direct examination of either accuracy (agreement) or precision, outside of its included documentation. Alfa Laval presented information on the relative potential for “false negative” results in the required and proposed alternative approaches. While false negatives in the required method (living organisms that are not motile and do not stain) and proposed alternative (viable autotrophic organisms that do not reproduce under the provided conditions and living heterotrophic organisms that are not motile) are contributors to potential differences in agreement and precision, they are not the sole sources of error.

Accuracy or Agreement

“Accuracy” means that the two methods should return the same result in terms of closeness to a known or “true” value. In this case, we have no way of knowing the “true” concentration that is independent of the methods being evaluated. In other words, how do we count the organisms without using the methods under consideration? In the absence of a way to evaluate accuracy, several approaches were taken to evaluate the degree of agreement between the proposed alternative and the required method in determining the number of living and viable organisms.⁸⁹

The applicants analyzed three subsamples of each of three concentrations of the cultured phytoplankter *Tetraselmis suecica*, using both the required and the autotroph (MPN) methods (Miller and Petri 2015).⁹⁰ In this experiment, the cultures of the alga were healthy and robust, and it could be expected that all or most of the living cells were likely also viable. To evaluate agreement between the two methods, the applicants calculated the mean of the three subsamples using the autotroph method divided by the mean concentration determined using the required method, multiplied by 100 (the required method was considered the benchmark for this comparison). The value (converted to a percent) would be 100% if both methods yielded the exact same concentration. Because the “true” concentration is unknown, this metric actually quantifies the agreement between the required and alternative methods (rather than accuracy, which is the term used in the proposal).

In this test, where most if not all the cells were both living and viable, the percent difference in measured concentration between the required and alternative methods ranged from 38% difference at a concentration of 1000 cells/mL to 412% difference at the lowest concentration of 10 cell/mL. In other words, in a circumstance where one would expect the results to be the same, the result using the alternative was at best 38% different, and at worst 412% different than the result using the required method. It is particularly concerning that the percent difference was so great at the lowest concentration, which is essentially at the level of the discharge standard. However, due to the high degree of variability in results (6 of 9 means for the required test had standard deviations less than 10, while 6 of the 9 means for the alternative test had standard deviations greater than 46), it is difficult to draw any further conclusions from this test regarding how agreement between methods might vary as a function of concentration.

The applicants also evaluated the agreement between the alternative method and the required method when all of the organisms in the samples were heterotrophic. Cultures of the rotifer *Brachionus plicatilis* were evaluated using the heterotrophic component of the alternative

⁸⁹ “Agreement” means that the proposed alternative method provides at least the same result when circumstances are such that the results should be the same. In the supporting material, the applicants provided results from an experiment in which the two methods were used to measure the concentration of samples in which all cells were likely both living and viable. In such case, the two methods should result in the same numbers. If, as in this case, accuracy cannot be evaluated because there is no independently derived “true” value against which to assess the methods, then agreement can be used to evaluate equivalency.

⁹⁰ *MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 µm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology*. Only autotrophs were used; no heterotrophs were used in this case. Since all organisms were phytoplankton and presumably the phytoplankton were capable of growth (since they were in culture, under favorable light and nutrient conditions), there was no need to perform the heterotroph method in addition to the autotroph method to determine the total number of organisms.

method and direct counts using a stereomicroscope; in both of which, movement of organisms was the parameter used to assess whether the organisms were living. There was no difference between the two methods in the concentration of rotifers. However, in this experiment, the alternative heterotrophic method was not compared to the actual required method, which uses epifluorescence microscopy to detect positive reactions of two vital stains and cell movement. For a legitimate comparison, both the heterotroph and required methods must be used.

When using the two methods to assess samples of ambient communities (Miller *et al.*, 2015b), the applicant examined the agreement between methods using a calculated “factor of agreement” (FOA, calculated as the average alternative concentration/average required concentration). The FOA varied widely among different experiments, from 0.47 to 33.8. Half of the 16 comparisons had an FOA of less than one, and half had values greater than one; six of the latter values were greater than 5.0. It is difficult to draw any conclusions from this experiment, given the possible sources of confounding effects: the MPN counts could have been reduced due to the presence of viable but non-culturable species, or increased due to the presence of organisms smaller than 10 μm , while the stain counts could have been depressed by the presence of non-motile, non-staining organisms that were nonetheless living (false negatives). These potentially confounding effects would have to be carefully partitioned in experiments using ambient organisms, and that would entail a significant amount of work. Alternatively, consistently culturable species known to take up the fluorescent stains of the required method and larger than 10 μm in size could be used, in laboratory tests using mono- and mixed cultures, to evaluate the degree of agreement between the two methods. The need for statistical adjustments for the presence of viable but non-culturable species would need to be evaluated and possibly developed and incorporated into the method, and the effect of contamination by organisms less than 10 μm in size would also need to be addressed. Even with pre-filtration to remove organisms less than 10 μm , some contamination is unavoidable in samples of natural assemblages, and controls for this source of error will need to be developed.

Given the findings discussed above, I cannot agree that the proposed alternative has been reasonably shown to be equivalent to the required method in terms of agreement.

Precision

A measure of equal precision means that the two methods should demonstrate the same result in terms of the variability in the measurement (e.g., variance around the mean). Unlike accuracy, the variance in result can be examined without having to know the “true” concentration. Repeatability, even if “wrong”, is the key. Precision can be examined systematically for known cultures, both singly and mixed, as well as for a variety of ambient assemblages, although the assumption that all of the living organisms are also viable gets more tenuous when samples of organisms from the environment are used, rather than organisms from known cultures in a healthy phase of population growth.

Problems arise when the ability to get organisms to consistently reproduce sufficient for detection by a method is unknown. When organisms are obtained from culture collections, these are, to a degree, “predisposed” to growing under culture during an MPN-based method. In those cases, organisms are likely to be detected by serial dilution and culturing. For ambient

organisms collected from the environment, one would have to run repeated culture experiments: either by selecting organisms and culturing from an initial “seed” individual, or by somehow demonstrating that every time organisms of that species are present in an ambient sample, they demonstrate increased concentration under the provided culture conditions.⁹¹ In the experiments conducted by the applicants, this is at least partly examined when taxonomic analyses are conducted for the post-incubation dilution tubes. However, the focus in the analysis was on species shown to increase in population size at least some of the time, rather than on species that were shown to be culturable in all tests. For a repeatable standard method, consistent culturability would be critical.

Laboratory experiments with monocultures of the autotrophic organism *Tetraselmis* were conducted (Miller and Petri 2015) to compare the precision of the autotrophic component of the alternative method to that of the required method.⁹² The results from this test showed the precision was better in the required method than in the alternative method: the coefficients of variation (CVs, a measure of precision, with larger CVs indicating lower precision; calculated from the data in Miller and Petri 2015) were 1-26% (average = 13%) for the required method and 20-135% (average = 50%) for the proposed alternative. The means of the CVs resulting from the two methods were significantly different ($p < 0.05$), when compared using a non-parametric Mann-Whitney U-test. In all but one case, the CV of the required method was lower than the CV of the alternative method. Thus, by this comparison, the required method has greater precision than the proposed alternative.

Alfa Laval also compared precision between the heterotrophic component of the alternative method and a similar microscopical method in experiments using monocultures of the rotifer *Brachionus* (Miller and Petri, 2015). In this comparison, there was no difference between the results produced by the two methods. However, while the two methods were shown to have similar precision (CVs of 23% and 30% respectively), the method against which the alternative was compared was not the required method. Thus, the comparison is invalid and irrelevant for proving equivalent precision with the required method.

Precision was not specifically examined in the experiments conducted with ambient assemblages at two test facilities (Miller *et al.*, 2015b).⁹³ However the experiments did report the results of comparisons between concentrations measured with the required method and with the proposed alternative method at the two locations and on two separate dates, providing 4 measurements each for five methods.⁹⁴ The applicant did not provide raw data for the results from the experiment for the required method. However, a summary figure was produced showing average concentrations of organisms obtained using the five methods, and also showing some measure of

⁹¹ This would potentially be confounded by inhibition or encouragement of reproduction by other organisms that would not be in effect if the target species was present as one individual in a MPN dilution tube.

⁹² In this case, all of the organisms were autotrophic, so the heterotrophic component of the alternative method was not pertinent.

⁹³ MPN Method Development Report No. 5; Miller, A. F. Norlin and B. Petri; 2015. These experiments were focused on evaluating the best culture conditions and in examining the Factor of Agreement (FOA) between the two methods.

⁹⁴ The 5 methods were the required method and four combinations of the proposed alternative method using two media and two temperatures.

the variance around the averages as “error bars.”⁹⁵ The degree of variance as indicated by the error bars is clearly greater for the alternative method results than for the required method. Additionally, the CVs for the measurements using the alternative method are provided in a table. Interestingly, the range of CVs was 19-145 for the 14 tests where “0” variance due to method problems was not an issue, and the average CV was 58. This is very similar to the range and average observed for the alternative method when used to measure concentrations of the single autotroph *Tetraselmis* (range: 20-135 and average: 50), as discussed above.

On the basis of these comparisons, the Coast Guard cannot agree that required and alternative methods are equivalent with respect to precision.

D. Does the request include a full description of the proposed alternative?

The request does not contain a full description of the proposed alternative. Overall, the proposed alternative method is clearly described and understandable. However, in several specific sections there are ambiguities or potential errors that must be addressed:⁹⁶

While the MPN statistical approach is a common microbiological assay for calculating concentrations based on observations of population growth in replicate serial dilution cultures, Alfa Laval has failed to justify why it is appropriate to use for BWMS.

Many microorganisms, such as bacteria, fungi, and algae, can reproduce via asexual cell division, and under the appropriate conditions for growth, a single cell can reproduce to concentrations that are easily detectable. For autotrophic organisms such as phytoplankton, a suspension containing a single organism, given ideal conditions (e.g., nutrients, substrate, temperature, and light), will undergo exponential population growth. After sufficient time for such population increase, bulk metrics can be used to indicate the presence of that population, indicating that at least one reproductive organism must have been present in the original suspension. Changes in common bulk metrics, including chl-a fluorescence for algae and turbidity for bacteria, between initial measurements and measurements after a period of culturing, denote the presence of at least one organism in the initial diluted sample capable of undergoing reproduction.

MPN’s success under these circumstances does not mean that it is appropriate for all circumstances, particularly for use with ambient, mixed assemblages of phytoplankton rather than a monoculture. In a given water body, the number of species varies geographically and seasonally, but it is on the order of dozens, if not hundreds, of species.⁹⁷ Applying MPN to such diverse communities may violate the assumptions of the method.

⁹⁵ It is unclear what the “error bars” represent. They could be standard deviations, standard errors, or CVs.

⁹⁶ These issues are separate from the problems related to lack of equivalence identified *supra*.

⁹⁷ For example, over a thousand phytoplankton species have been identified in the Chesapeake Bay estuary.

The following assumptions, which were written in reference to bacteria but are also applicable for phytoplankton, are “necessary to support the MPN method” and are stated up front in all specific versions of the MPN statistical approach:⁹⁸

- a) The organisms are distributed randomly within the sample.
- b) The organisms are separate, neither clustered together nor repelling each other.
- c) Every replicate (tube, plate, etc.) whose inoculum contains even one viable organism will produce detectable population growth or change.
- d) The individual tubes of the sample are independent.

A full description of the method must explain why apparent violations of these assumptions are either not critical, or are otherwise accounted for.

The most critical assumption of MPN as a statistical approach is that each viable cell is capable of reproducing, so its original presence in the diluted subsample is detectable through population growth and an increase in the measured parameter over the course of the MPN incubation. The ability of an organism to reproduce must be independent of other organisms, inhibitory factors, such as toxicity due to the presence of trace metals and viruses in the cultures. If every cell may not be capable of being cultured in MPN tubes, and in turn, may not be detected, then the mathematical underpinnings of the MPN calculations are not applicable and procedures that incorporate the MPN methodology will not return accurate estimates. One of the most important aspects of test procedures that incorporate MPN as a statistical approach is that all of the necessary conditions for reproduction and population increase be present during the incubation period. If this is not the case, the method will not detect the presence of organisms that do not reproduce sufficiently to result in a detectable population increase, and the procedure will not result in an accurate measurement of original concentration.

With the above fundamental requirement in mind, it is important to recognize that the information submitted in support of the proposed method includes data showing not all species of phytoplankton are capable of being consistently cultured, at least under the conditions that were used, during all tests. This point is not addressed in the application, other than to assert, without substantiation, that it will present a small bias. The applicants argue that the important issue is whether all species have been observed to reproduce under the provided conditions at least once, rather than consistently (i.e., every time culturing is attempted), and the species that are not known to have reproduced at least once approaches 0% when a long-term historical record is considered. However, the key issue is not whether a viable organism of a species has been observed to reproduce at least once in the past, but instead whether it is known to reproduce consistently whenever present during a test. Thus the percentage of organisms that may be viable but non-culturable is greater when the ability to grow in each test is considered.

The data submitted with the proposed alternative indicate that the proportion of species observed to consistently demonstrate reproduction and population increase varied between the two locations where the issue was examined. At one site, 20-44% of species present in the samples had consistently demonstrated population growth in culture, while at the other the range was 56-

⁹⁸ See, e.g., US Food and Drug Administration, *BAM Appendix 2: Most probable number from serial dilutions*, Washington, DC, (2010).

89%. Hence, the proportion of species present that had not been observed to reproduce consistently in tests intended to detect organisms capable of reproduction was 56-80% and 11-44% at the other. The conclusion is that the test is not capable of detecting whether members of these species are capable of reproduction.

The assumption that organisms do not aggregate is clearly called into question for mixed assemblages of phytoplankton because of the existence of many species that naturally occur as multi-celled colonies. It may be that this problem could be ameliorated by gentle agitation of the sample to break up the colonies into individual cells, but many of the colonial types adhere quite strongly, and the ability to disaggregate the colonies without causing mortality would need to be established for all the colonial species that might occur in test waters.

The ability of every cell to be cultured is a fundamental tenet of MPN, and a significant shortfall in meeting that criterion calls into question the use of the proposed alternative method. This critical shortfall needs to be resolved.⁹⁹

Additionally, there is further uncertainty regarding the estimated MPN in the proposal. Typically, the upper and lower values defining the confidence interval (CI) are reported with MPN estimates. Standard methods for MPN analyses, including methods published by the EPA (U.S. EPA 1978), the U.S. Food and Drug Administration (U.S. FDA 2010), and other scientific authorities (APHA et al. 1999), include tables that list the corresponding 95% CIs. These values must be included when reporting the outcome of an MPN analysis to assure data quality.¹⁰⁰ Likewise, the calculators evaluated in the submitted documents all report CIs. However, the proposed method does not require reporting the CIs, nor explain why. As for all calculations of standard errors (SE), of which the CI is an example, larger sample sizes (n) generally result in smaller SE. Take for example, a single dilution MPN with a sample volume of 0.1 mL and an original undiluted concentration of 2.23 viable organisms per mL. Varying the numbers of tubes in an MPN, while holding the percentage of sample tubes positive for growth in each test at 20%, results in a wide range of confidence interval sizes around the MPN. Using 5, 10 or 20 tubes per test results in confidence intervals of 0.31 – 15.9; 0.56 – 8.9; and 0.84 – 5.96, respectively. The precision of the estimate of a 5-tube MPN was limited: the range of CI spanned two orders of magnitude (from 0.31-15.9 organisms mL⁻¹). Higher numbers of sample tubes yield narrower CI ranges, and thus greater confidence in the calculated MPN value.

The proposed method stipulates an MPN matrix should consist of 3 dilutions x 5 tubes per dilution. As shown from the above example, a larger number of tubes must be used to ensure that an MPN value generated from an MPN table does not have a large CI. If the 5 tube case described above were observed in an approval test, even though the MPN is 2.23 viable organisms per mL, a value below the discharge standard, the upper confidence limit is 15.9 viable organisms, a value above the discharge limit.

⁹⁹ This is and remains a central issue of discussion within EPA's ETV Technical Panel formed to consider, among other things, the acceptability of an MPN-based viability assay. The data presented in support of the proposed alternative method have been the basis for many of the panel's discussions, and a generally accepted resolution has not yet been identified.

¹⁰⁰ See, e.g., EPA, *Soil sampling quality assurance user's guide*, Report number EPA/600/S4-84-043, Washington, DC.

Of further concern is that the proposed alternative method does not account for the CIs generated by MPN tables. The CI can be relatively large (as noted above), and excluding the CIs can potentially result in a BWMS being considered to meet the discharge standard on the basis of the MPN, whereas the BWMS may not meet the discharge standard if the upper CI was taken into consideration. The lack of consideration of the CI in the alternative method is not explained, other than by a statement that they are not used. The CI, and its meaning with respect to the calculated MPN, must be explained in the alternative method and it should be reported with all results.

Additionally, several sections of the method description require clarification, as discussed below:

- i. **Sampling:** The sampling scheme described in the method allows for two options: "...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken." Having two options allows for unnecessary and potentially disruptive differences among practitioners. Furthermore, the latter option (one sample with 3 subsamples) could greatly affect the outcome of the sampling and is inappropriate for use in this circumstance. This option results in "pseudoreplication,"¹⁰¹ in which the "replicates" are actually "subsamples" that violate the assumption of independence among samples. If the latter option were used, the statistical analyses would be flawed, potentially yielding results that were wrong. Thus, there must be only one recommended sampling scheme, it should be the first option (three replicate samples).¹⁰²
- ii. **Autotrophs – Filtering:** The proposed alternative method directs that samples for the autotroph method be filtered onto 10- μm filters. However, if organisms $<10\ \mu\text{m}$ are retained on the filter, as could be expected, estimates of organism numbers from the MPN analysis will be artificially inflated, because the final MPN number would include organisms that are regulated by the Coast Guard ($\geq 10\ \mu\text{m}$ and $<50\ \mu\text{m}$) as well as those that are not regulated by Coast Guard ($<10\ \mu\text{m}$). Similarly, organisms $\geq 50\ \mu\text{m}$ could also be retained on the filter, again, artificially inflating the MPN estimate. The applicant asserts this bias would be small, but in any case, it would be prudent for each test facility using this approach to examine the potential for these circumstances to bias the estimates from the MPN analysis. According to the method description, the filter may or may not be left in the MPN tube during the grow-out period. It is unclear if the practice of leaving a filter in the tube affects the potential for population growth due to smaller organisms entrained on the filter being included in the MPN tubes, or if the presence of the filter itself affects the fluorescence reading. The alternative method must provide unambiguous direction, and if the direction is to leave the filter in the tube, data showing that the practice does not affect the results must be presented.

¹⁰¹ Hurlbert SH, *Pseudoreplication and the design of ecological field experiments*, Ecol Monogr 54:187–211 (1984).

¹⁰² This does not apply to sample tubes used in the MPN approach; in such case, water for all dilution tubes (i.e., an array consisting of 3 dilutions, each with 5 replicates) should be drawn from the same population. Tubes are inoculated with water from a single, original sample.

- iii. Autotrophs – Measurements: To determine if reproduction and population growth has occurred in an MPN tube, the proposed method specified a minimum threshold fluorescence of four times the standard deviation (SD) of fluorescence measurements from method blanks (tubes containing no chlorophyll). In order for this threshold to be uniformly applied across laboratories, the fluorometers would need to be calibrated following the same, standardized procedure, and demonstrate consistency in measuring the threshold value. This specification must be included in the proposed method.
- iv. Heterotrophs – Detection: The heterotroph component of the proposed alternative method relies partly on the ability to detect the red autofluorescence of chlorophyll-a (chl-a) containing organisms using epifluorescence microscopy. However, the optical filter set specified in the method is optimized to detect the fluorescence from fluorescein, not chl-a; according to the wavelengths of the filter set specified in the heterotroph method (B-2E/C), the red autofluorescence of chl-a would not be visible (i.e., the optical filter would not serve to identify organisms with chl-a). Potentially, all organisms detected would not fluoresce, and would appear to lack chl-a and, therefore, if they were motile, they would be scored as heterotrophs. Filter sets are available that allow chl-a fluorescence to be detected and must be used. Additionally, the procedure used to quantify living heterotrophic organisms scores cells that are moving and do not show red autofluorescence (i.e., they do not contain chl a) as living heterotrophs. However, if organisms do not exhibit fluorescence and are not viewed with another light source (one is not stipulated in the proposal), they will, whether moving or stationary, be, at best, dimly illuminated by light and difficult to see. This potential problem should be addressed.
- v. Data Analysis: It is unclear how the uncertainties around the counts resulting from the autotroph and heterotroph components of the proposed alternative method are applied. Each set of replicate measurements will result in an estimate of the variance around the mean. If the variance estimates are to be added together, as are the means to arrive at a total number of organisms, there is no statistical justification provided for doing so. Instructions, and justifications for such, should be provided.

Conclusion

In sum, I deny your appeal and affirm MSC's decision denying Alfa Laval's request for a testing equivalency and type approval under 46 C.F.R. § 162.060-10. When promulgating its ballast water regulations, the Coast Guard explicitly rejected "viability" as part of its ballast water discharge standard. This policy decision was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations. MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). Because Alfa Laval's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying Alfa Laval's type approval application.

MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations. MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1). Because Alfa Laval's type approval application depended on tests which did not comply with the regulations and were not accepted as regulatory alternatives, MSC was correct in denying Alfa Laval's type approval application.

This decision constitutes final agency action on the issues raised in your appeal.

Sincerely,



Linda L. Fagan

Rear Admiral, U. S. Coast Guard

Deputy for Operations Policy and Capabilities

Technical References

1. (Miller 2015a) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Supplemental Information 02 MAR 2015
2. (Miller 2015b) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Updated Documentation 06 MAY 2015
3. (Trojan Marinex 2015a) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples 31 JAN 2015
4. (Trojan Marinex 2015b) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples, 05 MAY 2015
5. (Petri 2015a) Evaluating the MPN Dilution-Culture Method for the Enumeration of Viable Phytoplankton Cells
6. (Maurer and Welschmeyer 2015a) Flow Cytometric Analysis of the Relative Abundance of Heterotrophs and Autotrophs in the Regulated 10-50 μm Size Class
7. (DHI 2014) MPN Assay – Analyses of Algal Regrowth for Performance Evaluation of Ballast Water Management Systems Primary Validation
8. (Petri 2015b) MPN Method Development Experiments 1 to 3 Inter-Lab Comparison of the MPN Dilution-Culture Method and Fluorescein-Based Staining Methods for the Enumeration of Viable or Living Phytoplankton Cells
9. (Miller et al. 2015a) MPN Method Development Report Experiment 4
10. (Miller et al. 2015b) MPN Method Development Report Experiment 5
11. (Miller and Petri 2015) MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 μm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology
12. (Cullen and MacIntyre 2015) On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment
13. (Maurer and Welschmeyer 2015b) Rationale for the Use of Most Probable Number (MPN) Technique in the Evaluation of UV-based Ballast Water Management Systems
14. (MacIntyre et al. 2015) Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water

15. (Miller 2015c) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 03 JUN 2015
16. (Miller 2015d) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 30 JUN 2015
17. (Miller 2015e) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 23 JUL 2015
18. (Miller 2015f) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 26 JUL

U.S. Department of
Homeland Security

United States
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JUL 12 2016

Hyde Marine, Inc.
Attn: Mr. Mark Riggio
2000 McClaren Woods Drive
Coraopolis, PA 15108

Dear Mr. Riggio,

I refer to your appeal dated January 13, 2016, on behalf of Hyde Marine, Inc. (Hyde). You requested a formal administrative appeal of the decision of the Marine Safety Center (MSC) denying your request for a testing alternative to be used in the Coast Guard's ballast water management system (BWMS) type approval process.¹

This is an administrative appeal of an MSC decision or action taken pursuant to 46 Code of Federal Regulations (C.F.R.) § 162.060-10(b)(1) and is reviewed by my office under 46 C.F.R. § 159.001-2 and as provided in 46 C.F.R. § 1.03-15. In considering your request, I reviewed your appeal (including its appendix and the administrative appeals of DESMI Ocean Guard A/S, Alfa Laval Tumba AB, and Trojan Technologies, incorporated by reference via letter dated March 6, 2016), the administrative record (including MSC's denial of your request for reconsideration), and applicable laws, regulations, and policy.²

Based on this review, I hereby deny your appeal, affirming MSC's decision to deny Hyde's request for testing equivalency under 46 C.F.R. § 162.060-10(b)(1). This matter and your appeal are quite technical and detailed, and the issues raised will be discussed in more detail in this letter.³ To briefly summarize, during the development of the ballast water regulations, the Coast Guard explicitly rejected the use of BWMS that "may act to make organisms unviable or unable to reproduce rather than killing or removing them." This policy decision to reject "viability" was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations, and MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements

¹ Type testing and approval are used in equipment and manufacturing to determine that a specific "type" of equipment or process meets a minimum set of requirements. In this context, "type approval" is a vessel equipment approval process.

² I note that in the Trojan appeal, and, by incorporation by reference, Hyde, has reserved the right to "supplement" its appeal to address my response. That is unnecessary. This response constitutes final agency action on the issues raised in Hyde's appeal.

³ I have substantively responded to your appeal in the interest of transparency. This response does not waive any defenses the Coast Guard may have as to the timeliness of your assertions or failure to exhaust your administrative remedies.

of 46 C.F.R. § 162.060-10(b)(1). Your appeal is denied, and this decision constitutes final agency action.

Background

Marine environmental protection is one of the Coast Guard's core statutory and operational missions.⁴ As stewards of the marine environment, the Coast Guard maintains a robust environmental protection regulatory program and also assists other federal agencies in enforcing laws to protect, preserve, and remediate waters subject to the jurisdiction of the United States. The Coast Guard also leads and participates in initiatives at the International Maritime Organization (IMO), the intergovernmental organization specializing in commercial shipping safety, security, and environmental protection standards, to raise and standardize global shipping practices. These activities all have one desired end state: to help ensure the health and vitality of waters of the United States and its living marine natural resources.

The Coast Guard manages its marine environmental protection obligations through a well established network of Headquarters, regional and field offices. Within Coast Guard Headquarters, located in Washington, D.C., there are several organizations, or "programs," responsible for developing, promulgating, and enforcing marine environmental protection standards. The Office of Operating and Environmental Standards (CG-OES) and the Office of Design & Engineering Standards (CG-ENG) have the lead roles in promulgating and implementing (but not enforcing) Coast Guard environmental regulations, including requirements for approval of equipment installed on vessels. MSC's role focuses on regulatory compliance and policy development, generally related to plan reviews for domestic vessels and type approvals for vessel equipment. While MSC can be involved in the clearance process for rulemakings, MSC is not the lead office for environmental standards development. In other words, MSC applies environmental regulations but does not create them. All three programs are within the Directorate of Commercial Regulations and Standards (CG-5PS).

The Coast Guard's ballast water program is one of the Coast Guard's long-standing marine environmental protection programs. It is established under the authority of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, as amended by the National Invasive Species Act of 1996 (NANPCA/NISA).⁵ As mandated by NANPCA, the Coast Guard's program began in 1991 with voluntary ballast water management guidelines for the Great Lakes.⁶ Mandatory requirements for the Great Lakes followed these voluntary guidelines in 1993.⁷ In 1996, NISA amended NANPCA to, among other things, cover all navigable waters of

⁴ See Section 888, Homeland Security Act of 2002, Pub. L. No. 107-296 (H.R. 5005), 116 Stat. 2135 (2002), as amended, *classified to* 6 U.S.C. § 468.

⁵ Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (NANPCA/NISA), Pub. L. No. 101-646 (H.R. 5390), 104 Stat. 4761 (1990), *as amended; codified in* 16 U.S.C. §§ 4701, 4702, 4711, 4712-4714, 4721-4728, 4741, 4751. Congress enacted NANPCA in response to the disruption and damage caused by the introduction of nonindigenous zebra mussels into the Great Lakes, likely released via discharges of ships' ballast water. NANPCA originally focused on ballast water operations in the Great Lakes but was later amended by NISA to cover waters nationwide.

⁶ NANPCA/NISA, *supra*, n. 5, § 1101(a); *codified in* 16 U.S.C. § 4711(a). *See also* Ballast Water Management for Vessels Entering the Great Lakes, Final Rule, 58 Federal Register (Fed. Reg.) 18330 (April 8, 1993).

⁷ *Id.*

the United States.⁸ In the 1990s, the best method available for preventing the discharge of aquatic nuisance species from ballast water was an operational practice known as ballast water exchange. For this reason, NANPCA/NISA contained a specific requirement for certain vessels to conduct ballast water exchange.⁹ The Coast Guard updated its regulations to include nationwide, mandatory ballast water exchange requirements in 2004.¹⁰ However, ballast water exchange was an interim measure until more effective ballast water technology could be developed and verified. Under the NANPCA/NISA mandate to “ensure to the maximum extent practicable that aquatic nuisance species are not discharged into waters of the United States from vessels,” the Coast Guard collaborated with domestic and international partners to identify better methods and technology to prevent the introduction and spread of aquatic nuisance species. These efforts ultimately resulted in the Coast Guard’s 2012 Final Rule, Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters (2012 Final Rule).¹¹

NANPCA/NISA is a domestic legal authority and does not explicitly implement the international standard for ballast water discharges, which is found in the IMO International Convention for the Control and Management of Ships’ Ballast Water and Sediments, 2004 (BWM Convention).¹² The disconnect between U.S. domestic law and the international standard is partly timing: NANPCA was enacted in 1990, many years before the international community adopted the BWM Convention in 2004. Additionally, at this time, the United States is not a contracting government to the BWM Convention¹³ and the BWM Convention has not received enough ratifications to enter into force. The Coast Guard attempted to harmonize the 2012 Final Rule with the BWM Convention to the extent possible within its statutory authority.¹⁴ Ultimately, the

⁸ See NANPCA/NISA, *supra*, n. 5, § 1101(a) – (c); *codified in* 16 U.S.C. § 4711(a) – (c). This statutory bifurcation is the historical reason why Coast Guard ballast water regulations are promulgated in two subparts of 33 C.F.R. Part 151, despite there now being few differences between the ballast water management standards for the Great Lakes and those for the rest of the United States.

⁹ Ballast water exchange is a process by which a vessel replaces water, generally coastal water, in its ballast tanks. See definition of “exchange,” 33 C.F.R. § 151.2005(b).

¹⁰ Mandatory Ballast Water Management Program for U.S. Waters, 69 Fed. Reg. 44952 (July 28, 2004).

¹¹ 77 Fed. Reg. 17254 (March 23, 2012).

¹² IMO Doc. BWM/CONF/36.

¹³ If the United States became party to the BWM Convention, the Coast Guard could implement the convention through NANPCA/NISA even though there is no explicit reference. See NANPCA/NISA, *supra*, n. 5, § 1101(f)(3); *codified in* 16 U.S.C. § 4711(f)(3).

¹⁴ See, e.g., 77 Fed. Reg. 17260. I note that the Coast Guard has made some statements which could confuse the issue of whether the Coast Guard ballast water discharge standard is “identical” to the BWM Convention standard. From the Coast Guard’s perspective, as discussed further *infra*, the BWM Convention’s use of “viable” is synonymous with the Coast Guard’s use of “living,” and in that sense the standards are the same. However, there are some minor differences between the two regimes. That is why the Coast Guard has used certain language, such as “align with” and “equivalent to,” to show that the Coast Guard regime is *not* identical to the BWM Convention regime.

BWM Convention is not a treaty of the United States, and the Coast Guard has a mandate to implement NANPCA/NISA as written.¹⁵

The Coast Guard's ballast water regulations are codified into Titles 33 and 46 of the C.F.R. The regulations in 33 C.F.R. Part 151 Subparts C (Great Lakes) and D (Nationwide) are operational or performance standards, and the regulations in 46 C.F.R. Subpart 162.060 are equipment standards.¹⁶ In this case, the Title 33 operational requirements apply to vessels, vessel owners or operators, or other persons associated with the vessel, and the Title 46 equipment requirements apply to BWMS manufacturers seeking equipment approvals. However, as discussed *infra*, the two are still interrelated and should be read together.

The 2012 changes to 33 C.F.R. Part 151 introduced a scheduled phase-out of ballast water exchange as an accepted operational measure to reduce the introduction and spread of aquatic nuisance species and provided the new option of using a BWMS to treat ballast water prior to discharging it.¹⁷ 33 C.F.R. Part 151 now contains a numeric discharge standard for the maximum number of living organisms in ballast water, a standard which all Coast Guard-approved BWMS must meet.¹⁸ The 2012 changes also added other ballast water management options, including the use of water from U.S. public water systems and discharge to a reception facility. For a vessel meeting the discharge standard by using a BWMS to be in compliance with 33 C.F.R. Part 151, its BWMS must have received type approval under the standards located in 46 C.F.R. Subpart 162.060.¹⁹

I now address the issues raised in your appeal, as well as the principal arguments raised by the other manufacturers in their respective appeals, which have been incorporated by reference, as follows:

1. MSC did not have the discretion to approve Hyde's request

¹⁵ As discussed further, *infra*, the Coast Guard's regulatory standard is "to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels..." NANPCA/NISA, *supra*, n. 5, § 1101; *codified in* 16 U.S.C. § 4711. The Coast Guard believes the "practicability" element of this standard requires the U.S. ballast water discharge standard to take into account the relevant IMO standard, as most vessels engaged on international voyages will likely need to comply with the BWM Convention once it comes into force. If the U.S. domestic standard conflicts with or frustrates an international standard, the result could range from increased vessel costs to preventing the vessel from calling on certain ports (implementing whichever standard the vessel cannot meet).

¹⁶ Generally, for the C.F.R., the reason vessel operational standards appear in Title 33 and vessel equipment standards appear in Title 46 is because many Coast Guard statutory authorities are contained either in Title 33 of the United States Code (Navigation and Navigable Waters) or Title 46 (Shipping). Title 33 authorities are generally more "operational," such as the Ports and Waterways Safety Act (Pub. L. No. 92-340 (H.R. 8140), Tit.I, 86 Stat. 424 (1972), *as amended*; *codified in* 33 U.S.C. §§ 1221 – 1232) and the Act to Prevent Pollution from Ships (Pub. L. No. 96-478, 94 Stat. 2297 (1980), *as amended*; *codified in* 33 U.S.C. §§ 1901 – 1911). On the technical side, the Coast Guard maintains broad authority to regulate inspected vessel equipment under 46 U.S.C. § 3306, and its vessel equipment approval processes are correspondingly located in Title 46 of the C.F.R. This is true even when the underlying statutory authority is contained in a different title. In this case, the Coast Guard has overlapping statutory authority to set BWMS equipment standards, under both NANPCA/NISA and 46 U.S.C. § 3306.

¹⁷ 33 C.F.R. § 151.1510(a); 33 C.F.R. § 151.2025(a).

¹⁸ 33 C.F.R. § 151.1511; 33 C.F.R. § 151.2030.

¹⁹ *See, e.g.*, 33 C.F.R. § 151.2025(a)(1).

I agree with MSC that it lacked the discretion to approve Hyde's 46 C.F.R. § 162.060-10(b)(1) request, which sought approval of a testing method that measured "viable" rather than "living" organisms in ballast water. The Coast Guard's type approval regulations exclude "viability" as an option. This was an environmentally conservative policy decision, based on best scientific information available, which went through the public notice and comment process. Therefore, MSC could not grant an alternative request that circumvented the text and policy position of the Coast Guard's ballast water regulations.

Issue:

Hyde is a BWMS manufacturer who requested that the Coast Guard approve an alternative to the Coast Guard's BWMS type approval requirements. Hyde manufactures different models of BWMS, including those that use ultra-violet radiation (UV) to treat ballast water. Hyde submitted its application for a BWMS testing alternative under 46 C.F.R. § 162.060-10(b)(1), and the crux of this appeal revolves around the type of tests that can be used, under the Coast Guard regulations, to validate the efficacy of UV BWMS. Specifically, Hyde requested to use a "Most Probable Number assay" (MPN) in lieu of 5-chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) direct staining methods to test for certain organisms to meet type approval requirements.²⁰ The simplified distinction between these measurement methods is that Hyde's preferred measurement method measures "viability" of an organism, while the regulatory requirement method measures whether an organism is "living." This appeal concerns the testing method being used and not whether the Coast Guard can or will grant type approval to UV BWMS as a class of system.²¹ While Hyde has characterized the question as "whether the measurement method – MPN – is reliable and accurate," the main question before me is whether the ballast water regulations allowed MSC to approve MPN as a measurement tool.²²

Applicable regulatory standards and the meaning of "living":

46 C.F.R. § 162.060-10(b)(1) provides:

If an evaluation, inspection, or test required by this section is not practicable or applicable, a manufacturer or independent laboratory may submit a written request to the Commanding Officer (MSC), Attn: Marine Safety Center, U.S. Coast Guard Stop 7410, 4200 Wilson Boulevard Suite 400, Arlington, VA 20598-7410, or by email to msc@uscg.mil, for approval of alternatives as equivalent to the requirements in this section. The request must include the manufacturer's justification for any proposed changes and contain full descriptions of any proposed alternative tests.

²⁰ HYDE APPX-000419. Hyde characterized its submission as a request for a "pre-test variance," which is not a term used in the ballast water regulations. MSC treated it as a 46 C.F.R. 162.060-10(b)(1) alternative request.

²¹ Not all UV BWMS are designed like Hyde's: some UV BWMS are designed to kill organisms rather than to render them unviable. Thus, my decision should not be interpreted to mean that the Coast Guard will not grant type approval to any UV BWMS under the current regulatory standards.

²² A follow-on question, answered in Section 3 of this response, is whether Hyde met all elements of 46 C.F.R. § 162.060-10(b)(1).

The Coast Guard applies 46 C.F.R. § 162.060-10(b)(1) by considering the following four elements:

1. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?
2. Is that evaluation, inspection or test not practicable or applicable?
3. Is the proposed alternative equivalent to the regulatory requirement?
4. Does the request include a full description of the proposed alternative?

MSC's decision denying Hyde's application specifically addressed items 2 and 3 and found them both in the negative.²³ MSC found that Hyde's proposed alternative was not equivalent because

...it does not measure the efficacy of the ballast water treatment system to the performance standard required by the regulations. The regulations specifically require ballast water treatment systems to be evaluated based on their ability to kill certain organisms. Since the proposed MPN method assesses the viability of an organism to colonize after treatment, it measures to a different standard than that required by the regulations.²⁴

You contend for several reasons that this reasoning was unsound.

However, I believe MSC was correct that the Coast Guard's ballast water management regulations do not allow MSC to approve a method that measures viability in lieu of the regulatory standards.²⁵ I believe the reference, above, to the "performance standard required by the regulations" means the ballast water discharge standard contained in 33 C.F.R. Part 151.²⁶ As a general principle, a technical equipment standard in Title 46 of the C.F.R. would not be able to override a performance or operational standard in Title 33 of the C.F.R. In this case, the technical equipment standard and the operational standard are inextricably linked, and the meaning of "living" cannot be resolved by viewing a stark dichotomy between Titles 33 and 46 of the C.F.R.

To understand why, it is helpful to begin with the text of 46 C.F.R. § 162.060-10(b)(1). Hyde's request must meet the first prong of 46 C.F.R. § 162.060-10(b)(1), requesting an alternative to an "evaluation, inspection, or test required by this section." This is a reference to the requirements contained in 46 C.F.R. § 162.060-10(f) (emphasis below added):

A BWMS is eligible for approval if –

²³ Having found at least one of the elements in the negative, there was no need for MSC to opine on all of these elements. Additionally, I disagree that MSC did not "at least consider" Hyde's application. (Hyde appeal, page 15).

²⁴ Letter dated December 14, 2015, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Hyde Marine Inc., "Request for Approval of the Use of the Most Probable Number (MPN) Method to Determine Biological Efficacy of the Hyde Guardian Ballast Water Management System (BWMS)".

²⁵ This is not a matter of literal "equivalency" of testing methods, which is discussed *infra*, Section 3.

²⁶ See Letter dated February 2, 2016, from J. W. Mauger, Captain, U.S. Coast Guard, Marine Safety Center, to Hyde Marine Inc., "Request for Review and Reconsideration of December 14, 2105 Decision of Marine Safety Center Denying Application for Approval of Equivalent Test Method Under 46 C.F.R. § 162.060-10(b)(1); ("Therefore, in order to demonstrate compliance with the BWDS as set forth at 33 CFR §§ 151.1511 and 151.2030, a BWMS must be evaluated on the basis of counting living/dead vice viable/unviable" organisms.").

(2) It is evaluated, inspected, and tested under land-based and shipboard conditions in accordance with §162.060-26 and 162.060-28 of this subpart, respectively, and thereby **demonstrates that it consistently meets the ballast water discharge standard in 33 CFR part 151, subparts C and D;**

(3) All applicable components of the BWMS meet the component testing requirements of §162.060-30;

(4) The BWMS meets the requirements of §162.060-32 of this subpart if the BWMS uses an active substance or preparation...

This provision clearly delineates the operational standard contained in 33 C.F.R. Part 151 from the equipment standard in 46 C.F.R. Subpart 162.060 and supports MSC's reasoning that a Title 46 alternative or equivalency for vessel equipment testing cannot be used to override a Title 33 performance standard. However, the actual text in 33 C.F.R. Part 151 shows that the two standards are inextricably linked (emphasis below added):

(a) Vessels employing a Coast Guard-approved ballast water management system (BWMS) must meet the following BWDS by the date in §151.1512(b) of this subpart:

(2) For organisms less than 50 micrometers and greater than or equal to 10 micrometers: discharge must include fewer than 10 **living** organisms per milliliter (mL) of ballast water.²⁷

There is no definition of "living" or any other regulatory text in 33 C.F.R. Part 151 that explains this important detail of the discharge standard. The only way a ballast water manufacturer can understand the "living" organism standard in 33 C.F.R. Part 151 is by referring to 46 C.F.R. Subpart 162.060 and its technical requirements and reading the preamble of the final rule. Thus, while the discharge standard in 33 C.F.R. Part 151 is an operational standard which applies to vessels, it is inextricably intertwined with the technical equipment standards contained in 46 C.F.R. Subpart 162.060 which apply to ballast water manufacturers.²⁸ The substance of "living" remains within 46 C.F.R. Subpart 162.060, and MSC was not solely bound by the indeterminate "living" language contained in 33 C.F.R. Part 151.

46 C.F.R. Subpart 162.060 also has no definition of "living,"²⁹ but this subpart contains extensive efficacy requirements which constructively define the term. Specifically, the Coast

²⁷ 33 C.F.R. § 151.1511, emphasis added.

²⁸ For vessels engaged on international voyages, this interlinkage between performance standards and technical standards is common, as typically a vessel's operation of a type approved or certificated piece of equipment satisfies the operational requirement unless a Coast Guard inspector or investigator has reason to believe that the equipment is not operating or being operated properly.

²⁹ Hyde's sole focus on the definition of BWMS (46 C.F.R. § 162.060-3) is misplaced. The BWMS definition alone does not set a performance or technical standard for BWMS. It merely identifies a category of equipment that the Coast Guard is regulating. The performance and technical standards in Titles 33 and 46, respectively, set the requirements for BWMS, and meeting the broad definition of BWMS does not necessarily mean that the BWMS meets all of the technical and performance standards in the regulations. Specific requirements control general terms. For an analogy, see the definition of "tank vessel" contained in 46 U.S.C. § 2101. The fact that a vessel may meet this broad definition does not mean that it can be certificated as a tank vessel under the inspection requirements in C.F.R. Title 46. The definition identifies a broad category of thing that the Coast Guard is regulating. The vessel

Guard's testing regulations incorporate the Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV Protocol)³⁰ by reference.³¹ The ETV Protocol contains staining test requirements³² that evaluate the functioning of enzyme systems and cell membrane integrity of organisms, thereby defining "living" by virtue of these critical functions necessary for organisms to persist.³³ The ETV Protocol uses the term "viable," but defines it as "organisms and any life stages thereof that are living."³⁴ The ETV Protocol also explains why the ETV Technical Panel³⁵ decided to limit "viability":

Note that it is understood that many of the proposed regulatory discharge standards, and in fact the desired effect of BWTSs,³⁶ is that these technologies should render organisms unviable or incapable of reproduction. In other words, to "kill, remove or inactivate" is technically unnecessary when the objective is to eliminate the organism's capability for reproduction. However, as the introduction of "viability" as a measure of efficacy significantly complicates the Protocol and test methods, and since "kill, remove or inactivate" is a conservative approach, the latter has been adopted as the measure of biological efficacy in this Protocol.³⁷

would still need to meet the specific tank vessel inspection requirements to receive a tank vessel Certificate of Inspection.

³⁰ "Generic Protocol for the Verification of Ballast Water Treatment Technology," EPA/600/R-10/146, September 2010, available at https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=230926. The ETV Protocol is a document of the Environmental Technology Verification Program (ETV Program), funded in whole or in part by the U.S. Environmental Protection Agency.

³¹ See, e.g., 46 C.F.R. § 162.060-26(a), which refers to the Subpart's "Incorporation by Reference" section, § 162.060-5.

³² The ETV Protocol "requires" the determination of organism concentration, but it is true that the offered testing method – the dual stain method – is stated in voluntary terms. For example, the ETV Protocol states, "[t]his protocol recommends use a [sic] combination of two vital stains..." (paragraph 5.4.6.5). This is because the ETV Protocol recognizes that there are other stains and measurement methods that may be acceptable, as long as they measure the same thing: whether something is alive. If a manufacturer does not want to use the dual stain method to meet the ETV Protocol requirements, then a manufacturer uses the USCG approval process in 46 C.F.R. § 162.060-10(b)(1) to request an alternative for the purposes of complying with the USCG regulations.

³³ The appellants raise divergent arguments, sometimes claiming that the ETV Protocol trumps the regulatory text and other times claiming that the Coast Guard cannot rely on the final ETV Protocol at all because it was not properly incorporated into the ballast water NPRM. As to the latter argument, the Coast Guard fully explained in its response to comments the logical outgrowth basis of its decision to adopt the final ETV Protocol into the 2012 Final Rule. 77 Fed. Reg. at 17258. Additionally, FACA does not apply to collaborations or partnerships that gather and share information, provide individual or unsolicited advice, or that do not seek consensus. See Federal Advisory Committee Act of 1972, Pub. L. No. 92-463 (H.R. 4383), 86 Stat. 770 (1972), as amended, *classified to 5 U.S.C. App. §§ 1 – 16*.

³⁴ ETV Protocol, page xii.

³⁵ The ETV Technical Panel is a group "comprised of a subset of stakeholders and other individuals with a technical expertise in ballast water and environmental technology issues." Members include fresh water and marine biologists, environmental scientists, engineers, and ship architects. See ETV Protocol, page 4.

³⁶ ³⁶ The ETV Protocol uses the term 'Ballast Water Treatment System(s)' (BWTS); which the Coast Guard views as consistent with the term 'Ballast Water Management System(s)' (BWMS) in Coast Guard regulations. See, e.g., 33 C.F.R. § 151.1504.

³⁷ ETV Protocol, page 5.

The ETV Protocol is unequivocal on the viability issue.³⁸ “Living” does not mean “viable.”³⁹ The language of 46 C.F.R. § 162.060-10(b)(1) provides flexibility for the Coast Guard to accept a test method other than what is expressly enumerated in the ETV Protocol, but the alternative must be demonstrated to measure what the discharge standard requires.⁴⁰ A 46 C.F.R. § 162.060-10(b)(1) approval can be used to accept a different measurement tool (e.g., a 12 inch ruler versus a yard stick) but not to substitute the underlying measurement requirement (e.g., length versus weight).⁴¹ Thus, the efficacy standards in 46 C.F.R. Subpart 162.060 are a bar to MSC’s ability to accept the proposed viability measurement as a testing alternative.

Coast Guard policy and intent:

The preamble to the 2012 Final Rule is consistent with this ETV Protocol language. The Coast Guard responded to a comment directly on point regarding whether “living” meant or included “viable”:

One commenter stated that because some types of treatment processes, such as UV, may act to make organisms unviable or unable to reproduce rather than killing them outright, the Coast Guard should include viability as a criterion for determination of BWMS efficacy. The Coast Guard disagrees. This issue has been the point of much discussion both in the United States and internationally in association with the IMO BWM Convention. The Coast Guard has decided to use live/dead rather than viable/unviable, because the latter designations would require culturing potentially large numbers of different kinds of organisms to determine whether they were capable of reproduction. This would be made even more problematic by the fact that scientists are not able to culture many of the organisms in question. Finally, it is more conservative, and thus more protective, to base efficacy decision [sic] on the basis of live/dead, rather than viable/unviable.⁴²

³⁸ The ETV Protocol was developed independently from the USCG ballast water regulations. Its first two chapters describe an organizational process that is superseded by, or otherwise irrelevant to, the specific USCG equipment approval process contained in 46 C.F.R. § 162.060.

³⁹ This ETV Protocol language is a counterpoint to Hyde’s argument that viability was only addressed in the 2012 Final Rule preamble, but not in the regulation text. The ETV Protocol was incorporated by reference into the regulation text of 46 C.F.R. Subpart 162.060. *See, e.g.*, 46 C.F.R. § 162.060-26(a).

⁴⁰ I note the existence of ETV Protocol paragraph 5.4.8, which provides for the use of “alternative and emerging methods.” This section is expressly limited to methods relating to “living” organisms, not organisms capable of reproduction, and even if it were not, the Coast Guard’s process set forth in 46 C.F.R. § 162.060-10 provides the relevant process for seeking alternatives. The specific alternative procedures created by the Coast Guard supersede the ETV Protocol’s generalized procedures. Additionally, if there is a conflict between the ETV Protocol and the regulation text, the two must be read such that the ETV Protocol does not render 46 C.F.R. § 162.060-10 meaningless. Finally, the Coast Guard disagrees with any interpretation of paragraph 5.4.8 which would effectively eliminate agency oversight of a federal regulatory program.

⁴¹ I disagree that MSC’s denial required MPN to be “identical” to the staining methods identified in the ETV Protocol. Hyde Appeal, page 26. MSC has approved other 46 C.F.R. § 162.060-10(b)(1) requests.

⁴² 77 Fed. Reg. at 17274. I note the incongruity of Hyde’s argument that the Coast Guard cannot rely on this language to deny Hyde’s application while Hyde then quotes multiple preambular passages from multiple Coast Guard ballast water rulemakings in its favor.

This response to comment is unequivocal, and I find Hyde's arguments to discredit it unpersuasive. First, the commenter who submitted this UV-oriented, "viable/nonviable" comment was Hyde.⁴³ If Hyde believed that the standard clearly meant to include viable organisms, it would not have submitted this comment. In fact, it appears Hyde believed the opposite:

The characteristic "unviable" should be used in place of "dead" in determining the efficacy of BWMS... We wish to emphasize that the terms "kill" and "dead" should be replaced with "make unviable" and "unviable" throughout the proposed regulation.⁴⁴

At the time of Hyde's comment, the word "living" did not appear in the proposed discharge standard in either 33 C.F.R. § 151.1511 or 33 C.F.R. § 151.2030. The plain reading of the proposed discharge standard was that ballast water could contain only a maximum of *any* organism, living or dead. This is an extremely conservative and still practicably unachievable standard.⁴⁵ With such a conservative standard, it is understandable why Hyde submitted this comment to change the discharge standard.⁴⁶ Having made this comment, and being reasonably concerned about the practicability of such a discharge standard, Hyde cannot now argue that the language always meant something else.

Additionally, this Coast Guard response is consistent with another section of the 2012 Final Rule preamble, which provided:

One commenter requested that the proposed BWDS include language necessary for differentiation between living and nonliving organisms. Another said that the standard should allow for the presence of nonliving organisms since some treatment technologies act to kill living organisms without necessarily removing them from the ballast water.

The Coast Guard acknowledges that the proposed BWDS is slightly different in this respect from the IMO discharge standard, which uses the term "viable" instead of "living." It is important to note that, while the text of the IMO BWM Convention refers to "viable" organisms, the G8 guidelines define "viable" as "living." Therefore, the Coast Guard has decided that this issue is best addressed in the BWMS approval process, and will not alter the standard as suggested by these commenters. We note that the standard and approval process do allow for the presence of nonliving organisms. Additionally, we corrected a technical error

⁴³ Letter dated December 3, 2009, Hyde Marine, Inc. to U.S. Department of Transportation, "Reference: Docket # USCG-2001-10486."

⁴⁴ *Id.* at page 5.

⁴⁵ In response to comments, the Coast Guard corrected the intended discharge standard by adding the word "living."

⁴⁶ "For example, in Section 151.1504 '...BWMS should kill, *make unviable*, or remove organisms.'" Emphasis in original. USCG-2001-10486-0292. In the 2012 Final Rule, the Coast Guard added "render harmless" rather than "make unviable" to the BWMS definition. This shows that the Coast Guard considered inserting "make unviable" into the BWMS definition but deliberately did not.

present in the NPRM, which mistakenly omitted the term “living” from the proposed 33 CFR 151.1511(a). This final rule corrects that omission.⁴⁷

Like the ETV Protocol, this response explains that “viable” means “living” and not vice versa. As discussed, *supra*, the IMO BWM Convention provides the international standard for organisms discharged from vessels’ ballast water. The Coast Guard’s ballast water discharge standard is nearly identical to the BWM Convention discharge standard. One difference is that the IMO standard contains the adjective “viable” rather than “living” to modify “organisms.” This Coast Guard response shows that its deviation from the IMO standard text was deliberate. This response also tries to articulate that, from the Coast Guard’s perspective, the two standards are substantively the same.⁴⁸ The BWM Convention does not define “viable.” However, one BWM Convention guidance document, the Guidelines for approval of ballast water management systems (G8 Guidelines),⁴⁹ defines “viable” to mean “living.”⁵⁰ While Hyde flips the definition around, so that “living” means “viable,” that is not reflected in the text of the G8 Guidelines. Instead, the G8 Guidelines provide a more narrowed interpretation of the BWM Convention text, consistent with the Coast Guard’s discharge standard.⁵¹ That is why there was no need to alter the proposed discharge standard in 33 C.F.R. Part 151 to harmonize with the IMO standard.

The Coast Guard’s response goes on to fully respond to the comments by stating that “this issue” is “best addressed in the BWMS approval process...” This means the Coast Guard’s intent was to leave “living” in 33 C.F.R. Part 151 undefined while relying on the technical efficacy

⁴⁷ 77 Fed. Reg. 17266. Trojan argues that this language shows an intention by the Coast Guard to apply two different discharge standards to the Great Lakes and nationwide. However, the record shows that the Coast Guard intended both subparts (33 C.F.R. Subparts C and D) to have the same discharge standard, which was meant to align with the BWM Convention discharge standard. *See* 77 Fed. Reg. 17260 (“We corrected the BWDS in both subparts C and D to align with the IMO BWM Convention.”). If the nationwide discharge standard is read without the “living” modifier, the standard becomes *stricter* than the Great Lakes discharge standard. Such a result is nonsensical.

⁴⁸ I note the argument set forth by DESMI and Alfa Laval claiming that the Coast Guard failed in its rulemaking to consider the extra cost to foreign flag vessels which would be barred from using foreign-approved UV BWMS using an MPN-based test method. This argument confuses an environmental analysis with a Regulatory Analysis. The Coast Guard properly considered and calculated the costs to foreign flagged vessels in its Regulatory Analysis as a sensitivity analysis. Coast Guard developed a range of cost for a ballast water treatment systems based on potential technologies, calculating total costs based on the low end of the system cost estimates. Since UV BWMS are not the lowest cost option, the fact that some are not available for use in U.S. waters does not impact the Coast Guard’s analysis. Additionally, the reference to “more stringent measures” is to the “phase-two” ballast water discharge standard proposed in the 2009 NPRM but not promulgated as part of the 2012 Final Rule.

⁴⁹ Guidelines for approval of ballast water management systems, Resolution MEPC.174(58), adopted October 10, 2008. (Titles of IMO guidance documents are typically not capitalized except for the first word.)

⁵⁰ *Id.* at paragraph 3.12 (“Viable Organisms are organism and any life stages thereof that are living.”). This language should be read as it is written and not reversed, so that living means viable. At the most recent meeting of the IMO Marine Environment Protection Committee (MEPC), this specific issue – whether the G8 Guidelines should be amended to remove or change the definition of “viable” – was raised and considered. MEPC did not reach a conclusion either way (i.e., contrary to Hyde’s assertion, the international community is not agreed that the G8 Guidelines allow BWMS to be type approved for viability rather than live/dead). *See* IMO Report of the Marine Environment Protection Committee on its Sixty-Ninth Session, MEPC 69/21, May 13, 2016, paragraph 4.39.

⁵¹ Because “living” is broader than “viable,” the term covers more organisms, and thus a discharge standard incorporating the broader term into a maximum allowable concentration means that fewer organisms can remain in the discharged ballast water. That is why it is a more protective standard, and a “more narrowed” interpretation, despite “living” being a broader term than “viable.”

requirements in 46 C.F.R. Subpart 162.060 to set the standard by defining how the standard would be measured. Subpart 162.060 contains a myriad of equipment requirements, but its efficacy requirements incorporate the ETV Protocol, which does not include viability as a measurement of efficacy.⁵² “[B]est addressed in the BWMS approval process...” does not mean that 46 C.F.R. § 162.060-10(b)(1) can be used as a back door to insert viability into the discharge standard after it was deliberately excluded via rulemaking. It means that the ETV Protocol, or the regulation text, can be amended or updated to include viability as a measurement option once better scientific and technical capabilities are discovered. A new version of the ETV Protocol would still need to be incorporated by reference into the Coast Guard’s rulemaking, via the public notice and comment process.⁵³ At a minimum, the Coast Guard’s policy decision on viability was established through public notice and comment and would need to go back through public notice and comment to change.

Clarification of the administrative record:

I acknowledge some confusion in the administrative record regarding the interactions between Hyde and the Coast Guard on accepting viability as part of the discharge standard. I think more blame for this confusion falls on Hyde than it does on the Coast Guard. After the Coast Guard rejected Hyde’s request to include viability in the 2012 rulemaking, Hyde then voluntarily launched a campaign to change the Coast Guard’s position on the matter.⁵⁴ This campaign was not initially for an alternative to the new, existing standards but for the Coast Guard to reopen the rulemaking it had just completed and *change the discharge standard*. The Coast Guard was clear from the beginning that it was very unlikely to open up the rulemaking any time soon.⁵⁵ Hyde persisted in its campaign, however, and the drawn out process that followed is merely the result of a government agency trying its best to hear and try to accommodate a member of the regulated public, within the bounds of law and policy. Many of MSC’s procedural recommendations to Hyde were in direct response to Hyde’s insistence to be heard, by any

⁵² See, e.g., 46 C.F.R. § 162.060-26(a) and 46 C.F.R. § 162.060-28(j). I note the Coast Guard’s use of “viability” in § 162.060-28(j). While it is true that the Coast Guard often uses “viable” and “living” synonymously, it is not in a favorable way for Hyde. Both the ETV Protocol and the G8 Guidelines define “viable” as “living” and not the other way around. It should also be noted that the Coast Guard’s use of the term “viable” or “viability” has changed over time. In the 1990s and early 2000s, the Coast Guard used the term viability because of the state of available science. As technology and knowledge advanced, the Coast Guard developed a more narrow view of “viability” given the unknowns involved with trying to verify it. An agency is allowed to refine its policy position over time and is not bound to archaic or outdated ideas, particularly if the agency has a statutory mandate to base decisions on best scientific information available. I agree that the Coast Guard has considered UV BWMS as part of a suite of BWMS options for a long time, but under the current regulations, a UV BWMS must meet the prescribed technical requirements.

⁵³ 46 C.F.R. § 162.060-5(d)(1) incorporates a specific version of the ETV Protocol, and so this reference would need to be updated.

⁵⁴ I disagree with Hyde’s characterization of its interactions with the Coast Guard in late 2012, which paint a picture of dealing with an agency with no corporate knowledge of its own regulations. The Coast Guard had just completed what was a very labor intensive, very high profile rulemaking in which the viability issue was directly considered and addressed. In bureaucratic terms, “the ink wasn’t even dry yet.” The fact that there may have been internal Coast Guard uncertainty over how to procedurally address Hyde’s persistent requests is understandable considering the rulemaking – specifically the type approval program – was in the process of initial rollout and implementation.

⁵⁵ See, e.g., HYDE APPX-000002. Additionally, I find Hyde’s criticism, in footnote 3, of the Coast Guard’s “delay” to be misleading and without merit. The Coast Guard was waiting on the final decision of the ETV Technical Panel on the MPN issue.

means possible.⁵⁶ There were no guarantees that the process would work in Hyde's favor. Additionally, the Coast Guard does not prohibit the submission of applications even when a Coast Guard employee anticipates it will be denied on the merits.⁵⁷ How can the Coast Guard precisely know what the applicant is requesting if it does not see the actual request? In fact, if the Coast Guard refused to allow Hyde to submit an alternative request, that would have exposed the agency to claims of arbitrary and capricious behavior. Hyde includes a very similar argument in this appeal, when it claims MSC "refused to consider" Hyde's request. The record shows that Hyde was persistent in trying to change the Coast Guard's decision on viability, and the efforts Coast Guard employees went through to provide an answer to Hyde, at Hyde's insistence, should not be held against them now.

I will also clear up some confusion over the "ETV process," as Hyde refers to it. The "ETV process" can mean different things. One "ETV process" is the process by which Independent Laboratories (ILs) use the ETV Protocol to conduct testing pursuant to the ballast water regulation requirements.⁵⁸ A different "ETV process" is the process by which the ETV Technical Panel considers new developments in ballast water treatment technology and whether to update or amend the ETV Protocol. In this case, the ETV Technical Panel convened to consider general issues relating to the ETV Protocol, including whether the use of viability as a measurement and the MPN assay as a measurement tool was acceptable, independently of Hyde's November 2012 MPN campaign.⁵⁹ Since MSC knew that this work was underway, it is reasonable that MSC (or CG-OES) would mention it to Hyde and suggest that Hyde wait for the ETV Technical Panel to conclude its work.⁶⁰ If the ETV Technical Panel found in Hyde's favor, that decision would provide the basis for future Coast Guard action, including updating the regulations to include viability. It would also be very difficult for the Coast Guard to come to an

⁵⁶ See, e.g., MSC email, dated 12 February 2015, HYDE APPX 000417. This email begins, "Please submit your request for alternates as equivalent as stated. We'll review your request and determine if a meeting is warranted." This is in direct response to an email from Hyde, dated 10 February 2015, which ended "Alternately, please let us know of your continued refusal of this meeting and we will submit our official 162.060.10(A) and 162.060.10(B) letters by the end of the week." HYDE APPX-000416. MSC's remarks were not an endorsement but an attempt to be responsive to a direct Hyde demand.

⁵⁷ See HYDE APPX-000417. "If manufacturers do not want to wait until the ETV technical panel process plays out, they can submit a -10(b)(1) proposal for acceptance of an alternative method...however, that request would have to meet the -10(b)(1) requirements..." This is an objective, likely palliative, instruction that does not take into account specific facts or presuppose that the request will be granted. MSC's following comments provide the specific warning that a "-10(b)(1)" request for MPN would not be simple. Even after receiving a favorable opinion from the ETV Technical Panel (which has not occurred), Hyde would still need to come back to the Coast Guard for further consideration.

⁵⁸ See, e.g., 46 C.F.R. § 162.060-42.

⁵⁹ "Currently, the MPN remains an unapproved method for determining the biological efficacy of the BWMS. The method remains under review by the EPA tech panel and we have no outlook on when an answer may be reached or any indication as to what that answer may be. I would be cautious of conducting testing prior to approval of this method if your system will rely solely on this method to meet the discharge standards...MPN data may be accepted as existing data following testing provided that...the MPN method is accepted as an approved method." MSC email, dated 6 February 2015, HYDE APPX-000413. This warning from MSC is categorical.

⁶⁰ I also note that all four applicants relied on the recent work of the ETV Technical Panel in their respective 46 C.F.R. § 162.060-10(b)(1) requests. While the appellants characterize their MPN-based method as being singular (i.e., "the MPN method") and in use for decades, the record shows that the "MPN" method they submitted for alternative approval is the preliminary draft MPN-based method developed through the work of the ETV Technical Panel, which has yet to be validated (i.e., the specific submitted method was developed after the testing was conducted for their foreign type approvals).

independent conclusion on viability, as the Coast Guard does not have the same scientific and technical resources as the ETV Technical Panel. Additionally, the Coast Guard never suggested that the ILs or the ETV Technical Panel could decide or approve a 46 C.F.R. § 162.060-10(b)(1) request. However, information from either “ETV process” can certainly be submitted to support a 46 C.F.R. § 162.060-10(b)(1) request.

Finally, I reject Hyde’s characterization of the Coast Guard’s *Shipboard Technology Evaluation Program* (STEP) program and development of its current ballast water requirements as implicitly accepting the MPN method. As I mentioned earlier, the Coast Guard has regulated ballast water for decades. The Coast Guard ballast water requirements began with a Congressionally-mandated ballast water exchange requirement, discussed *supra*, certain best management practices, and reporting and record keeping requirements. During this time, and in accordance with its statutory mandate,⁶¹ the Coast Guard considered and reviewed various technological alternatives to ballast water exchange. These alternatives included BWMS based on UV technology. The STEP program is “intended to facilitate the development of effective [BWMS] technologies, to create more options for vessel owners/operators seeking alternatives to ballast water exchange...vessel owners/operators have expressed a reluctance to invest the resources to install and operate an experimental treatment system that might not meet discharge standards mandated by future regulations.”⁶² In other words, the point of the STEP program is that those BWMS are experimental. The fact that the Coast Guard, in 2008,⁶³ accepted a UV BWMS for use on a STEP-enrolled vessel does not mean that the particular UV BWMS, regardless of its efficacy, *meets the regulatory discharge standard*.⁶⁴ At the end of the day, MSC’s actions were to uphold a regulatory discharge standard in light of a proposed alternative test method and were not an opinion on UV BWMS efficacy.⁶⁵

In sum, I find that MSC did not have the discretion to approve viability as an alternative measurement to the regulatory standards under 46 C.F.R. § 162.060-10(b)(1). The ballast water discharge standard in 33 C.F.R. Part 151 must be read together with the BWMS type approval requirements found in 46 C.F.R. Subpart 162.060 to understand the meaning of the word “living”

⁶¹ NANPCA/NISA, *supra*, n. 5, § 1101(e); *codified in* 16 U.S.C. § 4711(e).

⁶² Navigation and Vessel Inspection Circular (NVIC) 01-04. The STEP program predates the 2012 Final Rule.

⁶³ Letter dated October 31, 2008, from M. L. Blair, Captain, U.S. Coast Guard, Office of Operating and Environmental Standards, to Princess Cruise Lines, no. 33.151.2035.0040. This acceptance pre-dated the 2009 NPRM’s publication.

⁶⁴ A similar reasoning dismisses the appellants’ assertions that the Coast Guard violated the National Environmental Policy Act, Pub. L. No. 91-190, §2, 83 Stat. 852 (1969), as amended, *codified in* 42 U.S.C. §§ 4321, 4331-4335, 4341-4346, 4346a, 4346b, 4347. The purpose and need of the Coast Guard’s 2012 Final Rule Final Environmental Impact Statement (FEIS) is to provide “an assessment of the potential environmental impacts associated with the proposed establishment of a ballast water discharge standard. The standard would be used to approve alternative ballast water management methods that are effective in preventing or reducing the introduction of nonindigenous species via discharged ballast water into the waters of the United States.” FEIS Appendix F, which lists BWMS enrolled in the STEP program, is meant to provide a “rational basis” that BWMS exist that could achieve the discharge standard. The FEIS was not intended to prove that any particular system met the type approval standards established in 46 C.F.R. Subpart 162.060. Additionally, it is clear from the description of each of the alternative concentration levels considered as being “living organisms (per volume)” and in the description of UV BWMS assessed, that the Coast Guard examined the impacts of the regulatory discharge standard in the context of killing organisms (*see, e.g.*, Pages 2-5 and 2-6 and Appendix F).

⁶⁵ In fact, the Coast Guard still considers UV BWMS a valid ballast water treatment technology and believes that UV BWMS can be type approved under the existing regulatory requirements.

in the Coast Guard's ballast water regulations. The type approval requirements do not allow "living" to be substituted with "viable," and therefore MSC did not have the discretion to approve a testing alternative that would insert viability into the discharge standard. While Hyde argues that certain Coast Guard employees agreed that 46 C.F.R. § 162.060-10(b)(1) could be used to insert viability into the discharge standard, I do not believe the administrative record definitively or specifically proves this assertion. Additionally, the Coast Guard's regulations and viability policy decision went through the public notice and comment process, and those decisions cannot be changed without returning to the public notice and comment process.

2. The Coast Guard has the statutory and regulatory discretion to reject alternative proposals

Hyde and the other appellants contend that MSC's rejection of their applications for regulatory alternatives was tantamount to failing to consider them and that such failure was arbitrary, capricious, and inconsistent with law. As an initial matter, I believe Hyde confuses "to consider" with "to approve." It is clear from the record, including MSC's rejection letter of December 14, 2015, that MSC considered Hyde's application. As discussed *supra*, MSC found that Hyde's application failed to meet the second and third prongs of 46 C.F.R. § 162.060-10(b)(1). The fact that MSC ultimately denied Hyde's application does not mean MSC did not consider it. If there were any defect in MSC's consideration,⁶⁶ I cure it now by independently finding that Hyde's application fails to meet the requirements of 46 C.F.R. § 162.060(b)(1). That reasoning is provided in Section 3, *infra*.

Hyde and the other appellants make various legal arguments asserting various levels of Coast Guard discretion or mandate to accept alternatives to the Coast Guard regulatory requirements. The Coast Guard does not dispute that it has the discretion, in theory, to accept viability as a BWMS efficacy measurement.⁶⁷ The Coast Guard also has not permanently rejected MPN as a BWMS measurement tool by a regulatory change. The 2012 Final Rule and its 2009 Notice of Public Rulemaking (2009 NPRM)⁶⁸ were very clear that the 2012 Final Rule is an interim phase of ballast water treatment and management. At that time, the Coast Guard did not have sufficient information to include viability as an approval criterion. However, NANPCA/NISA contains a mandate which requires the Coast Guard to periodically review and revise its ballast water

⁶⁶ MSC should have waited for the final results of the independent analysis before denying the application. We now have the final results, and my decision is based on those results.

⁶⁷ As a counterpoint to the appellants' arguments that NANPCA/NISA mandate that the Coast Guard accept viability by virtue of the NANPCA/NISA definition of "nonindigenous species" including the word "viable," Congress enacted the assumption that vessel water treatment systems should "kill" aquatic nuisance species: "provide an exemption from ballast water exchange requirements to passenger vessels with...treatment systems designed to kill aquatic organisms in ballast water..." NANPCA/NISA § 1101(c)(2)(K), 16 U.S.C. § 4711(c)(2)(K) (emphasis added). "Viable" is used in this definition to cover things like viruses, which are not universally considered to be "living." In any event, NANPCA/NISA is based on a precautionary rather than prescriptive framework. That means that the Coast Guard has the authority, under "maximum extent practicable," to regulate the concentration of both indigenous and nonindigenous, as well as invasive and noninvasive, species in ballast water in order to prevent the introduction and spread of aquatic nuisance species. For example, ballast water exchange does not discriminate among "viable" or "nonviable" organisms.

⁶⁸ See, e.g., Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters, Notice of Proposed Rulemaking, 74 Fed. Reg. 44632 at 44634 – 44635 (Aug. 28, 2009).

regulations based on the best scientific information available.⁶⁹ If and when the Coast Guard has such information, it can reconsider whether to include viability. As this criterion would differ from the existing regulatory text and policy established through public notice and comment, this change would need to go through public notice and comment rulemaking.

While I agree, in principle, about the Coast Guard's discretion to accept viability, there are some very important statutory and regulatory limitations that these arguments raise that must be addressed within this response.

The Coast Guard interprets NANPCA/NISA Section 1101 (16 U.S.C. § 4711) differently than Hyde and the other appellants. In particular, the Coast Guard disagrees with the applicability and interpretation of Section 1101(c)(2)(D) and believes Section 1101(e)(1) expressly or constructively "trumps" Section 1101(c)(2)(D).

Section 1101 can be difficult to understand if it is not read in the context in which it was originally enacted and subsequently amended.⁷⁰ When NANPCA was amended to include voluntary guidelines for the entire United States (Section 1101(c)(2)), the specific mandates in Section 1101(c)(2)(D) were for those initial voluntary guidelines:

The voluntary guidelines issued under this subsection shall-

- (D) direct a vessel that is carrying ballast water into waters of the United States after operating beyond the exclusive economic zone to-
- (i) carry out the exchange of ballast water of the vessel in waters beyond the exclusive economic zone;
 - (ii) exchange the ballast water of the vessel in other waters where the exchange does not pose a threat of infestation or spread of nonindigenous species in waters of the United States, as recommended by the Task Force under section 4712(a)(1) of this title; or
 - (iii) use environmentally sound alternative ballast water management methods, including modification of the vessel ballast water tanks and intake systems, if the Secretary determines that such alternative methods are at least as effective as ballast water exchange in preventing and controlling infestations of aquatic nuisance species...

In other words, there was no existing nationwide standard, and Congress provided an initial *minimum* or *floor* for the Coast Guard⁷¹ to meet. The Coast Guard's initial guidelines were based on the framework contained in Section 1101(c)(2)(D) and included guidance on conducting ballast water exchange for vessels carrying ballast water into waters of the United

⁶⁹ NANPCA/NISA, *supra*, n. 5, § 1101(e)(1)(D); *codified in* 16 U.S.C. § 4711(e)(1)(D). The Coast Guard also committed to a regulatory obligation to conduct a practicability review for a more stringent standard, initiating a rulemaking by 2017 if appropriate. *See, e.g.*, 33 C.F.R. § 151.1511.

⁷⁰ Please refer to the background section of this response for this discussion.

⁷¹ The Act refers to the "Secretary," defined as the Secretary of the department in which the Coast Guard is operating. For simplicity, I will instead refer to the Coast Guard, as the properly delegated entity of the Department of Homeland Security.

States after operating beyond the U.S. exclusive economic zone.⁷² Eventually, the Coast Guard converted its voluntary guidelines to mandatory, regulatory requirements under the mandate contained in Section 1101(f)(1).⁷³ These regulations were also based on the framework contained in Section 1101(c)(2)(D), as ballast water exchange remained the best available ballast water management option.

However, Section 1101(e)(1) requires the Coast Guard to consider revising its ballast water regulations no less than every three years. After conducting this periodic review, the Coast Guard is required under Section 1101(e)(1) to amend its ballast water regulations if, based on the best scientific information available, the existing guidelines and regulations implementing Section 1101(c) do not effectively reduce the introduction and spread of aquatic nuisance species by vessels. The Coast Guard's 2012 Final Rule was the result of an on-going review that began almost immediately after promulgation of the initial voluntary guidelines for ballast water exchange, and continued through participation in the development of the IMO ballast water management convention and development of test protocols for BWMS, and the best scientific information available showed that some BWMS were more effective than ballast water exchange in reducing the introduction and spread of aquatic nuisance species. While the Coast Guard initially characterized its new requirement of BWMS as an approval of an "environmentally sound alternative ballast water management method" under Section 1101(c)(2)(iii),⁷⁴ the Coast Guard later explained its reasoning that subparagraph (c)(2)(D) merely set forth initial ballast water requirements for certain vessels and it was acting under the broader mandates found in paragraphs (a) and (e).⁷⁵ To read Section 1101 as permanently binding the Coast Guard to the initial floor set by Section 1101(c)(2)(D) would render Sections 1101(c)(2)(A) and 1101(e)(1) meaningless.⁷⁶ The BWMS manufacturers' argument that the Coast Guard is bound to implement all of Section 1101(c)(2)(D) is even more perplexing considering it would mean the Coast Guard has no discretion to phase out ballast water exchange in favor of BWMS.⁷⁷ The

⁷² See Implementation of the National Invasive Species Act of 1996 (NISA), Interim Rule, 64 Fed. Reg. 26672 (May 17, 1999).

⁷³ See Mandatory Ballast Water Management Program for U.S. Waters, Final Rule, 69 Fed. Reg. 44952 (July 28, 2004).

⁷⁴ See, e.g., 2009 NPRM, 74 Fed. Reg. 44633.

⁷⁵ 77 Fed. Reg. 17282, 17286. This analysis also explains why the Coast Guard believes it has the authority to require all vessels equipped with ballast tanks – and not just those that have operated beyond the exclusive economic zone – to comply with its ballast water management requirements. The Coast Guard has previously, and publicly, rejected the legal argument that Section 1101(c)(2)(D) contains specific requirements which control "broader" requirements in Section 1101.

⁷⁶ I note Trojan's argument that NANPCA/NISA mandates the Coast Guard use "best science available," which Trojan evidently believes requires the Coast Guard to perpetually amend its equipment standards without going through a public notice and comment rulemaking. The Coast Guard properly incorporated the ETV Protocol into its regulations, as it does for many other vessel equipment standards such as those from the International Organization for Standardization. To suggest that the Coast Guard can "undo" a proper regulatory incorporation by reference without a notice and comment rulemaking is untenable. Additionally, the Coast Guard used "best science available" at the time it promulgated the 2012 Final Rule, four years ago. The Coast Guard is now completing its NANPCA/NISA-required periodic review, in which it is considering any new, properly validated scientific information. That information will inform whether to amend the Coast Guard regulations. Vessels must be able to keep up with changing equipment standards or the Coast Guard would not be maintaining NANPCA/NISA's "maximum extent practicable" mandate.

⁷⁷ Even reading Section 1101(c)(2)(D) alone, without reference to any other parts of NANPCA/NISA, cannot support this conclusion. Section 1101(c)(2)(D) is formed in the disjunctive, allowing the Coast Guard to choose all or only one of the options under it, while it was still operative. DESMI and Alfa Laval may have used a Coast

Coast Guard has moved beyond the initial mandate contained in Section 1101(c)(2)(D) to the more stringent mandate contained in Section 1101(c)(2)(A), which requires the Coast Guard to ensure, to the maximum extent practicable, that aquatic nuisance species are not discharged into the waters of the United States from vessels.⁷⁸

Thus, while I generally agree with the appellants' arguments that NANPCA/NISA gives the Coast Guard the discretion to consider and accept viability, I do not agree with all of their interpretations and analysis of NANPCA/NISA § 1101.⁷⁹ NANPCA/NISA does not "mandate" that the Coast Guard accept viability unless and until viability falls within the Coast Guard's responsibility to ensure to the maximum extent practicable that aquatic nuisance species are not discharged into the waters of the United States from vessels. Given the then-current state of science and technology, discussed *infra*, live/dead was the most environmentally conservative and practicably achievable standard, and viability does not yet fall within this mandate.

3. Hyde's application fails to meet all elements of 46 C.F.R. § 162.060-10(b)(1)

I find that Hyde's application did not meet the minimum requirements in 46 C.F.R. § 162.060-10(b)(1) and confirm MSC's denial of Hyde's alternative request.

This decision is based, in part, on the technical review and conclusions of the Naval Research Laboratory (NRL),⁸⁰ whom the Coast Guard contracted to review the technical aspects of the alternative request submitted by Trojan.⁸¹ The NRL review resulted in a comprehensive evaluation and the report includes more detailed descriptions and comments, not all of which were considered pertinent to the narrow issue of the 46 C.F.R. § 162.060-10(b)(1) requirement. I find the NRL report persuasive, and summarize my agreement with it as follows:

Guard preambular statement out of context on page 12 of their respective appeals. The quoted statement, from the ballast water NPRM, meant that any alternatives to ballast water exchange must be approved by the Coast Guard, not that the Coast Guard was required to approve all alternatives to ballast water exchange.

⁷⁸ For this reason, Trojan's argument about accepting a BWMS as an alternate management system (AMS) fails. There is a difference between the initial standard of "at least as effective as ballast water exchange," which is consistent with the purpose of AMS – a bridging strategy between ballast water exchange and BWMS – and an evolved "maximum extent practicable" standard. Type approved BWMS must meet the *discharge standard*, not meet the floor of "at least as effective as ballast water exchange."

⁷⁹ I also reject Trojan's argument that NANPCA/NISA does not apply to non-reproductive organisms, and therefore the Coast Guard lacks the authority to promulgate the existing discharge standard. (Trojan appeal, page 45 – 46). NANPCA/NISA's reference to aquatic nuisance species, of course, concerns the species prior to treatment in a BWMS.

⁸⁰ NRL, "Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(B)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{M}$ and $<50 \mu\text{M}$," Feb. 10, 2016, 3900 Ser 6130/1622.

⁸¹ The NRL reviewed only one 46 C.F.R. § 162.060-10(b)(1) application: Trojan. Nonetheless, the NRL report on Trojan is relevant to all four applicants. Three of the four applicants (Trojan, DESMI, and Alfa Laval) submitted a base set of the same six documents containing a description of the proposed test, an overview of the use of the most probable number approach for evaluating BWMS, and results of various experiments conducted during development of the method, including several in which the alternative and required methods were compared. Hyde submitted only three of the six documents. Trojan also submitted additional documentation. Thus, Trojan's application was the most comprehensive of the four and covered all of the evidence provided by all four applicants. If Trojan's application fails to meet 46 C.F.R. § 162.060-10(b)(1), then all four fail. I have also included a discussion of arguments (vice documentation) of the individual applicants.

The applicants request that Coast Guard approve as equivalent an alternative to the required test specified in the ETV Protocol for determining the concentration of living organisms in the 10-50 micrometers (μm)⁸² size range in samples of water during type approval testing. The proposed alternative method is composed of two separate procedures, one (based on “viable”) for autotrophic (photosynthetic) organisms, and one one (based on “living”) for heterotrophic organisms.

The autotroph procedure is based on using a “grow-out” approach, wherein samples are serially diluted, and replicate tubes at each dilution are cultured for a period of time, and then assayed for population growth of phytoplankton by detecting changes in the concentration of chlorophyll. The pattern of tubes with and without positive population growth is then used to estimate the probable original concentration, through a calculation termed MPN. The MPN statistical approach has been used for over a century to quantify monocultures (single species) of bacteria and phytoplankton, and several automated “calculators” are available for use. Significantly, the applicants propose to use MPN as the basis for quantifying concentrations of mixed assemblages of phytoplankton, composed of numerous different species in varying relative abundance.⁸³ This is a “new” use for MPN as a statistical approach under a regulatory context and has not been adequately validated for such purpose.

The heterotroph procedure, for heterotrophic organisms, uses microscopy to count numbers of non-photosynthetic organisms that are motile, where movement is the criterion for determining an organism is alive. This method is conceptually similar to the required method, in that direct counts of “living” (not “viable”) organisms are made using a microscope. However, the specific procedures for determining whether an organism is “living” and the type of microscope are different than specified in the required method.

Compliance with 46 C.F.R. § 162.060-10(b)(1):

A. Does the request involve an evaluation, inspection, or test required by Section 162.060-10?

I find that the applicant meets the first prong of 46 C.F.R. § 162.060-10(b)(1). Hyde’s request to use the MPN assay is a request for an alternative to the testing requirements of the ETV Protocol, incorporated by reference into 46 C.F.R. § 162.060-26 and 46 C.F.R. § 162.060-28, which are referred to in 46 C.F.R. § 162.060-10(f)(2).

B. Is that evaluation, inspection or test not practicable or applicable?

I find that Hyde has not provided sufficient justification that the testing requirement is not practicable or applicable.⁸⁴ Three applicants argued, in various ways, that the required method was not practicable and/or not applicable for evaluating the efficacy of UV-based BWMS. Some

⁸² A micrometer equals 0.001 millimeter.

⁸³ I will refer to the applicants’ approach as the “alternative method.” I note that there is no globally accepted “MPN method” for BWTS type approval. While the applicants used the same MPN-based method, it appears that MPN approaches vary across flag administrations.

⁸⁴ I agree that MSC’s conclusion on practicability was not sufficiently justified. For that reason, I vacate the practicability decision of MSC and reach my own decision.

of the arguments were not always clear whether they were being made on the basis of practicability or applicability, and so for this review I consider the two issues to have been combined and I review the appellants' arguments together. The applicants essentially make a two-part argument: first, that the phrasing of the discharge standard should be in terms of, or include, "viable" (or reproductive) organisms instead of, or in addition to, "living" organisms as currently phrased in the discharge standard; and second, that the required method for enumerating "living" organisms in the type approval requirements is not applicable to BWMSs intended to render organisms non-viable.

Only one applicant (Trojan) offered any objective basis to argue that the required method was not practicable (Miller 2015d). Trojan essentially argued that it is too expensive to use UV to kill organisms rather than render them non-reproductive, and so UV should be used to render organisms non-viable. This, according to Trojan, would provide an equivalent level of protection for the environment as killing them, because non-viable and dead organisms represented the same level of risk reduction with respect to biological invasions. Rather than supporting an argument that the required method is not practicable for evaluating numbers of living organisms, I view this as an argument that UV is not practicable for killing organisms in ballast water. Under the current type approval requirements, BWMS are required to be tested to demonstrate efficacy in reducing numbers of "living" organisms below concentrations in the discharge standard, and to do so using specific test methods that measure numbers of "living" organisms. As explained, *supra*, the Coast Guard cannot grant a waiver to the "living" requirement under the current regulations. -

Furthermore, the proposed alternative method includes an assessment of "living" organisms in the case of heterotrophic organisms, which undermines the contention that the required method, which assesses "living" organisms is impracticable or inapplicable. In the proposed alternative method, a culture-based viability assay is used to assess viable autotrophic organisms (organisms capable of synthesizing their own food from inorganic substances using light or chemical energy; e.g., plants and algae) while a "living" assay is used for heterotrophic organisms (organisms that cannot manufacture their own food and instead obtain food and energy by taking in organic substances, usually plant or animal matter; e.g., animals and fungi).⁸⁵ Hyde does not explain why it is practicable and applicable to evaluate "living" organisms when assessing the efficacy of UV systems in treating heterotrophic organisms, but it is not when the focus is autotrophic organisms. This inconsistency must be explained.⁸⁶

⁸⁵ The required method is used to enumerate both of these two components of the size group.

⁸⁶ DESMI and Alfa Laval make a similar argument against the Coast Guard, stating that for organisms in the <10 μm size class, tests for only 3 indicator species are allowed. This argument conflates the ETV Protocol with the overarching Coast Guard regulatory standard, found in 33 C.F.R. §§ 151.1511 and 151.2030. There is no "<10 μm size class." The Coast Guard regulations set forth specific requirements for those three indicator microorganisms, and they are not 'indicators' for a size class. Alfa Laval also points to the Coast Guard's incorporation of the option to use water from a U.S. public water system for ballast water management as evidence that MPN is acceptable. However, this point proves the opposite: the Coast Guard *limited* the option to *only* U.S. sources because of the difficulties in determining water quality from foreign sources. In the case of the United States, the regulatory requirements for these facilities are known, uniform, and verifiable. Additionally, water treatment facilities use MPN-based methods to estimate abundances of *specific* well known species, not the thousands in ballast water.

Specific to Hyde, I disagree that the required method for assessing the concentration of living organisms in treated ballast water is not applicable to UV-based BWMS. To make this assertion, Hyde is forced to define the term “living” in the discharge standard to mean viable or reproductive. As I explained, *supra*, I disagree that this is an appropriate definition of “living.” The Coast Guard intentionally used the broader term “living” because Coast Guard considered it less difficult and problematic to distinguish living organisms from dead organisms than to distinguish between reproductive and non-reproductive living organisms. While Hyde argues that conceptually, a non-reproductive organism represents the same risk of a biological invasion as does a non-living organism, and I do not disagree with that conceptual perspective, Hyde does not successfully argue that a reliable method exists for determining the reproductive ability of the thousands of species occurring in ballast water (this is discussed further below regarding the technical aspects of the proposed method). For these general reasons, I find that the applicant’s assertion that the required method is not applicable to evaluating UV-based BWMS is unsubstantiated.

I also address the following specific points raised by Hyde (HMI 2015):

- i. *“The inherent nature of the proposed staining technique measures the capability of organisms to hold fluorescence rather than to provide a measurement of “living/dead”...”* The fluorescent markers in the required method, as well as many other fluorescent markers specific for other biochemical characteristics of living organisms, are widely used in biology to differentiate living from dead.
- ii. *“...all scientific definitions of the word “living” include the ability to reproduce.”* This assertion is a gross mischaracterization of the definition of “living” in the context under discussion, which is how one can differentiate between a living organism and a dead organism. The context for the assertion is the higher-level issue of differentiating between “living” things (i.e., organisms) such as animals, plants, fungi, bacteria, etc and “non-living” things (e.g., rocks, water, fire, etc), wherein living things are defined in part as those things that exhibit reproduction at some time. Such an overarching definition is not pertinent to the issue at hand, as the required method is used to differentiate “living” organisms from “dead” organisms, not organisms from rocks (although the fluorescent “signal” from the marker does assist in seeing living organisms within the jumble of nonliving material such as bits of rock, shell fragments, and plant detritus.) Many “living” organisms are not capable of reproduction (adult humans who have undergone sterilization procedures; sterile castes in social insects, mules, female mammals that have reached menopause, etc). Viable organisms are by definition included in the larger sub-set of “living” organisms (because an organism must be alive to reproduce). If a reliable procedure can be identified by which viable individuals of thousands of different species can be consistently discriminated from non-viable individuals, then such methods could be included in the approved methods for determining numbers of living organisms, through amendment of the regulations. To avoid reducing the protectiveness of the discharge limits, any such viability assessment must also have levels of accuracy and precision equivalent to that of the method used to determine the number of living organisms.

- iii. *“UV as a disinfection product sterilizes organisms by disrupting their DNA and interrupting their ability to reproduce.”* The use of UV as a disinfection process is not the issue. The issue is whether UV has been, or can be, demonstrated to be an effective disinfection process for the vast number of different species in the 10-50 μm size group found in ships’ ballast water. UV is generally used to render organisms non-reproductive because it is cheaper than using UV to “kill.” Using UV to render organisms non-reproductive, rather than to kill, depends on a good understanding of the specific UV dose necessary to render specific organisms incapable of reproduction. This has been done for a relatively small subset of organisms that are either human pathogens (e.g., *Vibrio cholera*, several species of the genus *Cryptosporidium*, *Hepatitis A*, etc.) or indicators of poor water quality due to unsanitary conditions (e.g., *Escherichia coli* and several species of *Enterococcus*) that can be associated with the presence of such pathogens. By contrast, there are many thousands of species carried in ships’ ballast water, and little or nothing is known about the specific UV doses required to render these permanently incapable of reproduction, nor is there a good understanding of how these many species could be consistently cultured in the laboratory to detect viability.
- iv. *“In order not to eliminate UV disinfection in ballast water, a reproductive measurement assay must be used.”* This is an argument based on economics, not the question of whether the required method is applicable to testing whether UV-based technologies are effective in achieving the limits on concentrations of living organisms.
- v. *“...in any disinfection enumeration, looking at the reproductive capability of the organisms studied is a more accurate representation of effectiveness than looking simply at their ability to hold a stain.”* This statement is only true for those disinfection processes intended to render organisms non-reproductive. For a process intended and designed to kill organisms, the appropriate approach in measuring effectiveness would be to identify the numbers of living organisms. The assertion that the required method “simply” looks at the ability of organisms to “hold stain” is a gross over-simplification and ignores completely the broad use of vital markers to identify “living” cells.
- vi. *“The international testing community and US EPA have historically used reproductive assays in lieu of staining to demonstrate the effectiveness of UV systems in ballast and other applications.”* The “international testing community,” meaning the test facilities conducting tests of BWMS for foreign administrations, may have used, collectively, a range of methods that involved reproductive assays, but these have not been made generally available to the public, including in particular the documentation demonstrating the careful validation of culture-based methods. Several different “methods” have been used by different facilities, and the validations that these methods were able to measure what they were intended to measure, or the comparisons among methods, have never been made public; indeed, at least one test facility has long asserted that the details of its methods are “proprietary.” When the

ETV panel first began to consider the issue of whether an acceptable viability assessment could be identified for use in testing BWMS, the various test facilities and manufacturers were unable to provide or point to any specific validations, and had to subsequently conduct much of the validation work submitted (after much testing of BWMS had been completed) in support of the method being developed within the ETV Program, a draft of which was submitted to the Coast Guard for consideration under the -10(b)(1) provision.

I address the following specific points raised by DESMI:

- i. *UV treatment of ballast water does not outright kill organisms in the water, but destroys their DNA and RNA making them incapable of reproduction.* This is a decision made by treatment system manufacturers on the basis of economic considerations, and reflects the specific, and differentiated from shipboard, circumstances of drinking water, food safety and waste water concerns. UV can have a biocidal (i.e., killing) effect. Currently the type approval testing requirements specify measurement of numbers of living organisms as determined by the specific method described in the ETV protocol. This method is as appropriate for enumerating living organisms in UV-treated water as it is for the same purpose in water treated by other means.
- ii. *Reproductive capability is central to the definition of invasive species laid out by the US Federal Aquatic Nuisance Species Task Force in 2012. It follows that organisms incapable of reproduction cannot become invasive species.* A 46 C.F.R. § 162.060-10(b)(1) analysis does not consider the definition of “invasive species” or whether organisms that are incapable of reproduction cannot become invasive species. Rather, this section is concerned with the method(s) accepted by the Coast Guard under these equipment type approval regulations.
- iii. *Destruction of organisms’ ability to reproduce therefore grants the same protection against aquatic invasive species as is obtained by killing the organisms.* The crux of the matter is the degree to which “ability to reproduce” can be determined for all of the different kinds of organisms in ballast water. I do not believe that there is currently an acceptably validated method that can be used to consistently assess whether organisms in treated ballast water are capable of reproduction.
- iv. *The FDA/CMFDA staining method prescribed in the ETV protocol for testing of ballast water treatment does not show whether an organism is capable of reproducing or not.* I do not dispute this assertion. However, if it is not practicable to adequately determine whether living organisms in treated water are able to reproduce, then limiting the number of “live” organisms includes both viable and non-viable individuals, and so provides a conservative, more protective limit.⁸⁷

⁸⁷ 77 F.R. 17274 (“Finally, it is more conservative, and thus more protective, to base efficacy decision on the basis of live/dead, rather than viable/unviable.”).

- v. *Therefore, the FDA/CMFDA method is not well suited for assessment of the effectiveness of a UV based ballast water treatment system.* The relevant question is whether the required FDA/CMFDA method in the ETV Protocol is suitable for assessing effectiveness of a BWMS in meeting the “live” criterion in the USCG discharge standard, not whether it is well suited for a BWMS meeting some other discharge standard.
- vi. *On this basis it is concluded that:*
- a) *The method contained in the ETV protocol for organisms in the 10-50 micron size category is not practicable or applicable to evaluate the performance of UV based ballast water treatment systems.* I disagree. DESMI frames practicability in terms of measuring something (“viable”) that is not pertinent to the actual standard, which is framed in terms of “live” organisms. Again, the required method is practical for enumerating the number of living organisms in a sample of ballast water. The applicant desires to be held to a different end-point – not “living” but instead “able to reproduce”.
- b) *The proposed alternative test method provides same level of protection against invasive species, and is equivalent to existing requirements in terms of accuracy.* This is irrelevant to the issue of whether the required method is practical or applicable in the case of assessing the effectiveness of a BWMS in meeting the discharge standard. This point is addressed, *infra*, in regard to whether the proposed alternative is equivalent to the requirement.

I address the following specific points raised by Trojan (Miller 2015d):

- i. *The existing method is not practicable for evaluation of UV-based BWTS. The current requirement for analysis of the organisms in the 10-50 μ m size class in the ETV protocol requires the use of FDA/CMFDA stains to categorize organisms as being alive or dead. The stains evaluate the functioning of an organism’s esterase system as a proxy for cell death. Treatment with UV irradiation, causes damage that prevents cell replication (and thus precludes invaders from colonizing), but esterases are not directly affected at UV doses typically employed for disinfection, and thus effective treatment by UV is not evaluated by the existing method.* Trojan asserts that the required method is not practicable for evaluation of UV-based BWMS that are intended to render organisms non-reproductive rather than dead. I do not dispute that the staining methods evaluate the functioning of an organism’s esterase system rather than the ability of an organism to reproduce, but the issue is that the discharge standard is not phrased in terms of “non-reproductive” or “non-viable” organisms, but instead is phrased in terms of “living” organisms. At the time of the 2012 Final Rule, the Coast Guard believed that there were significant difficulties associated with determining the reproductive ability of the thousands of species of organisms found in ships’ ballast water. Because of those difficulties, the Coast Guard determined that it was more practical and protective of the environment to phrase the standard in terms of living organisms. Concurrently with establishing the discharge standard, the Coast

Guard established the required methods by which numbers of living organisms would be determined during type approval testing. The required method for organisms in the 10-50 μm size class is the FDA/CMFDA fluorescent marking method specified in the ETV Protocol. The applicant has not provided an adequate argument that the required method is not practicable for evaluating the number of *living* organisms in ballast water.

- ii. *A BWTS designed for UV doses equivalent to ten (10) times the current UV doses employed in the industry would require 10X the number of UV lamps, and would therefore be approximately 10X the footprint and require 10X the electrical power for operation. This increase would render UV systems impractical for implementation on board vessels.* Rather than making an argument that the required method is not practicable for enumerating living organisms after UV treatment, Trojan essentially argues that the required method is not applicable to UV treatment when such treatment is intended to render organisms non-reproductive. In essence, Trojan does not present an argument that the required method is not practicable, but rather that it is not practicable to use UV treatment to achieve the required discharge standard. Trojan argues that the UV doses commonly used in BWMS that are intended to render organisms non-reproductive are not great enough to induce mortality during type approval testing. Increased UV doses would not only prevent reproduction or cellular division, but if sufficient, also induce mortality that is detected following treatment using the required method. Thus, the required method would indicate treatment efficacy in meeting the “live” discharge standard because the dead cells would not fluoresce green. An alternative method would not be needed.
- iii. The applicant asserts that it is not practicable to increase the UV dose so treated cells are dead (as determined by the required method) rather than not viable (as determined by the alternative method). The applicant provides no data on the actual UV dosages necessary to kill the many species of organisms found in ballast water. Using derived ratios of UV doses needed to kill to doses needed to render organisms non-reproductive (no actual doses or dose-response curves were provided) from a study using 12 species of algae, the applicants argued that a UV dose sufficient to damage cells’ non-specific esterases—the foundation of the required method—and reduce concentrations of algae by 100-fold (from 1000 cells mL^{-1} to 10 cells mL^{-1}) would be “extremely high”. The study found that for the 12 species, on average, a 10-fold increase in UV dose was needed to kill cells using the required method compared to the dose needed to show cells were non-viable using a culture-based viability assay. However, no data on actual dosages required to kill organisms or the power required to achieve those dosages were provided, so it is not possible to objectively evaluate this claim.

Consequently, I find that none of the applicants provided an adequate justification that the required method was not practicable and/or not applicable for its intended purpose when used to evaluate the efficacy of BWMS that used UV to treat organisms in the 10-50 μm size range in ballast water to meet the discharge limits set in the Coast Guard’s March 2012 Final Rule.

C. Is the proposed alternative equivalent to the regulatory standard?

I find that the applicants have not justified the proposed alternative's equivalency to the requirement. "Equivalence" among testing methods, in a literal sense, entails different methods of achieving the same measurement. A simple example would be measuring the length of an object by using a ruler in a specific manner or taking a photo of the object and analyzing length using an image analysis software program (in which case the program converts pixels along the delineation into length, based on a user-supplied calibration between pixels and length). In such a case, the methods result in measurement of the same thing (in the example, length). In a more technical sense, "equivalence" among testing methods⁸⁸ means that the methods must have the same or very similar accuracy (closeness to true value) and precision (degree of repeatability under unchanged conditions).⁸⁹

I have already found that the proposed alternative is not "equivalent" in the literal sense, and MSC did not have the discretion to use 46 C.F.R. § 162.060-10(b)(1) to rewrite the discharge standard. Even if the Coast Guard could accept viability without going through public notice and comment, which it cannot, the UV applicants' alternative would still fail for technical reasons.

To determine if the alternative "viable" and required "live" methods are equivalent, a proposal for the alternative must demonstrate equivalent accuracy and precision of measurement – even if the actual parameters being measured are different. Precision can be assessed within a method, but accuracy is usually evaluated through comparison with a "true" value.

Accuracy or Agreement

"Accuracy" means that the two methods should return the same result in terms of closeness to a known or "true" value. In this case, we have no way of knowing the "true" concentration that is independent of the methods being evaluated. In other words, how do we count the organisms without using the methods under consideration? In the absence of a way to evaluate accuracy, several approaches were taken to evaluate the degree of agreement between the proposed alternative and the required method in determining the number of living and viable organisms.⁹⁰

⁸⁸ I will assume for the sake of argument that "viable" and "living" relate equivalently to the risk of biological invasion. However, I do not reach a conclusion on that issue.

⁸⁹ Neither Hyde nor Alfa Laval included any additional arguments for accuracy or precision in their respective alternative requests, outside of the included documentation. DESMI did not provide direct examination of either accuracy (agreement) or precision, outside of its included documentation. DESMI presented information on the relative potential for "false negative" results in the required and proposed alternative approaches. While false negatives in the required method (living organisms that are not motile and do not stain) and proposed alternative (viable autotrophic organisms that do not reproduce under the provided conditions and living heterotrophic organisms that are not motile) are contributors to potential differences in agreement and precision, they are not the sole sources of error.

⁹⁰ "Agreement" means that the proposed alternative method provides at least the same result when circumstances are such that the results should be the same. In the supporting material, the applicants provided results from an experiment in which the two methods were used to measure the concentration of samples in which all cells were likely both living and viable. In such case, the two methods should result in the same numbers. If, as in this case, accuracy cannot be evaluated because there is no independently derived "true" value against which to assess the methods, then agreement can be used to evaluate equivalency.

The applicants analyzed three subsamples of each of three concentrations of the cultured phytoplankter *Tetraselmis suecica*, using both the required and the autotroph (MPN) methods (Miller and Petri 2015).⁹¹ In this experiment, the cultures of the alga were healthy and robust, and it could be expected that all or most of the living cells were likely also viable. To evaluate agreement between the two methods, the applicants calculated the mean of the three subsamples using the autotroph method divided by the mean concentration determined using the required method, multiplied by 100 (the required method was considered the benchmark for this comparison). The value (converted to a percent) would be 100% if both methods yielded the exact same concentration. Because the “true” concentration is unknown, this metric actually quantifies the agreement between the required and alternative methods (rather than accuracy, which is the term used in the proposal).

In this test, where most if not all the cells were both living and viable, the percent difference in measured concentration between the required and alternative methods ranged from 38% difference at a concentration of 1000 cells/mL to 412% difference at the lowest concentration of 10 cell/mL. In other words, in a circumstance where one would expect the results to be the same, the result using the alternative was at best 38% different, and at worst 412% different than the result using the required method. It is particularly concerning that the percent difference was so great at the lowest concentration, which is essentially at the level of the discharge standard. However, due to the high degree of variability in results (6 of 9 means for the required test had standard deviations less than 10, while 6 of the 9 means for the alternative test had standard deviations greater than 46), it is difficult to draw any further conclusions from this test regarding how agreement between methods might vary as a function of concentration.

The applicants also evaluated the agreement between the alternative method and the required method when all of the organisms in the samples were heterotrophic. Cultures of the rotifer *Brachionus plicatilis* were evaluated using the heterotrophic component of the alternative method and direct counts using a stereomicroscope; in both of which, movement of organisms was the parameter used to assess whether the organisms were living. There was no difference between the two methods in the concentration of rotifers. However, in this experiment, the alternative heterotrophic method was not compared to the actual required method, which uses epifluorescence microscopy to detect positive reactions of two vital stains and cell movement. For a legitimate comparison, both the heterotroph and required methods must be used.

When using the two methods to assess samples of ambient communities (Miller *et al.*, 2015b), the applicant examined the agreement between methods using a calculated “factor of agreement” (FOA, calculated as the average alternative concentration/average required concentration). The FOA varied widely among different experiments, from 0.47 to 33.8. Half of the 16 comparisons had an FOA of less than one, and half had values greater than one; six of the latter values were greater than 5.0. It is difficult to draw any conclusions from this experiment, given the possible sources of confounding effects: the MPN counts could have been reduced due to the presence of

⁹¹ *MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 µm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology.* Only autotrophs were used; no heterotrophs were used in this case. Since all organisms were phytoplankton and presumably the phytoplankton were capable of growth (since they were in culture, under favorable light and nutrient conditions), there was no need to perform the heterotroph method in addition to the autotroph method to determine the total number of organisms.

viable but non-culturable species, or increased due to the presence of organisms smaller than 10 um, while the stain counts could have been depressed by the presence of non-motile, non-staining organisms that were nonetheless living (false negatives). These potentially confounding effects would have to be carefully partitioned in experiments using ambient organisms, and that would entail a significant amount of work. Alternatively, consistently culturable species known to take up the fluorescent stains of the required method and larger than 10 um in size could be used, in laboratory tests using mono- and mixed cultures, to evaluate the degree of agreement between the two methods. The need for statistical adjustments for the presence of viable but non-culturable species would need to be evaluated and possibly developed and incorporated into the method, and the effect of contamination by organisms less than 10 um in size would also need to be addressed. Even with pre-filtration to remove organisms less than 10 um, some contamination is unavoidable in samples of natural assemblages, and controls for this source of error will need to be developed.

Given the findings discussed above, I cannot agree that the proposed alternative has been reasonably shown to be equivalent to the required method in terms of agreement.

Precision

A measure of equal precision means that the two methods should demonstrate the same result in terms of the variability in the measurement (e.g., variance around the mean). Unlike accuracy, the variance in result can be examined without having to know the “true” concentration. Repeatability, even if “wrong”, is the key. Precision can be examined systematically for known cultures, both singly and mixed, as well as for a variety of ambient assemblages, although the assumption that all of the living organisms are also viable gets more tenuous when samples of organisms from the environment are used, rather than organisms from known cultures in a healthy phase of population growth.

Problems arise when the ability to get organisms to consistently reproduce sufficient for detection by a method is unknown. When organisms are obtained from culture collections, these are, to a degree, “predisposed” to growing under culture during an MPN-based method. In those cases, organisms are likely to be detected by serial dilution and culturing. For ambient organisms collected from the environment, one would have to run repeated culture experiments: either by selecting organisms and culturing from an initial “seed” individual, or by somehow demonstrating that every time organisms of that species are present in an ambient sample, they demonstrate increased concentration under the provided culture conditions.⁹² In the experiments conducted by the applicants, this is at least partly examined when taxonomic analyses are conducted for the post-incubation dilution tubes. However, the focus in the analysis was on species shown to increase in population size at least some of the time, rather than on species that were shown to be culturable in all tests. For a repeatable standard method, consistent culturability would be critical.

Laboratory experiments with monocultures of the autotrophic organism *Tetraselmis* were conducted (Miller and Petri 2015) to compare the precision of the autotrophic component of the

⁹² This would potentially be confounded by inhibition or encouragement of reproduction by other organisms that would not be in effect if the target species was present as one individual in a MPN dilution tube.

alternative method to that of the required method.⁹³ The results from this test showed the precision was better in the required method than in the alternative method: the coefficients of variation (CVs, a measure of precision, with larger CVs indicating lower precision; calculated from the data in Miller and Petri 2015) were 1-26% (average = 13%) for the required method and 20-135% (average = 50%) for the proposed alternative. The means of the CVs resulting from the two methods were significantly different ($p < 0.05$), when compared using a non-parametric Mann-Whitney U-test. In all but one case, the CV of the required method was lower than the CV of the alternative method. Thus, by this comparison, the required method has greater precision than the proposed alternative.

The applicant also compared precision between the heterotrophic component of the alternative method and a similar microscopical method in experiments using monocultures of the rotifer *Brachionus* (Miller and Petri, 2015). In this comparison, there was no difference between the results produced by the two methods. However, while the two methods were shown to have similar precision (CVs of 23% and 30% respectively), the method against which the alternative was compared was not the required method. Thus, the comparison is invalid and irrelevant for proving equivalent precision with the required method.

Precision was not specifically examined in the experiments conducted with ambient assemblages at two test facilities (Miller *et al.*, 2015b).⁹⁴ However the experiments did report the results of comparisons between concentrations measured with the required method and with the proposed alternative method at the two locations and on two separate dates, providing 4 measurements each for five methods.⁹⁵ The applicant did not provide raw data for the results from the experiment for the required method. However, a summary figure was produced showing average concentrations of organisms obtained using the five methods, and also showing some measure of the variance around the averages as “error bars.”⁹⁶ The degree of variance as indicated by the error bars is clearly greater for the alternative method results than for the required method. Additionally, the CVs for the measurements using the alternative method are provided in a table. Interestingly, the range of CVs was 19-145 for the 14 tests where “0” variance due to method problems was not an issue, and the average CV was 58. This is very similar to the range and average observed for the alternative method when used to measure concentrations of the single autotroph *Tetraselmis* (range: 20-135 and average: 50), as discussed above.

On the basis of these comparisons, the Coast Guard cannot agree that required and alternative methods are equivalent with respect to precision.

D. Does the request include a full description of the proposed alternative?

⁹³ In this case, all of the organisms were autotrophic, so the heterotrophic component of the alternative method was not pertinent.

⁹⁴ MPN Method Development Report No. 5; Miller, A. F. Norlin and B. Petri; 2015. These experiments were focused on evaluating the best culture conditions and in examining the Factor of Agreement (FOA) between the two methods.

⁹⁵ The 5 methods were the required method and four combinations of the proposed alternative method using two media and two temperatures.

⁹⁶ It is unclear what the “error bars” represent. They could be standard deviations, standard errors, or CVs.

The request does not contain a full description of the proposed alternative. Overall, the proposed alternative method is clearly described and understandable. However, in several specific sections there are ambiguities or potential errors that must be addressed:⁹⁷

While the MPN statistical approach is a common microbiological assay for calculating concentrations based on observations of population growth in replicate serial dilution cultures, Hyde has failed to justify why it is appropriate to use for BWMS.

Many microorganisms, such as bacteria, fungi, and algae, can reproduce via asexual cell division, and under the appropriate conditions for growth, a single cell can reproduce to concentrations that are easily detectable. For autotrophic organisms such as phytoplankton, a suspension containing a single organism, given ideal conditions (e.g., nutrients, substrate, temperature, and light), will undergo exponential population growth. After sufficient time for such population increase, bulk metrics can be used to indicate the presence of that population, indicating that at least one reproductive organism must have been present in the original suspension. Changes in common bulk metrics, including chl-a fluorescence for algae and turbidity for bacteria, between initial measurements and measurements after a period of culturing, denote the presence of at least one organism in the initial diluted sample capable of undergoing reproduction.

MPN's success under these circumstances does not mean that it is appropriate for all circumstances, particularly for use with ambient, mixed assemblages of phytoplankton rather than a monoculture. In a given water body, the number of species varies geographically and seasonally, but it is on the order of dozens, if not hundreds, of species.⁹⁸ Applying MPN to such diverse communities may violate the assumptions of the method.

The following assumptions, which were written in reference to bacteria but are also applicable for phytoplankton, are "necessary to support the MPN method" and are stated up front in all specific versions of the MPN statistical approach:⁹⁹

- a) The organisms are distributed randomly within the sample.
- b) The organisms are separate, neither clustered together nor repelling each other.
- c) Every replicate (tube, plate, etc.) whose inoculum contains even one viable organism will produce detectable population growth or change.
- d) The individual tubes of the sample are independent.

A full description of the method must explain why apparent violations of these assumptions are either not critical, or are otherwise accounted for.

The most critical assumption of MPN as a statistical approach is that each viable cell is capable of reproducing, so its original presence in the diluted subsample is detectable through population growth and an increase in the measured parameter over the course of the MPN incubation. The

⁹⁷ These issues are separate from the problems related to lack of equivalence identified *supra*.

⁹⁸ For example, over a thousand phytoplankton species have been identified in the Chesapeake Bay estuary.

⁹⁹ See, e.g., US Food and Drug Administration, *BAM Appendix 2: Most probable number from serial dilutions*, Washington, DC, (2010).

ability of an organism to reproduce must be independent of other organisms, inhibitory factors, such as toxicity due to the presence of trace metals and viruses in the cultures. If every cell may not be capable of being cultured in MPN tubes, and in turn, may not be detected, then the mathematical underpinnings of the MPN calculations are not applicable and procedures that incorporate the MPN methodology will not return accurate estimates. One of the most important aspects of test procedures that incorporate MPN as a statistical approach is that all of the necessary conditions for reproduction and population increase be present during the incubation period. If this is not the case, the method will not detect the presence of organisms that do not reproduce sufficiently to result in a detectable population increase, and the procedure will not result in an accurate measurement of original concentration.

With the above fundamental requirement in mind, it is important to recognize that the information submitted in support of the proposed method includes data showing not all species of phytoplankton are capable of being consistently cultured, at least under the conditions that were used, during all tests. This point is not addressed in the application, other than to assert, without substantiation, that it will present a small bias. The applicants argue that the important issue is whether all species have been observed to reproduce under the provided conditions at least once, rather than consistently (i.e., every time culturing is attempted), and the species that are not known to have reproduced at least once approaches 0% when a long-term historical record is considered. However, the key issue is not whether a viable organism of a species has been observed to reproduce at least once in the past, but instead whether it is known to reproduce consistently whenever present during a test. Thus the percentage of organisms that may be viable but non-culturable is greater when the ability to grow in each test is considered.

The data submitted with the proposed alternative indicate that the proportion of species observed to consistently demonstrate reproduction and population increase varied between the two locations where the issue was examined. At one site, 20-44% of species present in the samples had consistently demonstrated population growth in culture, while at the other the range was 56-89%. Hence, the proportion of species present that had not been observed to reproduce consistently in tests intended to detect organisms capable of reproduction was 56-80% and 11-44% at the other. The conclusion is that the test is not capable of detecting whether members of these species are capable of reproduction.

The assumption that organisms do not aggregate is clearly called into question for mixed assemblages of phytoplankton because of the existence of many species that naturally occur as multi-celled colonies. It may be that this problem could be ameliorated by gentle agitation of the sample to break up the colonies into individual cells, but many of the colonial types adhere quite strongly, and the ability to disaggregate the colonies without causing mortality would need to be established for all the colonial species that might occur in test waters.

The ability of every cell to be cultured is a fundamental tenet of MPN, and a significant shortfall in meeting that criterion calls into question the use of the proposed alternative method. This critical shortfall needs to be resolved.¹⁰⁰

¹⁰⁰ This is and remains a central issue of discussion within EPA's ETV Technical Panel formed to consider, among other things, the acceptability of an MPN-based viability assay. The data presented in support of the proposed

Additionally, there is further uncertainty regarding the estimated MPN in the proposal. Typically, the upper and lower values defining the confidence interval (CI) are reported with MPN estimates. Standard methods for MPN analyses, including methods published by the EPA (U.S. EPA 1978), the U.S. Food and Drug Administration (U.S. FDA 2010), and other scientific authorities (APHA et al. 1999), include tables that list the corresponding 95% CIs. These values must be included when reporting the outcome of an MPN analysis to assure data quality.¹⁰¹ Likewise, the calculators evaluated in the submitted documents all report CIs. However, the proposed method does not require reporting the CIs, nor explain why. As for all calculations of standard errors (SE), of which the CI is an example, larger sample sizes (n) generally result in smaller SE. Take for example, a single dilution MPN with a sample volume of 0.1 mL and an original undiluted concentration of 2.23 viable organisms per mL. Varying the numbers of tubes in an MPN, while holding the percentage of sample tubes positive for growth in each test at 20%, results in a wide range of confidence interval sizes around the MPN. Using 5, 10 or 20 tubes per test results in confidence intervals of 0.31 – 15.9; 0.56 – 8.9; and 0.84 – 5.96, respectively. The precision of the estimate of a 5-tube MPN was limited: the range of CI spanned two orders of magnitude (from 0.31-15.9 organisms mL⁻¹). Higher numbers of sample tubes yield narrower CI ranges, and thus greater confidence in the calculated MPN value.

The proposed method stipulates an MPN matrix should consist of 3 dilutions x 5 tubes per dilution. As shown from the above example, a larger number of tubes must be used to ensure that an MPN value generated from an MPN table does not have a large CI. If the 5 tube case described above were observed in an approval test, even though the MPN is 2.23 viable organisms per mL, a value below the discharge standard, the upper confidence limit is 15.9 viable organisms, a value above the discharge limit.

Of further concern is that the proposed alternative method does not account for the CIs generated by MPN tables. The CI can be relatively large (as noted above), and excluding the CIs can potentially result in a BWMS being considered to meet the discharge standard on the basis of the MPN, whereas the BWMS may not meet the discharge standard if the upper CI was taken into consideration. The lack of consideration of the CI in the alternative method is not explained, other than by a statement that they are not used. The CI, and its meaning with respect to the calculated MPN, must be explained in the alternative method and it should be reported with all results.

Additionally, several sections of the method description require clarification, as discussed below:

- i. Sampling: The sampling scheme described in the method allows for two options: "...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken." Having two options allows for unnecessary and potentially disruptive differences among practitioners. Furthermore, the latter option (one sample with 3 subsamples) could greatly affect the

alternative method have been the basis for many of the panel's discussions, and a generally accepted resolution has not yet been identified.

¹⁰¹ See, e.g., EPA, *Soil sampling quality assurance user's guide*, Report number EPA/600/S4-84-043, Washington, DC.

- outcome of the sampling and is inappropriate for use in this circumstance. This option results in “pseudoreplication,”¹⁰² in which the “replicates” are actually “subsamples” that violate the assumption of independence among samples. If the latter option were used, the statistical analyses would be flawed, potentially yielding results that were wrong. Thus, there must be only one recommended sampling scheme, it should be the first option (three replicate samples).¹⁰³
- ii. Autotrophs – Filtering: The proposed alternative method directs that samples for the autotroph method be filtered onto 10- μm filters. However, if organisms $<10\ \mu\text{m}$ are retained on the filter, as could be expected, estimates of organism numbers from the MPN analysis will be artificially inflated, because the final MPN number would include organisms that are regulated by the Coast Guard ($\geq 10\ \mu\text{m}$ and $<50\ \mu\text{m}$) as well as those that are not regulated by Coast Guard ($<10\ \mu\text{m}$). Similarly, organisms $\geq 50\ \mu\text{m}$ could also be retained on the filter, again, artificially inflating the MPN estimate. The applicant asserts this bias would be small, but in any case, it would be prudent for each test facility using this approach to examine the potential for these circumstances to bias the estimates from the MPN analysis. According to the method description, the filter may or may not be left in the MPN tube during the grow-out period. It is unclear if the practice of leaving a filter in the tube affects the potential for population growth due to smaller organisms entrained on the filter being included in the MPN tubes, or if the presence of the filter itself affects the fluorescence reading. The alternative method must provide unambiguous direction, and if the direction is to leave the filter in the tube, data showing that the practice does not affect the results must be presented.
 - iii. Autotrophs – Measurements: To determine if reproduction and population growth has occurred in an MPN tube, the proposed method specified a minimum threshold fluorescence of four times the standard deviation (SD) of fluorescence measurements from method blanks (tubes containing no chlorophyll). In order for this threshold to be uniformly applied across laboratories, the fluorometers would need to be calibrated following the same, standardized procedure, and demonstrate consistency in measuring the threshold value. This specification must be included in the proposed method.
 - iv. Heterotrophs – Detection: The heterotroph component of the proposed alternative method relies partly on the ability to detect the red autofluorescence of chlorophyll-a (chl-a) containing organisms using epifluorescence microscopy. However, the optical filter set specified in the method is optimized to detect the fluorescence from fluorescein, not chl-a; according to the wavelengths of the filter set specified in the heterotroph method (B-2E/C), the red autofluorescence of chl-a would not be visible (i.e., the optical filter would not serve to identify organisms with chl-a). Potentially, all organisms detected would not fluoresce, and would appear to lack chl-a and, therefore, if they were motile, they would be scored as heterotrophs. Filter sets are available that allow chl-a fluorescence to be detected and must be used.

¹⁰² Hurlbert SH, *Pseudoreplication and the design of ecological field experiments*, Ecol Monogr 54:187–211 (1984).

¹⁰³ This does not apply to sample tubes used in the MPN approach; in such case, water for all dilution tubes (i.e., an array consisting of 3 dilutions, each with 5 replicates) should be drawn from the same population. Tubes are inoculated with water from a single, original sample.

Additionally, the procedure used to quantify living heterotrophic organisms scores cells that are moving and do not show red autofluorescence (i.e., they do not contain chl a) as living heterotrophs. However, if organisms do not exhibit fluorescence and are not viewed with another light source (one is not stipulated in the proposal), they will, whether moving or stationary, be, at best, dimly illuminated by light and difficult to see. This potential problem should be addressed.

- v. Data Analysis: It is unclear how the uncertainties around the counts resulting from the autotroph and heterotroph components of the proposed alternative method are applied. Each set of replicate measurements will result in an estimate of the variance around the mean. If the variance estimates are to be added together, as are the means to arrive at a total number of organisms, there is no statistical justification provided for doing so. Instructions, and justifications for such, should be provided.

Conclusion

In sum, I deny your appeal and affirm MSC's decision denying Hyde's request for a testing equivalency under 46 C.F.R. § 162.060-10(b)(1). When promulgating its ballast water regulations, the Coast Guard explicitly rejected "viability" as part of its ballast water discharge standard. This policy decision was based on the best scientific information available and was the most appropriate, environmentally protective decision within the Coast Guard's statutory mandate. MSC was therefore correct in denying your proposal because your proposal requests approval of a test method that assesses viability of organisms rather than using criterion for counting 'living' organisms as required by the regulations. MSC lacked the discretion to accept your proposed testing method. Even if MSC did have the discretion to accept viability, your application failed to meet the requirements of 46 C.F.R. § 162.060-10(b)(1).

This decision constitutes final agency action on the issues raised in your appeal.

Sincerely,



Linda L. Fagan

Rear Admiral, U. S. Coast Guard

Deputy for Operations Policy and Capabilities

Technical References

1. (Miller 2015a) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Supplemental Information 02 MAR 2015
2. (Miller 2015b) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060 10(b)(1)) Updated Documentation 06 MAY 2015
3. (Trojan Marinex 2015a) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples 31 JAN 2015
4. (Trojan Marinex 2015b) An Alternative Method for Determining the Number of Living Organisms in the 10-50 μm Size Class for Ballast Water Management System Test Samples, 05 MAY 2015
5. (Petri 2015a) Evaluating the MPN Dilution-Culture Method for the Enumeration of Viable Phytoplankton Cells
6. (Maurer and Welschmeyer 2015a) Flow Cytometric Analysis of the Relative Abundance of Heterotrophs and Autotrophs in the Regulated 10-50 μm Size Class
7. (DHI 2014) MPN Assay – Analyses of Algal Regrowth for Performance Evaluation of Ballast Water Management Systems Primary Validation
8. (Petri 2015b) MPN Method Development Experiments 1 to 3 Inter-Lab Comparison of the MPN Dilution-Culture Method and Fluorescein-Based Staining Methods for the Enumeration of Viable or Living Phytoplankton Cells
9. (Miller et al. 2015a) MPN Method Development Report Experiment 4
10. (Miller et al. 2015b) MPN Method Development Report Experiment 5
11. (Miller and Petri 2015) MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 μm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology
12. (Cullen and MacIntyre 2015) On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment
13. (Maurer and Welschmeyer 2015b) Rationale for the Use of Most Probable Number (MPN) Technique in the Evaluation of UV-based Ballast Water Management Systems
14. (MacIntyre et al. 2015) Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water

15. (Miller 2015c) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 03 JUN 2015
16. (Miller 2015d) 1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 30 JUN 2015
17. (Miller 2015e) 1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 23 JUL 2015
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From: Commanding Officer, Naval Research Laboratory
To: Commandant, United States Coast Guard (CG-OES-3 R. Bergner)

Subj: REVIEW OF A REQUEST FOR APPROVAL OF AN ALTERNATIVE METHOD FOR BALLAST
WATER TESTING (46 CFR 162.060-10(B)(1)): TROJAN MARINEX'S METHOD FOR
ASSESSING ORGANISMS $\geq 10 \mu\text{M}$ AND $< 50 \mu\text{M}$

Encl: (1) Two copies of subject report

1. Enclosure (1), entitled "Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(b)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$," provides a technical review of a request to use an alternative method to quantify organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ (nominally protists).
2. This work was sponsored by and guidance was provided by the United States Coast Guard Office of Environmental Standards (CG-OES-3).
3. The NRL points of contact are Lisa Drake, Code 6136, 305-293-4215, e-mail: lisa.drake@nrl.navy.mil; and Edward Lemieux, Code 6130, 202-404-2123, email: edward.lemieux@nrl.navy.mil.


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Review of a Request for Approval of an Alternative Method for Ballast Water Testing (46 CFR 162.060-10(b)(1)): Trojan Marinex's Method for Assessing Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$

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EXECUTIVE SUMMARY

In response to international and national actions to reduce the transport and delivery of aquatic nuisance species (ANS) in ballast water, an industry of commercial ballast water management systems (BWMS) using a variety of technical approaches has developed over the past decade. To gain U.S. Type Approval (TA) for these systems, they must be tested according to regulations in 46 CFR 162.060—Ballast Water Management Systems, which incorporate by reference the Environmental Protection Agency (EPA) Environmental Technology Verification (ETV) Program “Generic Protocol for the Verification of Ballast Water Treatment Technology” (U.S. EPA 2010). Within the ETV Protocol, procedures and conditions for land-based verification testing of BWMS are described.

Trojan Marinex submitted a series of documents (Proposal) to the U.S. Coast Guard (USCG) requesting approval under regulation 162.060–10(b)(1) to use an Alternative Method to quantify concentrations of living organisms ≥ 10 and < 50 μm (nominally protists, which includes autotrophic and heterotrophic organisms, as discussed below). The currently Required Method prescribed in the ETV Protocol is a microscopy-based approach combining cellular movement and two fluorescent markers, fluorescein diacetate (FDA) and 5-chloromethylfluorescein diacetate (CMFDA). Here, organisms in the ≥ 10 and < 50 μm size class—which includes autotrophs (in this case, photosynthetic organisms known as phytoplankton or microalgae) and heterotrophs (organisms, such as amoeba, that derive energy from other organisms)—are quantified. The Required Method uses movement and the two fluorescent markers to assess a cell’s active enzymes (specifically, esterases) to measure if an organism is *living* (or dead, if no enzyme activity is visible and no cellular movement is detected). The proposed Alternative Method consists of two parts: (1) the Autotroph Method, which uses the Most Probable Number (MPN) method to quantify photosynthetic organisms, and (2) the Heterotroph Method, which uses direct microscope counts to quantify heterotrophic organisms (identified by movement and absence of chl *a* fluorescence). The MPN method is a culturing technique adapted from the field of food microbiology for use in evaluating phytoplankton communities, and it uses a 14-day (d) grow-out period to quantify *viable* (= able to reproduce) organisms. This approach is in contrast to the Required Method, which quantifies *living* organisms.

The Ballast Water Science and Technology Program at the Naval Research Laboratory (NRL) was tasked by USCG to review the Proposal and provide their technical opinion. This report (Review) is the outcome of that work. The following paragraphs summarize how the Proposal addressed the requisite elements of a 10(b)(1) request—specifically its practicability and applicability argument, completeness of description, and equivalency with the Required Method—as well as the applicability of the Alternative Method to compliance testing.

- The Proposal partly addressed the practicability criterion by stating the Required Method used to quantify living organisms is not practicable for ultraviolet (UV)-based BWMS. The Proposal asserted that, as systems are currently designed, it is not practicable to increase the UV dose to kill organisms (so that the Required Method would show cells were dead following UV treatment), thus obviating the need for the Alternative Method, which uses a 14-d grow-out period to show if cells are viable. Using a summary of data submitted to a peer-reviewed journal (the data are unpublished at present), the Proposal

argued that: a UV dose sufficient to reduce cell numbers by 100 fold and damage cells' enzyme systems to render them dead following treatment—the foundation of the Required Method—would necessitate a 10-fold increase in the UV dose. Such an increase would incur a concomitant 10-fold increase in the footprint of the BWMS and its electrical power requirements. The argument, however, contained no supporting data (e.g., the engineering and economic calculations to show the increase in power and corresponding number of BWMS that would be needed to use the Required Method were lacking). This information, as well as the data and dose-response curves (demonstrating the US dose required to kill organisms in this size class) from the unpublished study, should be provided.

- As a fundamental principal, the Proposal contended that the goal of the USCG ballast water regulations is to prevent the spread of potentially invasive organisms. Hence, a treatment that renders organisms unable to reproduce (rendering organisms “non-viable”, such as using UV treatment as currently applied by the BWMS manufactured by Trojan Marinex) meets the intent of the regulation. This reasoning addresses the “applicability” aspect of the 10(b)(1) request. The authors of this Review concur that such an outcome meets the intent, although not the letter, of the USCG final rule.

- The method described in the Proposal was, for the most part, clear and understandable. Field and laboratory experiments were summarized (and the raw data were provided) to support the conclusions made in the Proposal regarding development of the method. Further, the sequence of experiments was clear as the proposers gathered more information to refine the MPN portion of the Alternative Method. This Review identified several concerns in this portion of the evaluation; most stem from a lack of data to validate steps in the method or a lack of standardization. The items and their potential outcomes are listed below in decreasing order of importance.
 - The threshold for determining growth in an MPN tube is quite low, and it will be dependent on the fluorometer's calibration. These items should be specified and standardized in the Proposal.
 - The filter set described in the Heterotrophic Method is not optimized to detect chl *a*, the target molecule.
 - Additional data are required to determine if filters should be removed from the MPN tubes.
 - From calculations prepared for this Review, it appears the number of MPN tubes used in testing should be increased; this point should be investigated.
 - MPN values may be inflated if organisms <10 μm are inadvertently added to the MPN tubes as may occur if filters are left in the dilution tubes; this potential should be investigated.
 - The confidence intervals (CIs) around the MPN results should be explained in the Alternative Method and should be reported with all results; for that matter, all data using the Required Method should also include CIs.
 - Similarly, the means to combine the CIs for the Autotroph and Heterotroph Methods should be provided.

- The Alternative Method allows the Testing Organization (TO) to determine the approach in which samples are collected, and that should not be the case: the Alternative Method should stipulate how samples are collected.
 - The means to categorize organisms by size (as part of the Heterotroph Method) should be stated explicitly in the Alternative Method.
 - The number of samples analyzed for autotrophs and heterotrophs in tests of the Alternative Method's Initial Precision and Accuracy (IPA) and Ongoing Precision and Accuracy (OPA) should be increased from one to at least three. Likewise, an upper limit of the Factor of Agreement (FOA) between the Required and Alternative Methods should be defined in the Proposal.
- The Proposal did not provide adequate justification that the proposed Alternative Method is equivalent to the Required Method for three reasons:
- (1) The equivalence between dead and non-viable organisms was not demonstrated.
 - The equivalence between dead and non-viable organisms was not demonstrated using empirical data, and it should be provided to show, first, that dead and non-viable cells are equivalent, and second, that non-viable cells remain so. These issues could be addressed in an experiment using phytoplankton that are treated to induce immediate mortality without the possibility of repair (such as electrochlorination) or treated by UV radiation, and comparing the outcomes using the Required and Autotroph (MPN) Methods over a period >14 d (to assess the capacity of cells to undergo repair). This longer assessment period is important for organisms that are capable of repair but do not reproduce to detectable levels within the span of the 14-d incubation identified in the Alternative Method. These results could be augmented or replaced with similar experiments already described in the peer-reviewed literature.
 - (2) The equivalence between the precision of the Required and Alternative Methods was not demonstrated.
 - Laboratory experiments showed the precision was better in the Required Method than in the Alternative Method: the coefficients of variation (CVs) were 1-26% (average = 13%) for the Required Method and 20-135% (average = 50%) for the Autotroph (MPN) method. In all but one case, the CV of the Required Method was better (lower) than the CV of the Autotroph (MPN) Method. Thus, using a metric typically used to compare methods, precision, the Alternative Method was shown—using the data provided in the Proposal—to be less precise than the Required Method. Further, empirical measurements of the FOA (using ambient communities) between the two methods varied widely among different experiments. At times, the Alternative Method yielded a count at least five times greater than the Required Method. This result is surprising, since the water samples in these trials were not treated with UV radiation; in untreated samples, the Required and Alternative Methods would be expected to have similar results (FOA of ~1). This result could be due to the inclusion of organisms <10 μm in the samples that are not removed by

the filtration through the 10- μ m pre-filter step prior to analysis with the Alternative Method.

- In addition, in the Proposal, the Alternative Heterotrophic Method was not compared to the Required Method (it was instead compared to a microscopy-based method that was different from the epifluorescence-microscopy method outlined in the Required Method). For a legitimate comparison, the Heterotroph and Required Methods should be compared.

(3) Statistical questions remain unresolved.

- Regarding the use of the Autotroph (MPN) Method, most critically, the percentage of non-culturable taxa is not accounted for in the calculation of the number of viable cells. This percentage should be known at each test facility (TF) using the Alternative Method, and upon the advice of statisticians, somehow incorporated into the calculations of cell densities. It is unclear how this factor would be incorporated into tests for shipboard verification of BWMS, because it would be nearly impossible to characterize the culturable and non-culturable taxa in every shipping port. Likewise, it is unclear how such “false negatives” would be incorporated into the Required Method during shipboard trials (that is, how organisms that are living but not moving or fluorescing would be addressed).
 - Other statistical concerns are more easily addressed: the MPN estimates are provided without measures of uncertainty (e.g., CIs), and they should be reported with all results. Additionally, at times, MPN estimates may be undefined, e.g., results may be reported as “greater than” (>) or “less than” (<) the detection limit, and in these instances, it is unclear how these results should be used, such as when calculating the sample mean. Finally, the process for combining the uncertainty in the autotroph concentration (based on probability from the MPN Method) and the uncertainty in the heterotrophic concentration (based on cell counts) is unclear (i.e., how the CIs for both numbers are used—are they combined in a straightforward fashion, by adding them together, or are additional calculations required?). These omissions should be rectified.
- For a number of reasons, it seems impracticable that the Alternative Method (specifically, the MPN portion of it) could be used for compliance testing to determine ships’ adherence to the ballast water discharge standard. If the Alternative Method was used for TA testing of BWMS and another method was used for compliance testing, it would be necessary to determine the correspondence between the two methods. At this point, it appears that variable fluorescence of photoautotrophs in unfiltered (whole) water samples may be used to determine compliance with the discharge standard. It would be prudent to begin a validation study to determine how the Alternative Method and variable fluorescence method relate to one another.

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ACRONYMS, ABBREVIATIONS, AND SYMBOLS

Term	Definition
<i>A</i>	Aliquot volume
ANS	Aquatic nuisance species
BP	Band pass
BWM	Ballast water management
BWMS	Ballast water management system
C	Celsius
<i>C</i>	Concentrated sample volume
CFR	Code of Federal Regulations
cfu	Colony forming unit
chl <i>a</i>	Chlorophyll <i>a</i>
CI	Confidence interval
CMFDA	Chloromethylfluorescein diacetate
CV	Coefficient of variation
d	Day
<i>D</i>	Dilution factor
DHI	Danish Hydrological Institute
DNA	Deoxyribonucleic acid
EPA	United States Environmental Protection Agency
ETV	Environmental Technology Verification Program
FDA	Food and Drug Administration or Fluorescein diacetate
FOA	Factor of Agreement
FR	Final Rule
G8	Guidelines for Approval of Ballast Water Management Systems
GSI	Great Ships Initiative
<i>I</i>	Individual count
IL	Independent Laboratory
IMO	International Maritime Organization
IPA	Initial Precision and Accuracy
ISO	International Organization for Standardization
L	Liter
LP	Long pass
<i>m</i>	Number of dilutions

Term	Definition
m ³	Cubic meter
min	Minute
mL	Milliliter
MLML	Moss Landing Marine Laboratories
MPN	Most Probable Number
MSC	Marine Safety Center (United States Coast Guard)
<i>n</i>	Number of subsamples
NIVA	Norwegian Institute of Water Research
NRL	Naval Research Laboratory
NRLKW	Naval Research Laboratory, Key West Florida
OPA	Ongoing Precision and Accuracy
<i>P</i>	Population concentration
PAR	Photosynthetically active radiation
<i>S</i>	Sample volume
SD	Standard deviation
SE	Standard error
SOP	Standard operating procedure
SP	Short pass
RNA	Ribonucleic acid
TA	Type Approval
TF	Test facility
TO	Testing Organization
USCG	United States Coast Guard
UV	Ultraviolet
UV-B	Ultraviolet radiation with wavelength of 290-320 nm
UV-C	Ultraviolet radiation with wavelength of 100-290 nm
<i>v</i>	Volume of subsample
δ	Density of organisms
μm	Micrometer

1 INTRODUCTION

Actions have been taken to reduce the transport of aquatic nuisance species (ANS) in ships' ballast water. At the largest—international—scale, the International Maritime Organization (IMO) adopted the Ballast Water Management Convention (BWM Convention; IMO 2004), which has yet to be ratified sufficiently to enter into force. At a national—US—scale, several statutorily authorized executive actions governing ballast water discharges were promulgated by the U.S. Coast Guard (USCG) and the Environmental Protection Agency (EPA) between 1990 and 2013 (e.g., USCG 2012, U.S. EPA 2013). Both the IMO and US actions aim to limit the number of organisms discharged in ballast water that could potentially establish new populations, and they have similar discharge standards (Table 1); most ships will use a ballast water management system (BWMS) to meet the numeric limits.

Table 1. Ballast water discharge standards.

Organization and standard	Organisms $\geq 50 \mu\text{m}$ in minimum dimension ^A	Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ in minimum dimension ^B	Toxigenic <i>Vibrio cholerae</i> ^C	<i>Escherichia coli</i>	Intestinal enterococci
US Discharge Standard	$< 10 \text{ m}^{-3}$ (“living”)	$< 10 \text{ mL}^{-1}$ (“living”)	$< 1 \text{ cfu}$ 100 mL^{-1}	$< 250 \text{ cfu}$ 100 mL^{-1}	$< 100 \text{ cfu}$ 100 mL^{-1}
IMO Regulation D-2 Ballast Water Performance Standard	$< 10 \text{ m}^{-3}$ (“viable”) ^D	$< 10 \text{ mL}^{-1}$ (“viable”) ^D	$< 1 \text{ cfu}$ 100 mL^{-1} or $< 1 \text{ cfu g}^{-1}$ (wet weight zoopl.)	$< 250 \text{ cfu}$ 100 mL^{-1}	$< 100 \text{ cfu}$ 100 mL^{-1}

^ANominally zooplankton. ^BNominally protists. ^CSerotypes O1 and O139. ^DWhile the discharge standard in the Ballast Water Management Convention is framed in terms of “viable” organisms, the Guidelines for Approval of Ballast Water Management Systems (G8) specify that for purpose of testing the efficacy of BWMS, “viable” means “living”; cfu = colony forming unit, IMO = International Maritime Organization, and zoopl. = zooplankton.

Notably, the US standard applies to organisms that are “living” (which may include organisms unable to reproduce) rather than “viable” (able to reproduce). This distinction is due to (1) the inability to culture all potential organisms in the laboratory to determine their viability, and (2) the more protective and conservative criterion set by requiring cells to be *dead* rather than *non-viable* (e.g., Federal Register 2012). While the BWM Convention is framed in terms of “viable” organisms in regulation D-2, the IMO Guidelines for Approval of Ballast Water Management Systems (G8)—which were finalized after the BWM Convention was adopted—specify that for purposes of testing the efficacy of BWMS, “viable” is defined “organisms and any life stages thereof that are living” (IMO 2008). That said, in practice, viability assessments, (rather than live-dead assessments) have been made in Type Approval (TA) testing of BWMS under the BWM Convention. Here, the Most Probable Number (MPN) technique, developed for single

strains of bacteria, is used to quantify organisms in the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class (nominally protists). The bacteria for which the MPN technique was developed are readily culturable under laboratory conditions, whereas not all species present in the diverse community of ambient protists can be grown in the laboratory, which presents a difficulty, which is discussed below.

In the decade following the adoption of the BWM Convention, an industry for commercial BWMS has developed. In the US, under the USCG regulations, BWMS must be type approved by the USCG, and the process for granting TA for BWMS requires testing (following a rigorous quality management system) in accordance with standard, published protocols. The USCG Marine Safety Center (MSC) is responsible for reviewing, accepting, and approving applications for TA. Under this process, BWMS manufacturers are required to work with a USCG-accepted Independent Laboratory (IL), which will conduct land-based, shipboard, and environmental testing, as well as submit the TA test reports in support of the manufacturer's TA application to MSC.

The requirements for the TA application and verification testing are codified in the US Code of Federal Regulations (CFR; 46 CFR 162.060). Testing and test documentation must be in accordance with regulatory references for quality management (International Organization for Standardization, ISO 17025:2005(E)) (ISO 2005). Specifically, land-based testing will proceed according to the "Generic Protocol for the Verification of Ballast Water Treatment Technology" (U.S. EPA 2010). The Protocol was developed—with the input of stakeholders and technical experts—under the Environmental Technology Verification (ETV) Program in a joint effort between the USCG and the EPA. The draft protocol was tested at the Naval Research Laboratory, Key West, FL (NRLKW) (Lemieux et al. 2008), modified as needed, finalized (henceforth, ETV Protocol), and, later, incorporated by reference into the USCG final rule (FR) for the "Standards for Living Organisms in Ships' Ballast Water Discharged in US Waters" (USCG 2012). A shipboard protocol is now being drafted, and it may ultimately be incorporated into the existing ETV Protocol. In addition to land-based and shipboard verification testing under well-documented biological and water quality conditions, USCG regulations require all BWMS components, the marine suitability and safety of the BWMS, and its manufacturing processes to be assessed.

1.1 Basis for Approval of Alternative Methods Changes

The procedures used in sampling and analysis for US TA testing of BWMS are complicated, not only due to the engineering aspects (relatively large volumes of water are used, e.g., 200 m³ or greater), but also due to the biological aspects: a system's biological efficacy must be carefully measured to accurately determine adherence to a very strict numerical standard, e.g., < 10 organisms $\geq 50 \mu\text{m}$ in 1 m³ of ballast water. Accordingly, deviations in testing are allowable through regulation 162.060–10(b)(1), with requests addressed to and approved by USCG MSC. Here, this formal process allows for *pre-approval* for any deviations from the evaluations, inspections, or tests prescribed by the regulations. The request for a deviation must be justified and an Alternative approach validated:

“46 CFR § 162.060–10 Approval procedures. (b)(1) If an evaluation, inspection, or test required by this section is not practicable or applicable, a

manufacturer may submit a written request ... for approval of Alternatives as equivalent to the requirements in this section. The request must include the manufacturer's justification for any proposed changes and contain full descriptions of any proposed Alternative tests.”

1.2 Goals and Objectives

On 05 FEB 2015, Trojan Marinex submitted a 10(b)(1) request (Proposal, a series of documents) to USCG to use an Alternative Method to quantify living organisms in the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class. On 23 APR 2015, the Ballast Water Science and Technology Program at NRL was tasked by the USCG to review the Proposal and provide a technical evaluation on the following aspects of the application:

1. The justification that the requirement in 162.060 is not practicable or applicable,
2. The degree to which the method description provides a clear presentation of test procedures,
3. The justification that the proposed Alternative Method is equivalent to the Required Method, and
4. The potential for conflict or incompatibility between TA testing and compliance testing; that is, if efficacy during TA testing is based on viability and compliance assessment during vessel inspections is based on relationships between concentrations of living photosynthetic organisms and fluorescence of their photosynthetic pigment systems.

This report (Review) addresses those goals by first considering the elements of practicability and applicability. Next, the Alternative Method and its supporting documents are considered, followed by a discussion of the validation of the Alternative and Required Methods. Afterwards, the two methods' equivalency is discussed. Finally, the compatibility between the Alternative Method's use in TA and compliance testing is considered.

2 JUSTIFICATION THE REQUIREMENT IS NOT PRACTICABLE OR APPLICABLE

The foundation for a 10(b)(1) request is that a requirement in 162.060 is not “practicable or applicable”. That is, the requirement is (1) not possible to practice or perform or (2) not relevant, suitable, or appropriate for use in evaluating the performance of the BWMS. In this section, the practicability of using the Required Method to assess ballast water treated by ultraviolet (UV) radiation is addressed, followed by the Required Method's applicability in this instance.

The Required Method prescribed in the ETV Protocol quantifies living organisms in the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class, which includes autotrophs (here, photosynthetic organisms known as phytoplankton or microalgae) and heterotrophs (organisms, such as amoeba, that derive energy from other organisms). The assay quantifies living organisms (at the time of interrogation) and does not assess reproductive ability. This method combines two molecular, membrane-permeable markers, fluorescein diacetate (FDA) and 5-chloromethylfluorescein diacetate (CMFDA or CellTracker™ Green [Invitrogen]). These markers are widely used in many fields of biology to identify living cells. Initially as the Required Method was developed, trials were

conducted using the FDA and CMFDA markers singly; later, the markers were combined because they have similar emission spectra and could be combined and used simultaneously to stain a larger taxonomic range of organisms than either marker used singly would cover. When the colorless markers enter a living cell, non-specific esterases in the cell hydrolyze the probes to create a non-permeable product (i.e., it is trapped within the cell that has an intact cell membrane). The product fluoresces green when excited with blue light; the green signal is visible using an epifluorescence microscope. When an organism exhibits this green fluorescence or it is motile (even if it does not show green fluorescence), it is scored as “living”. Organisms that are motile (and thus are clearly living) that do not fluoresce green represent “false negative” results: they are living, so they should fluoresce, but they do not; regardless, due to their motility, they are enumerated as living cells. Another form of false negative results is organisms that are living but are not motile and do not fluoresce green—they are not scored as living cells. This error represents a shortcoming in the assay and is analogous to organisms that do not grow in MPN assays.

The argument is made by the Proposal that the Required Method will not “evaluate” organisms in the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class that are alive but treated with UV that “prevents cell replication (and thus precludes invaders from colonizing)” (Miller 2015a). Hence, the Proposal infers (but does not directly state) the Required Method is not applicable to assess UV treatment efficacy. Instead, the Proposal recommends an Alternative Method that combines two approaches to quantify organisms in this size class: (1) the Autotroph Method, which uses the MPN method to quantify photosynthetic organisms, and (2) the Heterotroph Method, which uses direct microscope counts to quantify heterotrophic organisms (identified by movement and absence of chlorophyll *a* [chl *a*] fluorescence). The Autotroph Method uses an MPN culturing technique with a 14-day (d) grow-out time to assess the number of *viable* organisms, that is, the number able to reproduce. This approach is in contrast to the Required Method, which assesses the number of *living* organisms.

The idea that the Required Method is not applicable to UV treatment is really an issue of practicability in achieving specific endpoints when using UV as a treatment. The UV doses *commonly used* in BWMS are not great enough to induce mortality during the 1-5 d hold time following treatment during TA testing. Increased UV doses would not only prevent reproduction or cellular division, but also induce mortality that is detected following treatment using the Required Method. If so, cell membranes would be compromised and cytosolic enzymes would be denatured or lost, and the fluorescent markers would not be hydrolyzed within cells. Thus, the Required Method would indicate treatment efficacy in meeting the “live” discharge standard because the dead cells would not fluoresce green. An Alternative Method would not be needed.

An assertion was made in the Proposal that it is not practicable to increase the UV dose so cells are dead (as determined by the Required Method) rather than not viable (as determined by the Alternative Method) (Miller 2015d). Using summary data (no dose-response curves were provided) from an unpublished study using 12 species of algae, the Proposal argued that a UV dose sufficient to damage cells’ non-specific esterases—the foundation of the Required Method—and reduce concentrations of algae by 100-fold (from 1000 cells mL^{-1} to 10 cells mL^{-1}) would be “extremely high”. On average, a 10-fold increase in UV dose was needed to kill cells using the Required Method compared to the dose needed to show cells were non-viable with the

Alternative method. It should be noted that in three of the 12 algal species, the study found no threshold at which cells were dead when scored using the Required method. In the remaining nine species, the average increase in dose required (i.e., 10-fold) was skewed by two species, which required an 18- to 34-fold increase. Seven of the 12 species required a dose <10-fold greater than that used with the Alternative Method to kill them. The doses were not specified. The Proposal further asserted such an increase would incur a concomitant 10-fold increase in the footprint of the BWMS and its electrical power requirements. Thus, requiring ballast water treated with UV BWMS to meet the discharge standard for organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ (i.e., < 10 living organisms mL^{-1}), as assessed using the Required Method, would not be practicable for this technology, essentially excluding UV treatment from the BWMS market. The Proposal, however, contained no supporting data to support the assertion of 10-fold increases in space or energy requirement. For example, calculations illustrating the increase in power and footprint that would be needed to meet the “live” criterion assessed by the Required Method were not included. The Proposal did not state why the practicability argument pertains to the autotrophic portion of the ballast water community (which is assessed with a viability method) but not the heterotrophic portion (which is assessed with a live-dead method).

As a fundamental principal, the Proposal contends that the goal of the USCG ballast water regulations is to prevent the spread of potentially invasive organisms. Therefore, a treatment that renders organisms unable to reproduce (rendering organisms “non-viable”, such as using UV treatment as currently applied by the BWMS manufactured by Trojan Marinex) meets the intent of the regulation. The authors of this Review concur that such an outcome meets the intent, although not the letter, of the USCG final rule. Further, any Alternative methods must be shown to be equivalent to the Required Method; the purpose of this report is to assess equivalency.

3 PROPOSAL DOCUMENTS AND DESCRIPTION OF THE ALTERNATIVE METHOD

3.1 Documents Submitted

Eighteen documents were submitted as part of the Proposal (Table 2). The first document in the table (1511138 – Request for Approval of an Alternative Method) provides an overview of the request and the initially submitted supporting documents; six documents were received after the initial submission.

Table 2. Documents submitted as part of the Proposal.

#	Title and Authors	Type of Submission
1	1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1)) Supplemental Information 02 MAR 2015 (Miller 2015a)	Correspondence: cover letter accompanying supplemental information (this correspondence references the letter accompanying the original request for approval [dated 05 FEB 2015, Reference 1511138], which was not provided to the authors of this Review)
2	1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1)) Updated Documentation 06 MAY 2015 (Miller 2015b)	Correspondence: cover letter accompanying the updated Alternative Method Description (see row 4 of this table)
3	An Alternative Method for Determining the Number of Living Organisms in the 10-50 µm Size Class for Ballast Water Management System Test Samples (Trojan Marinex 2015a)	Alternative Method Description: detailed protocol describing the Alternative Method
4	An Alternative Method for Determining the Number of Living Organisms in the 10-50 µm Size Class for Ballast Water Management System Test Samples, 05 MAY 2015 (Trojan Marinex 2015b)	Alternative Method Description: updated detailed protocol describing the Alternative Method, which was the version used for this Review; at the same time, an additional version was submitted concurrently with the “track changes” feature in Microsoft Word; this document was not assigned a separate document number
5	Evaluating the MPN Dilution-Culture Method for the Enumeration of Viable Phytoplankton Cells (Petri 2015a)	Supporting information: paper that describes the MPN dilution culture method
6	Flow Cytometric Analysis of the Relative Abundance of Heterotrophs and Autotrophs in the Regulated 10-50 µm Size Class (Maurer and Welschmeyer 2015a)	Supporting information: presentation that describes the relative abundance of heterotrophs and autotrophs at several US West Coast locations and at DHI Denmark

#	Title and Authors	Type of Submission
7	MPN Assay – Analyses of Algal Regrowth for Performance Evaluation of Ballast Water Management Systems Primary Validation (DHI 2014)	Supporting information: paper that describes the validation of the MPN assay at DHI Denmark
8	MPN Method Development Experiments 1 to 3 Inter-Lab Comparison of the MPN Dilution-Culture Method and Fluorescein-Based Staining Methods for the Enumeration of Viable or Living Phytoplankton Cells (Petri 2015b)	Supporting information: paper that describes experimental results and analyses of BWMS samples obtained from a test at DHI Denmark and then analyzed at three locations: DHI Denmark, MLML, and BallastTech-NIVA AS
9	MPN Method Development Report Experiment 4 (Miller et al. 2015a)	Supporting information: paper that describes experimental results and analyses of samples that were collected at three laboratories: DHI Denmark, MLML, and NIVA. Samples were analyzed to determine the MPN results with different growth media, temperature, and filtration
10	MPN Method Development Report Experiment 5 (Miller et al. 2015b)	Supporting information: paper that describes with experimental results and analyses of samples collected at DHI and NIVA. The Autotroph Method, Heterotroph Method, and Required Method were used to analyze the samples
11	MPN Method Development Experiment 6 Generating Method Performance Data for the Alternative Method for Analyzing 10-50 µm Organisms in the ETV Generic Protocol for the Verification of Ballast Water Treatment Technology (Miller and Petri 2015)	Supporting information: paper that describes experimental results and analyses of samples for a workshop in London, Canada. This evaluation used standard test organisms, <i>Tetraselmis spp.</i> (phytoplankton) and <i>Brachionus spp.</i> (a rotifer, a heterotroph), to assess the Autotroph and Heterotroph Methods at various cell concentrations and compared the results to those obtained using the Required Method. The goal was to determine the precision and accuracy of the Alternative Method

#	Title and Authors	Type of Submission
12	On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment (Cullen and MacIntyre 2015)	Supporting information: article that was accepted by the Journal of Applied Phycology that describes the use of MPN assay for assessing viability of organisms; the final version of this document was later sent but not assigned a separate document number
13	Rationale for the Use of Most Probable Number (MPN) Technique in the Evaluation of UV-based Ballast Water Management Systems (Maurer and Welschmeyer 2015b)	Supporting information: paper that analyzes data from GBF over a 2-year period to compare the FDA counting technique to the MPN assay that was used during testing of UV-treated ballast water samples at GBF
14	Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water (MacIntyre et al. 2015)	Supporting information: presentation that describes false positives that may be encountered when the markers used in the Required Methods are compared to the MPN growth assay
15	1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 03 JUNE 2015 (Miller 2015c)	Correspondence: letter that provides an overview of the publication by Cullen and MacIntyre et al. (2015)
16	1511138—Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 30 JUNE 2015 (Miller 2015d)	Correspondence: letter that explains why the Required Method is not practicable
17	1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 23 JULY 2015 (Miller 2015e)	Correspondence: letter that explains the equivalent environmental protection of the Alternative Method from a quantitative and experimental perspective
18	1511138 - Request for Approval of Alternative Method (Ref 46 CFR 162.060-10(b)(1) Supplemental Information 26 JULY (Miller 2015f)	Correspondence: letter with an overall summary of the accuracy of the proposed Alternative Method

DHI = Danish Hydrological Institute, ETV = Environmental Technology Verification, FDA = fluorescein diacetate, GBF = Golden Bear Facility, MLML = Moss Landing Marine Laboratories, MPN = most probable number, and NIVA = Norwegian Institute of Water Research

3.2 Overview of Alternative Method

The Proposal describes a two-part Method that evaluates autotrophs and heterotrophs in the ≥ 10 μm and < 50 μm size class (it is assumed that organisms ≥ 50 μm are removed in the BWMS prior to treatment with UV, and thus, this size class is not considered in the Proposal or this Review). The terms “Autotroph Method” and “Heterotroph Method” were used throughout the Proposal, and for clarity, this Review will employ consistent terminology; the sum of the two methods, i.e., the overall method, is deemed the Alternative Method. Note that the Alternative Method sometimes referred to the largest size class as “10-50 μm ”, which this Review considers equivalent to “ ≥ 10 μm and < 50 μm ”, or it discussed organisms > 50 μm , which this Review considers equivalent to ≥ 50 μm (following the size classes delineated in Table 1).

3.2.1 Sample Collection

In the Alternative Method, triplicate subsamples are collected and analyzed. The Alternative Method allows the testing organization (TO) some latitude in procedure. For example, to determine how the triplicate subsamples are collected, the available choices are: “...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken” (Trojan Marinex 2015b). This process is discussed further below (Section 3.3.1 Drawbacks—Sampling).

3.2.2 Autotroph Method—Most Probable Number (MPN) Approach

The Autotroph Method uses a chl *a*-based MPN culture dilution technique to evaluate the concentration of reproductive, photoautotrophic organisms (phytoplankton). While autotrophs may derive energy from other sources than oxygenic photosynthesis, such as chemoautotrophy, the most abundant autotrophs expected to be encountered in ballast water are photoautotrophs (phytoplankton or microalgae). The methods in the Proposal were developed to evaluate UV treatment, although, in theory, the methods should be applicable to water treated by any type of BWMS. Regardless, the Proposal discussed the damage rendered by UV treatment to organisms’ deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which can prevent the organism’s ability to reproduce. The Proposal also contended that organisms incapable of reproduction were the same (from the standpoint of the risk for biological invasions) as organisms that are scored as “dead” in the Required Method. The Proposal considered “viable cells” the same as the cells scored as “living” using the Required Method, and in several instances, interchanged the term “viable” with “living”. This distinction is critical because the current U.S. Ballast Water Discharge Standard sets a limit on the allowed concentration of *living* organisms, and this Proposal would equate living, non-reproducing cells to dead cells. This point is discussed further below (Section 4.1 Ability of the Alternative Method to Measure the Same Proximate Aspect or Function of the BWMS).

In the Autotroph Method, a whole water sample of ballast water is collected, filtered on a 10- μm filter to remove microorganisms < 10 μm in size, and the material retained on the filter is serially diluted. An array of culture tubes are filled with media (Guillard’s for marine water, and presumably [although not stated], also for brackish water; Bold Modified Basal media for

freshwater). Three dilutions are created, and each dilution has 5 replicate culture tubes (thus, a total of 15 MPN tubes is used). The initial fluorescence of the chl *a* is measured in each tube using a fluorometer, the tubes are incubated in continuous irradiance (nominally 50-150 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation [PAR]) for 14 days (d), and then the fluorescence is measured again.

The tubes are scored as + (showing population growth [growth]) or - (showing no growth) based on an increase (or the lack of an increase) in fluorescence; an increase in fluorescence from the start of the incubation period to the end of the incubation period shows an increase in the amount of chl *a*, which corresponds to an increase in the number of cells. Note that the term “growth” is used in this Review, and it applies to the growth of the population, not an increase in the size of an individual organism. In addition, growth refers to culturability over the duration of the MPN assay and does not imply the organisms must be maintained in perpetuity.

The threshold for growth is set at “4 times the standard deviation of the fluorescence of a set of method blanks”, with the justification that it would “provide the easiest (minimal fluorescence increases) scoring of growth, leading to higher MPN values, and thus the most environmental protection when used to evaluate equipment performance.” (Trojan Marinex 2015b). This idea should be formalized with data, e.g., an experiment to demonstrate this threshold is appropriate. Note that for the purposes of this Review, we assume that the fluorometer is properly calibrated and yields stable, reproducible readings across its range of detection. The scoring pattern is then entered into an MPN “calculator” (e.g., <http://www.i2workout.com/mcuriale/mpn/index.html>) to determine the most probable concentration of viable autotrophs in the original sample. In the Proposal, three MPN calculators were evaluated, with no difference among their outputs of cell concentration (Trojan Marinex 2015b). The confidence intervals (CI) were different, but the Proposal dismissed this difference, as the CIs are not used in the Alternative Method (this idea is discussed in Section 3.7.1.2 Confidence Intervals in the Autotroph Method).

3.2.3 Heterotroph Method—Epifluorescence Microscopy

Because heterotrophs do not convert inorganic carbon to organic compounds using photosynthesis, and therefore do not contain chl *a*, the Autotroph (MPN) Method is not applicable to this group of organisms. The proposed Heterotroph Method uses a microscopic technique to evaluate if an organism is a living heterotroph by assessing two criteria, motility and chl *a* autofluorescence. Moving cells or organisms are clearly living and are scored as such. Obligate heterotrophs lack chl *a* (the primary light-harvesting pigment in photosynthesis). When excited with blue light, chl *a* fluoresces red, naturally exhibiting red autofluorescence; no fluorescent markers are added to the sample. Thus, using this criterion, organisms *lacking* chl *a* do not exhibit red autofluorescence and are heterotrophs.

In the Heterotroph Method, a 1-mL subsample is loaded into a Sedgewick Rafter counting chamber, which is examined on an epifluorescence microscope, and living heterotrophs are identified as organisms that *do* show motility but *do not* show red autofluorescence (i.e., they lack chl *a*). Notably, this method differs from the one used in the Required Method that is used to enumerate living heterotrophs in this size class of organisms. In the Required Method, samples exhibiting green fluorescence from FDA and CMFDA markers, motility, or both

approaches are scored as living. The Required Method is used to enumerate all living organisms (both autotrophs and heterotrophs) in the size class. In addition to the methodological differences used to quantify heterotrophs using the Required and Alternative Methods, the methods also differ in their fundamental approaches. The Required Method solely enumerates living organisms (autotrophs and heterotrophs), whereas the Alternative Method enumerates living organisms (heterotrophs) and viable organisms (autotrophs). It is unclear (and unexplained in the Proposal) why it would be appropriate to enumerate the living organisms in the heterotrophic portion of the protist community when UV treatment (at the doses currently used in ballast water applications) is intended to render cells non-viable. Following the argument that non-viable cells are equivalent to dead cells, the viability of the heterotrophs would be determined as well.

3.2.4 Determining the Number of Total Living Organisms

Under the Alternative Method, the mean of three subsamples analyzed by the Autotroph Method is added to the mean of three subsamples analyzed by the Heterotroph Method to obtain the total living organism concentration in the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class. This value is used to evaluate the performance of a BWMS compared to the ballast water discharge standard, or to quantify organisms in the uptake water or control discharge. The combination of these values is discussed below (Section 3.3.4 Drawbacks—Data Analysis).

3.3 Potential Biases and Drawbacks of the Alternative Method

The Proposal includes a discussion of possible interferences, biases, limitations, and mitigating strategies that may affect the data collected using the Alternative Method. Therein, these were grouped by autotrophs and heterotrophs, and for this Review, they are excerpted and summarized (Table 3). The biases seem more impactful in the Autotroph Method than the Heterotroph Method, but since both carry potential risk for bias, both parts of the Alternative Method will be considered. Below (in this section and others), these items, and additional concerns, are discussed.

Table 3. Potential interferences, biases, and limitations as listed verbatim in the Alternative Method (Trojan Marinex 2015b); additional text added for this Review is indicated in square brackets.

[Description]	Bias on Number of Living 10-50 μm Organisms	Proposed Magnitude [and Mitigation Strategy from this Review]
Autotrophs [Autotroph Method]		
Inclusion of >50 μm Autotrophs	Overestimate	Small, <3%
Inclusion of <10 μm Autotrophs	Overestimate	[Not listed]
Removal of >10 μm Autotrophs	Underestimate	Can be minimized [with gentle filtration]
Filtration mortality of >10 μm Autotrophs	Underestimate	Can be minimized [with gentle filtration]
Non-Growing Autotrophs	Underestimate	Small, near 0% by abundance [when detailed taxonomy was used]
Slow-Growing Autotrophs	Underestimate	Small
Chain-Forming Autotrophs	Under- and Overestimate	[Can be reduced when chains are “shortened by disruptions like pumping and tank agitation”*]
Grazing by Heterotrophs	Underestimate	Small
Heterotrophs [Heterotroph Method]		
Inclusion of >50 μm Heterotrophs	Overestimate	Can be minimized [as they will be excluded in microscopic counts]
Inclusion of <10 μm Heterotrophs	Overestimate	Can be minimized [as they will be excluded in microscopic counts]
Removal of >10 μm Heterotrophs	Underestimate	Can be minimized [with gentle filtration]
Filtration mortality of >10 μm Heterotrophs	Underestimate	Can be minimized
Assessment of Live-Dead Status	Overestimate	Large for UV treatment

*Cullen and MacIntyre (2015) recommend “relatively gentle mixing” of 100 inversions to disrupt chains or colonies.

3.3.1 Drawbacks—Sampling

Recall that the sampling scheme allowed for two options: “...either 3 replicate samples can be collected with a subsample taken from each, or a single sample can be taken with 3 replicate subsamples taken” (Trojan Marinex 2015b).” As above, it would be preferable if only one sampling scheme was put forward. More worrisome, this choice can greatly affect the outcome of the sampling. The latter approach results in “pseudoreplication” (Hurlbert 1984), in which the

“replicates” are actually “subsamples” that violate the assumption of independence, which is the foundation of most commonly used statistical analyses. That is, the statistical analyses would be flawed, yielding results that were wrong. Thus, the first approach (three replicate samples) should be used for statistical comparisons, e.g., between the control and treatment tanks upon discharge. This advice does not apply to sample tubes used in the MPN approach; in this case, water for all dilution tubes (i.e., an array consisting of 3 dilutions, each with 5 replicates) should be drawn from the same population, so here, tubes are inoculated with water from a single, original sample. Regardless, the procedures should be unambiguous in the Proposal.

3.3.2 Drawbacks—Autotrophs

A small number of steps within the Alternative Method invite ambiguity. Others are not fully validated. These potential drawbacks are discussed below.

3.3.2.1 Drawbacks—Autotrophs: Filtering

Samples for the Autotroph (MPN) Method are filtered onto 10- μm filters, and if organisms $<10\ \mu\text{m}$ are inadvertently retained on the filter, estimates of organism numbers will be artificially inflated. In effect, the final MPN number would include organisms that are regulated by USCG ($\geq 10\ \mu\text{m}$ and $<50\ \mu\text{m}$) as well as those that are not regulated by USCG ($<10\ \mu\text{m}$). This question could be addressed by filtering cultures or ambient organisms that are $<10\ \mu\text{m}$ and using microscopy to measure the organisms retained on the filter.

Organisms $\geq 50\ \mu\text{m}$ could also be retained on the filter, again, artificially inflating the MPN estimate. The Proposal indicates this bias would be small, with a note that phytoplankton $\geq 50\ \mu\text{m}$ tend to be rare. While this is generally true, it would be prudent for each test facility (TF) using this approach to determine the fraction of relatively large phytoplankton at the test facility. In shipboard testing, a whole water sample could be collected and the cells measured by microscopy to ensure the community of microalgae did not include cells $\geq 50\ \mu\text{m}$.

According to the Alternative Method, the filter may or may not be left in the MPN tube during the grow-out period. This is a concern because it is unclear if leaving a filter in the tube affects the fluorescence reading (as discussed above) or if the filter itself serves as a barrier, reducing the fluorescence reading. If this issue has been addressed, it was not included in the Proposal. Regardless, the Alternative Method should be updated to provide unambiguous direction, and if the direction is to leave the filter in the tube, data showing that the practice does not affect the results should be presented.

3.3.2.2 Drawbacks—Autotrophs: Measurements

To determine if an MPN tube has growth (and is scored as positive) a threshold was set in the Proposal as four times the standard deviation (SD) of method blanks. In order for this threshold to be uniformly applied across laboratories, the fluorometers would need to be calibrated following the same, standardized procedure and demonstrate consistency in measuring the threshold value. This specification is not included in the proposed method, and it should be.

3.3.3 Drawbacks—Heterotrophs

The Heterotroph Method relies partly on the ability to detect the red autofluorescence of chl *a*-containing organisms using epifluorescence microscopy. The basis of epifluorescence microscopy is that a series of optical filters is arranged to select visible wavelengths to illuminate organisms in the field of view. Fundamentally, fluorescing molecules will emit light at a different (longer) wavelength than the wavelength used to excite them, and thus, using a given set of filters for excitation and emission, fluorescing biomolecules can be excited and detected.

For chl *a* (or any target fluorescent substance), excitation and emission filters should correspond to the maximum excitation and emission wavelengths (here, 430 nm and 662 nm, respectively, for chl *a*). However, in the Heterotroph Method, the optical filter set is optimized to detect the fluorescence from fluorescein, not chl *a*; the guidance was developed to accommodate commonly used filter sets, so this one was chosen because green-fluorescing markers, such as fluorescein, are very common (B. Petri, pers. com.). According to the wavelengths of the filter set specified in the Heterotroph Method (B-2E/C), however, the red autofluorescence of chl *a* would *not* be visible (Table 4). This arrangement could lead to an overestimate of the number of heterotrophs, as all organisms detected would appear to lack chl *a* and, therefore, if they were motile, they would be scored as heterotrophs. It is unknown if this method was used in TA applications submitted to USCG. If it was, that would indicate the overestimation of heterotrophs was small enough not to exceed the discharge standard, since TA applications indicate the discharge standard is not exceeded.

Table 4. Specifications of two optical filter sets optimized for fluorescein detection. The filter set names are specific to Nikon microscopes, but other, similar configurations are available.

Filter Set	Excitation Filter	Dichromatic Mirror	Emission Filter	Red Fluorescence
B-2E/C*	465-495 nm BP	505 nm LP	515-555 nm BP	Not visible
B-2A	450-490 nm BP	500 nm LP	515 nm LP**	Visible

*The filter specified in the Alternative Method. **Wavelengths longer than 515 nm, such as 662 nm (the peak emission for chl *a*), can be detected. BP = Band Pass, a filter that transmits wavelengths of light within the range specified, dichromatic mirror = a type of LP filter that reflects light of wavelengths shorter than the nominal value and transmits longer wavelengths, LP = Long Pass, a filter that attenuates wavelengths of light shorter than a nominal value to transmit longer wavelengths, nm = nanometer, and SP = Short Pass, an optical filter that attenuates wavelengths of light longer than a nominal value so that shorter wavelengths are transmitted).

Encouragingly, filter sets are available that detect fluorescein *and* may also allow chl *a* fluorescence to be detected. One such filter set (B-2A) is listed in Table 4 for comparison to the filter set identified in the Alternative Method. While the excitation filter in B-2A is not optimal (it is not well aligned with the peak excitation of chl *a*, 430 nm), the emission filter would allow red fluorescence to be detected. If this filter set (B-2A in Table 4) does not allow sufficient detection of chl *a*, other, chl *a*-specific, filter sets exist, and they could be specified.

Another concern is the procedure used to detect non-fluorescing organisms to quantify living heterotrophic organisms. Recall that epifluorescence microscopy is used, and if cells are moving and do not show red autofluorescence (i.e., they do not contain chl *a*), organisms are scored as living heterotrophs. The Alternative Method states that organisms are detected by movement, but if they do not exhibit fluorescence and are not viewed with another light source (one is not stipulated in the Proposal), organisms (whether moving or stationary) would be, at best, dimly illuminated by light and difficult to see. Perhaps a step to initially detect moving organisms (via brightfield or phase-contrast microscopy) is implicit in the method, but this point should be explicitly stated and clearly described.

3.3.4 Drawbacks—Data Analysis

From the materials provided, it is unclear how the uncertainties around the Autotroph and Heterotroph Methods are applied. Each will have an estimate of the mean's variance. If they are to be added together, there is no statistical justification provided for doing so. These instructions should be provided in the Alternative Method.

3.4 The degree to which the Method Description Provides a Clear Presentation of Test Procedures

Overall, the Alternative Method is clear and understandable. In several instances, however, it allows the TO to make potentially impactful decisions. It is best, in the opinion of this Review's authors, if a method prescribes steps to allow as little differential implementation as possible. Following that idea, in some instances (enumerated above), data are needed to justify the use of a particular procedure, e.g., leaving the filter in the MPN tube for analysis. These data should be provided.

3.5 Validation of the Alternative Method

In this section, first, the means to validate *any* method will be discussed. Next, the validation that was completed by the proposers for the Alternative Method will be described. Then, for completeness, the validation of the Required Method will be summarized. Finally, the statistical foundation of the Alternative Method will be reviewed.

Before the validation is discussed, it is useful to consider two parameters that are commonly used to assess any method: precision and accuracy. If two methods are compared, the relationship between them can be illustrated in a conceptual figure (Figure 1), with the key metrics of precision (Figure 1A) and accuracy (Figure 1B) of the method relative to a known value (or a reference method). In a comparison between the Alternative and the Required Methods, the *true* cell concentration is not—and cannot be—known, so methods can be compared based upon their precision (the variation around the mean value of each method) and the difference between the two means (a proxy for accuracy, since the true value is not known) (Figure 1C). Ideally, the two methods will have a similarly small variation around the mean. Notably, the Alternative and Required Methods measure different metrics: the Alternative Method measures non-photosynthetic organisms by movement and autofluorescence, and it measures viable

phytoplankton amenable to culturing, whereas the Required Method measures phytoplankton and non-photosynthetic organisms in this size class by the examining active enzyme systems.

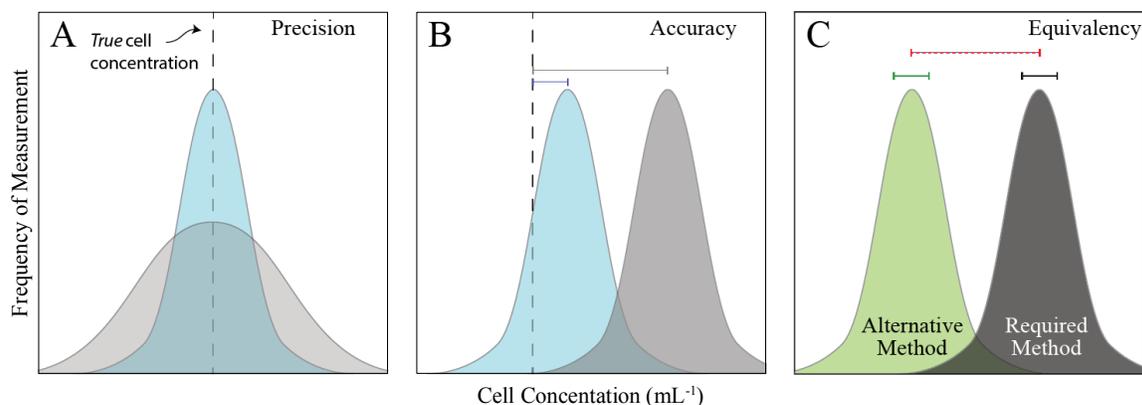


Figure 1. Theoretical comparison between two methods. The panels show conceptual frequency distributions of measurements of cell concentrations. The two methods can be compared based upon their precision (i.e., the spread of the data used to calculate the mean value, Panel A) and their accuracy (i.e., the correctness of the estimated mean value relative to the true value, Panel B). The true cell concentration is shown as a dotted, vertical line. The two methods may have the same measurements of the mean cell concentration, but the precision (variation around the mean) may differ, as shown in Panel A. The two methods may have the same precision, but the mean values may differ in accuracy (Panel B). In a comparison between the Alternative and the Required Methods, the *true* cell concentration is not known, so methods can be compared based upon their precision (the variation within a set of readings, shown as the solid error bars above the measurement sets) and the difference between their means, shown as the red dotted line (panel C).

3.5.1 Considerations in Validating any Method

The validation of a method requires considering numerous parameters, such as those outlined in a widely used paper describing the validation of chemical assays (Green 1996). While this framework applies to chemical methods, it identifies attributes of a method that are useful in any application. Note that while the Proposal addresses many of these elements explicitly, it also includes additional parameters, in particular, the Factor of Agreement (FOA)—the approach defined in the Proposal that measures the agreement between the Required and the Alternative Methods:

- **Accuracy:** The difference between estimated and actual values; since standards with known concentrations of living organisms are not available, accuracy is determined by a comparison estimates of the Alternative Method to the Required Method
- **Precision:** The variation among replicate readings or analyses; several factors contribute to the overall precision of a method:

- ***Volumetric precision***: The precision of the device used to aliquot, transfer, or inject the sample
- ***Inter-replicate precision***: A measure of variation among readings of multiple subsamples from a single sample
- ***Inter-analyst or inter-instrument precision***: A measure of the variation among readings of subsamples among different analysts or different instruments
- ***Reproducibility***: A measure of variation in the readings of a single sample across multiple laboratories or locations
- ***Specificity***: A measure of whether the assay is completely inclusive of the target population (in this case, living organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$) and exclusive of non-target organisms
- ***Linearity***: A measure whether the response of an assay (i.e., its estimates of concentrations) vary proportionally to the actual organism concentrations or aliquot volumes
- ***Detection range***: A numerical range indicating the upper and lower limits of detection for the assays; since organism $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ can be concentrated or diluted prior to analysis, the detection range can span several orders of magnitude; some specific factors for consideration are described below:
 - ***Detection limit***: The lowest detectable concentration; for a direct microscope count, the detection limit is one single organism. In the Alternative Method, organisms are concentrated on a mesh filter prior to preparing an array of dilutions, each with replicate tubes. In this case, the limit of detection hinges upon the volume concentrated on the filter and the set of dilutions
 - ***Quantitation limit***: The lowest concentration that can be accurately and precisely measured
- ***Sensitivity (Robustness)***: The intensity of change in an assay's response to small changes in the assay conditions; for example, what is the difference in concentration estimates if a sample is incubated for eight minutes (min) (rather than 10 min) with fluorescent labels? Or does a change in the incubation light regime affect the final result due to consequent effects on autotroph reproductive rates?

3.5.2 Appraisal of the Alternative Method Validation

In this Review, the Alternative Method used to quantify living organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ is evaluated by the guidance for analytical method validation (Green 1996). Here, the Alternative Method is compared to the Required Method. Both approaches face similar complexities in the analysis of living organisms (which differ from the features and attributes of chemical assays appraised by Green [1996]). This Review first considers the acceptance criteria listed in the Proposal. Next, the results from the Alternative Method validation are considered (beginning in Section 3.5.3 Results of the Validation of the Alternative Method)

3.5.2.1 Establishment of Acceptance Criteria

A key step in method validation is to establish minimum acceptance criteria to evaluate the performance of the method (Green 1996). The criteria used to gauge whether the Alternative Method is valid are defined and described in Trojan Marinex (2015b) (in the following sections, experiment [Exp.] numbers, or table numbers from [Trojan Marinex 2015b] are cited where appropriate). Because the Alternative Method combines two parts (the Autotroph Method and Heterotroph Method), they are discussed separately below.

3.5.2.2 Acceptance Criteria—Autotroph (MPN) Method

To determine the Initial Precision and Accuracy (IPA; this term was defined and used in the Proposal) of the Required and Alternative Methods, three subsamples of each of three concentrations of the cultured phytoplankter *Tetraselmis suecica* were analyzed by the proposers using both the Required and the Autotroph (MPN) Methods (Trojan Marinex 2015b). Since all organisms were phytoplankton (autotrophs; no heterotrophs were used in this case) and presumably the phytoplankton were capable of growth (since they were in culture, under favorable light and nutrient conditions), there was no need to perform the Heterotroph Method in addition to the Autotroph Method to determine the total number of organisms.

To determine accuracy, the Proposal showed it should be calculated as the mean of the three subsamples using the Autotroph approach divided by the mean concentration determined using the Required Method, multiplied by 100 (the Required Method was considered the benchmark for this comparison). The value (converted to a percent) would be 100% if both methods yielded the exact same concentration. Because the “true” concentration is unknown, this metric actually shows the agreement between the Required and Alternative Methods (rather than accuracy, which is the term used in the Proposal). For consistency with the Proposal, “accuracy” will be used in this Review.

Precision for the Autotroph Method was calculated in the Proposal as the coefficient of variation (CV). Specifically, the SD of three subsamples was divided by the mean of the three subsamples and multiplied by 100. This calculation is typically used to quantify precision.

3.5.2.2.1 Acceptance Criteria—Autotroph (MPN) Method—Initial Precision and Accuracy Values

The acceptance criteria for accuracy and precision defined in the Proposal were self-derived, based on empirical work conducted at the Danish Hydrological Institute (DHI) and the Norwegian Institute of Water Research (NIVA) in support of the Alternative Method development (Trojan Marinex 2015b) (Table 5 in this Review; Miller and Petri 2015). According to the Proposal, these criteria will be used to determine the IPA, and if the thresholds are met, analyses of field samples can proceed by organizations using the Alternative Method. For example, at a concentration of 10 *T. suecica* cells mL⁻¹, the CV must be less than 48% and the accuracy must fall within 61 and 168%. The accuracy and precision values in Table 5 would be used to determine acceptable precision and accuracy; in this manner, these criteria serve as data quality indicators. This Review suggests the number of samples per concentration should be increased from one to three (and the subsamples per concentration decreased from three to one), because, with few exceptions, at least three independent measurements are taken in

scientific endeavors to provide a reasonable estimation of the uncertainty around the result. Additionally, the OPA measurements should also be applied to ambient communities, since they are the communities used in BWMS testing, and it is expected that the community composition will change temporally (see Section 3.5.2.4 Factor of Agreement (FOA)).

Table 5. Initial acceptance criteria for the Autotroph Method as defined and listed in the Proposal for the comparison of the Alternative Method and the Required Method. This table copies the data in Trojan Marinex 2015b (Section 10.2.2.1.4, Table 4 therein). CV = coefficient of variation.

Concentration of <i>Tetraselmis suecica</i> (mL ⁻¹)	Precision (CV)	Accuracy*
10	<48%	61 – 168%
100	<55%	109 – 166%
1000	<63%	107 – 161%

*As defined in Section 3.5.2.2 Acceptance Criteria—Autotroph (MPN) Method of this Review: (mean concentration using the Autotroph approach)/(mean concentration using the Required Method) * 100.

Here, precision is dependent upon sample concentration. Unexpectedly, measurements of higher concentrations (100 and 1000 mL⁻¹) are *less* precise (the CV is higher) than measurements of the lowest concentration (10 mL⁻¹). This is surprising, as CV (a measure of precision) is sensitive to small mean values, and as the mean approaches zero, CV approaches infinity.

Regarding the accuracy of the acceptance criteria, for high concentrations (100 and 1000 mL⁻¹), it appears that MPN estimates are expected to be greater than estimates from the Required Method (as the accuracy range is entirely >100%). Note that these criteria were established by conducting empirical experiments, and the criteria reflect the empirical findings from a study the proposers conducted using a single, cultured alga. Such conditions should yield highly accurate and precise measurements. Presumably, this is the justification for using these values as acceptance criteria.

3.5.2.2.2 Acceptance Criteria—Autotroph (MPN) Method—Other Precision and Accuracy Values

For context, an empirical study using cultured organisms and the Required Method was conducted at NRLKW (this previous work was unrelated to the Proposal or this Review). The precision (CV) of microscope counts of an organism $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$, the dinoflagellate *Prorocentrum micans*, was 19% for samples with organism concentrations of approximately 10 mL⁻¹, and it was 12% for samples with concentrations of approximately 1000 mL⁻¹ (First et al., in prep.). Thus, the NRLKW study had better precision. Even given that biological data tend to be “messy”, the precision criteria stipulated in the Autotrophic Method are uncomfortably broad (<48 to <63%). Particularly in the best-case scenario, such as using cultured organisms in controlled, laboratory conditions (Table 5), a better precision should be achievable. Other organizations set more stringent thresholds for the precision of ambient organisms $\geq 10 \mu\text{m}$ and

<50 µm: <20% (the Great Ships Initiative [GSI] test facility, which has a long history of ballast-water testing [Drake et al. 2012]) or <25% (the Golden Bear test facility [Drake et al. 2012]).

Regarding accuracy, a threshold for this parameter is not set by test facilities, but in water quality parameters (dissolved and particulate organic carbon), GSI set their accuracy in recovering “spikes” (known concentrations of a given parameter) at 75-125% (Drake et al. 2012). Of course, making measurements of carbon is more straightforward than measuring living organisms. Regardless, it would be desirable for the accuracy in the Autotrophic Method (61-168%, Table 5) to be lower (e.g., 80-120%).

3.5.2.2.3 Acceptance Criteria—Autotroph (MPN) Method—Ongoing Precision and Accuracy

The Proposal indicates that Ongoing Precision and Accuracy (OPA) should be measured at the start of the BWMS testing season and every six months. One sample of *T. suecica* at a concentration of 1000 cells mL⁻¹ is prepared, and three subsamples are analyzed according to procedures for the Initial Precision and Accuracy. The Proposal lists acceptance criteria as precision (CV) <63% and accuracy of 107-161%.

3.5.2.3 Acceptance Criteria—Heterotroph Method

To establish Initial Precision and Accuracy for the Heterotroph portion of the Alternative Method, 10 subsamples of a sample containing the rotifer *Brachionus plicatilis* (10 mL⁻¹) were analyzed using the Alternative Method and a comparison method: stereomicroscopy, i.e., a microscope employing white light (Trojan Marinex 2015b). Note that the latter (comparative) approach differs from the Required Method, which uses the two fluorescent markers and an epifluorescence microscope). In the Proposal, accuracy and precision were calculated as described for the Autotroph Method.

3.5.2.3.1 Acceptance Criteria—Heterotroph Method—Initial Precision and Accuracy Values and Ongoing Precision and Accuracy

For heterotrophic analysis, the Initial Precision and Accuracy acceptance criterion was set for precision <46%, and the acceptance criterion for accuracy was set at 85-95% (Miller 2015b, Table 4 therein). In the Proposal, these values were presented, and they are based on the results from NIVA (Miller and Petri 2015), which were updated in the final Alternative Method. The precision is greater than desirable (<25%, see Section 3.5.2.2.2 *Acceptance Criteria—Autotroph (MPN) Method—Other Precision and Accuracy Values*), but the range of accuracy (agreement) is acceptable (<20%), although it does not allow for values >100%. Notably, the comparison of the Alternative Heterotrophic Method was not to the Required Method, so while these data are encouraging, it is difficult to interpret them in the context of the Required Method. For a robust comparison, the Heterotroph and Required Methods should be compared.

Similar to the Autotroph Method, OPA of the Heterotroph Method was indicated. One sample with 10 subsamples is to be analyzed at the start of testing and at six-month intervals, using the

accuracy and precision thresholds in the preceding paragraph. This Review suggests the number of samples should be increased to three samples.

3.5.2.4 Factor of Agreement (FOA)

The Alternative Method stipulates an FOA between the Required and Alternative Methods for field samples (not laboratory cultures, which were used for the Initial and Ongoing Precision and Accuracy determinations). Note that this parameter was not defined by Green (1996) but was developed for the Alternative Method to assess treated discharge samples. These comparisons are similar to those used in the acceptance criteria, but the FOA is used to measure agreement of the methods in field samples—which have more variability than laboratory cultures. The FOA is calculated as the mean of the Alternative Method divided by the mean of the Required Method (but *not* multiplied by 100). Here, the Required Method is used as the benchmark. For both uptake and discharge control samples, the FOA (as defined in the Proposal) should be ≥ 0.5 , which indicates that the Alternative Method estimate is at least 50% of the estimate of the Required Method. If that threshold is met, the analyses can continue; thus, although not explicitly stated, it appears this analysis would be conducted at all test facilities.

There is no upper limit on the FOA specification, and in the Proposal, there was no justification for the value of ≥ 0.5 , other than the Required and Alternative Methods differ in their means to assess organisms and “a general expectation is that the methods should agree within a factor of two (2), with no expectation of the direction of bias. As more comparative data is collected, this factor of agreement can be refined.” (Miller 2015b). Thus, the FOA is a safety factor with a wide allowance for differences, and given the breadth of acceptable agreement between the methods, it is unclear how valuable this assessment could be. Values >1 (i.e., $>100\%$) indicate the Alternative Method yielded an estimate greater than the Required Method, which for treated discharge, would be a conservative estimate. However, since accurate concentrations are necessary to assure that the test criteria are met for the uptake and untreated discharge, an upper limit of the FOA should be defined in the Proposal.

3.5.3 Results of the Validation of the Alternative Method

The empirical experiments used to measure the accuracy, precision, and reproducibility of the Alternative Method were described within the Proposal documents (see Table 2 and Table 6 of this Review). Note that the Proposal was not framed in the format developed by Green (1996), but the work done to support the Proposal and the resulting data do allow an examination of the parameters identified by Green (1996). **Accuracy** was addressed by comparing the results combined from the Autotroph and Heterotroph Methods to the Required Method for both ballast water samples (Petri 2015b [Exp. 1-3]) and target concentrations of cultured organisms (Miller and Petri 2015 [Exp. 6]) (although, as noted above, because the true answer is unknown, these manipulations point to agreement between methods rather than accuracy). **Precision** was measured by assessing the variation among replicate readings, and **reproducibility** was addressed by conducting comparisons at different locations by different analysts. Both precision and reproducibility were addressed in all six experiments. **Specificity** was addressed by evaluating the presence and growth of culture-amenable species relative to the presence of non-culture amenable species (the specificity also affects accuracy and precision in that species that are not amenable to culture will show lower values of accuracy and precision). Changes in culturing

conditions (e.g., temperature and growth media) were used to determine the *sensitivity* of the method (Miller et al. 2015a [Exp. 4]; Miller et al. 2015b [Exp. 5]).

Table 6. Documents in the Proposal describing the validation experiments.

Parameter*	Document Title	Experiment Description	Experimental Goals [Implied in the Proposal]	Identifier
Accuracy, precision, reproducibility	MPN Method Development Experiments 1-3.pdf	Inter-laboratory comparison of the MPN dilution-culture method and fluorescein-based staining methods for the enumeration of viable or living phytoplankton cells	Measure accuracy, precision, reproducibility of ballast water samples	Petri 2015a (Exp. 1-3)
Robustness, specificity	MPN Method Development Experiment 4.pdf	MPN Method development report	Evaluate different culturing conditions (temperature and media) and growth efficiency	Miller et al. 2015a (Exp. 4)
Accuracy, specificity	MPN Method Development Experiment 5.pdf	MPN Method development report	Categorize culture-amenable species to measure the method specificity; measure accuracy by comparing the MPN Method to the Required Method	Miller et al. 2015b (Exp. 5)
Accuracy, precision, reproducibility	MPN Method Development Experiment 6.pdf	Generating method performance data for the Alternative Method for analyzing 10-50 μm organisms in the ETV generic protocol for the verification of ballast water treatment technology	Measure accuracy, precision, reproducibility of cultured organisms	Miller and Petri 2015 (Exp. 6)

*From Green (1996). MPN = Most Probable Number, ETV = Environmental Technology Verification, and Exp. = Experiment.

3.5.3.1 Method Accuracy and Precision

Frequently, results of the Autotroph (MPN) Method were reported in the Proposal as greater or less than the limit of detection. This occurs in instances where all replicates of the different dilutions showed either growth or no growth. In this case, values may be, for example, <0.18 cells mL^{-1} (when *no* replicates exhibit growth) or >1600 cells mL^{-1} (when *all* replicates show growth). These values are based upon the number of dilutions and number of replicate tubes.

When no tubes at any dilution show growth, the value $0.18 \text{ cell mL}^{-1}$ represents the concentration when only one of five tubes in the lowest dilution showed growth. Thus, no growth in any tube is below the limit of detection ($<0.18 \text{ cells mL}^{-1}$). Likewise, when all tubes show growth, the value $>1600 \text{ cells mL}^{-1}$ indicates that cell concentration exceeds the upper quantitation limit. In these instances, the values are considered by the Proposal to be categorical so they can be used for calculations (Petri 2015b [Exp. 1-3]). This is an unusual approach, and using this categorical result complicates mathematical operations, such as adding the results of the Autotrophic and Heterotrophic Methods.

The results from the comparison of the two methods differed when untreated or UV-treated ballast water was assessed: in untreated (control) samples, all estimates by the Autotrophic Method were greater than estimates of the Required Method (>1 order of magnitude, in several instances), but in UV-treated samples, all MPN estimates were lower than estimates of the Required Method (Petri 2015b [Exp. 1-3]). Note that this work was done prior to the finalization of the Alternative Method, and the methodologies used at the three laboratories were not standardized at the time. Nonetheless, the results illustrate the different outcomes of the Autotroph (MPN) approach and the Required Method.

Possibly, overestimations by the Autotroph (MPN) Method in untreated samples were due to the inclusion of organisms $<10 \mu\text{m}$ in the samples that are not removed by the filtration through the $10\text{-}\mu\text{m}$ pre-filter; these organisms would be excluded in the Required Method, as the microscopist would not include them in the count based on organism size. On the other hand, this overestimation should also affect the UV-treated samples, although this assessment is complicated by the fact that the Required Method will enumerate all living organisms, even those rendered non-viable by UV treatment. Indeed, the observation that the MPN numbers were lower than the Required Method in the UV-treated samples was expected according to Petri (2015b), as the Required Method can detect living cells that are not able to reproduce. Additionally, non-culturable organisms in the Autotrophic Method would decrease the counts relative to the Required Method. Note that if the two methods worked perfectly, the Required Method would *always* yield a concentration equal to or greater than the Alternative Method: the two methods should measure the same populations of living heterotrophic organisms, i.e., those displaying movement without chl *a* autofluorescence (in the Alternative Method) or those displaying FDA/CMFDA fluorescence, or movement, or both (in the Required Method). The two methods, however, measure different populations of autotrophic organisms (living vs. viable). The population of autotrophic organisms capable of reproduction will be $<100\%$ of the total number of living organisms (from the data discussed in the next Section, 3.5.3.2 Method Specificity), and, therefore, the Required Method (assuming no false negatives occur) will always yield a concentration greater than or equal to the Alternative Method. This difference between the two methods should be greatest in samples treated by UV radiation.

3.5.3.2 Method Specificity

The specificity of the Autotrophic Method was determined by the ability of phototrophic organisms ≥ 10 and $<50 \mu\text{m}$ to exhibit measurable population growth under laboratory conditions. At two test sites (DHI and NIVA), an inventory was conducted of organisms at the

test sites that were (1) found in ambient water samples, and (2) capable of demonstrating growth under laboratory conditions.

The results varied between the two locations, where 20-44% or 56-89% of species in the ambient untreated samples consistently demonstrated growth (i.e., they grew in *all* tests) using the MPN procedure at DHI and NIVA, respectively (Miller et al. 2015a [Exp. 4]). However, both test sites reported higher numbers of species that were capable of growth in cultures, when *any single test* was considered, that is, the percentages of species that have been able to grow in *at least one test* were 80-89% and 70-94% at DHI and NIVA, respectively; this higher number represents the historical record, that is, if a species could grow in *any* test over the history of testing (no reference to the extent of the historical testing was provided, e.g., 10 samples per year over 5 years). When the number of *individuals* that could grow was considered (the number was extrapolated from relative abundances within a sample), the trends were similar, with DHI reporting lower percentages than NIVA: 37-43% and 95-100% of individuals were capable of growth in all tests at DHI and NIVA, respectively, and 66-70% and 97-100% of individuals were capable of growth in any test at DHI and NIVA, respectively (Miller et al. 2015a [Exp. 4]). The difference between the facilities' results was ascribed to their methods for assessing the taxonomic diversity in the MPN arrays, as DHI (where the percent culturable was lower) analyzed one MPN tube (the middle dilution), whereas NIVA analyzed all dilutions in the MPN array. Regardless, the ability of organisms to be cultured is central to the usefulness of the MPN Method. If cells do not grow, and that lack of growth is attributed—incorrectly—to ballast water treatment, then the efficacy of the BWMS is overestimated. This issue is addressed below (Section 3.7.1.1 Key Assumptions of the MPN Method).

3.5.3.3 Agreement between the Required and Alternative Methods

Empirical measurements of FOA were reported in the Proposal from ambient samples collected and analyzed at two test facilities (Table 7 in this Review; data from Miller et al. 2015b [Exp. 5], Table 5 and the text within). In these trials, two culture media types were used (Guillard's and Keller) and two temperatures were used (10° and 20°C), with two samples of ambient water analyzed at each facility for each of the four possible media and temperature combinations. This discussion (Table 7) will focus on the results using Guillard's media, since that media is recommended in the Proposal for marine facilities, such as those participating in these trials. The FOA ranged from 0.47 to 33.88, and in 7 of 8 samples, it was >0.5. In 3 of 8 samples, the FOA was >5, meaning the Alternative Method yielded a count at least five times greater than the Required Method. This result is unexpected, since the water samples were not treated with UV radiation; in untreated samples, the Required and Alternative Methods would be expected to have similar results (FOA of ~1). It may be that the conditions (temperature, media) were not fully optimized, but the ambient community will likely change (at least seasonally), so selecting one set of conditions would be difficult, as these data show.

The highest FOA value, 33.88, occurred when the ambient temperature (8°C) was similar to the incubation temperature (10°C). This result could be due to the inclusion of organisms <10 µm in the samples that were not removed by the filtration through the 10-µm pre-filter (Section 3.3.2.1 Drawbacks—Autotrophs: Filtering). Conversely, the second-highest FOA value, 9.30, occurred

when the ambient temperature (8°C) was less than half of the incubation temperature (20°C). Regardless, these results point to the need for each TF to validate the use of the media and incubation temperature, although it is unclear how the media could be validated for shipboard testing, given the variety of potential phytoplankton. In shipboard testing, the incubation temperature could be close to the ambient water temperature, as stipulated in the Alternative Method.

Table 7. Factor of Agreement between the Required Method and the Autotroph Method (data from Miller et al. 2015b [Exp. 5], data from Table 5 therein; the column of ambient water temperature is from the text of Miller et al. 2015b [Exp. 5]).

Laboratory	Sample	Ambient Water Temperature (°C)	Guillard's 20°C	Guillard's 10°C
DHI	5:1	19	5.59	0.53
	5:2	10	0.47	0.64
NIVA	5:1	16	0.51	0.57
	5:2	8	9.30	33.88

DHI = Danish Hydrological Institute and NIVA = Norwegian Institute of Water Research.

Accuracy and precision were assessed by measuring three concentrations (10, 100, 1000 mL⁻¹) of cultured algae (*Tetraselmis suecica*) with the Required and Autotrophic (MPN) Methods (Miller and Petri 2015 [Exp. 6, Table 2]). Samples were analyzed at two locations: NIVA (with two samples, labeled NIVA-1 and NIVA-2) and DHI. The raw data were provided in Miller and Petri (2015), and for this Review, the CV, a measure of precision, was calculated (Table 8). All concentrations are reported in cells of *T. suecica* per mL. Also for this Review, the difference between the two methods (in %) was calculated as the absolute difference in concentration between the Alternative and Required Methods divided by the concentration reported by the Required Method, multiplied by 100. This value is another indication of the agreement of the two methods. The percent differences in concentrations between the methods ranged from 7 to 413%, although the difference of 413% was observed in a sample with the lowest (and most difficult to measure) target concentration, 10 cells mL⁻¹ (DHI). In this instance, the Required and Autotroph (MPN) method reported mean concentrations of 7 and 36 cells mL⁻¹, respectively. The CVs ranged from 1-26% (average = 13%) and from 20-135% (average = 50%) for the Required Method and Autotroph (MPN) Method, respectively (Table 8). The means of the CVs were significantly different ($p < 0.05$, using a non-parametric Mann-Whitney U-test [SigmaPlot, V11, Systat Software, San Jose, CA]). In all but one case, the CV of the Required Method was lower than the CV of the Autotroph (MPN) Method, meaning the Required Method had greater precision.

Table 8. Agreement and precision of the Required and Autotroph (Most Probable Number, MPN) Methods using *Tetraselmis suecica* (raw data from Miller and Petri 2015 [Exp. 6]). All values were rounded to the nearest integer. The “Difference between the Mean Values” was calculated for this Review by subtracting the Required Method Concentration mean from the Autotroph (MPN) Method Concentration mean, taking the absolute value of that number, dividing it by the Required Method Concentration mean, and multiplying it by 100.

Location and Sample	Target Conc. (mL ⁻¹)	Required Method Concentration			Autotroph (MPN) Method Concentration			Difference between the Mean Values (%)
		Mean (mL ⁻¹)	SD (mL ⁻¹)	CV	Mean (mL ⁻¹)	SD (mL ⁻¹)	CV	
NIVA-1	1000	698.0	8.7	1%	863.3	209.8	24%	24%
NIVA-1	100	60.7	2.5	4%	66.0	15.4	23%	9%
NIVA-1	10	14.0	3.6	26%	8.6	4.1	48%	39%
NIVA-2	1000	931.0	66.6	7%	993.3	630.1	63%	7%
NIVA-2	100	109.3	12.1	11%	129.7	46.4	36%	19%
NIVA-2	10	5.3	1.2	23%	8.9	1.8	20%	68%
DHI	1000	776.7	110.6	14%	1,030.0	467.7	45%	33%
DHI	100	66.3	5.0	8%	109.7	60.5	55%	65%
DHI	10	7.0	1.7	24%	35.9	48.6	135%	413%

Conc. = concentration, CV = coefficient of variation, DHI = Danish Hydrological Institute, NIVA = the Norwegian Institute of Water Research, and SD = standard deviation.

The accuracy and precision were also determined for the Heterotroph Method. Accuracy was measured in comparison to a stereomicroscope count, and precision was measured as the variability among replicate readings. The percent difference between the stereomicroscope method and the Heterotroph Method was low (5%), and the CVs were comparable (23 and 30%, respectively; data not shown; Miller and Petri 2015 [Exp. 6, Table 3]). Neither method yielded concentrations that were consistently higher, thus indicating no systematic, or directional, bias. Notably, the comparison of the Heterotroph Method was not to the Required Method, which uses epifluorescence microscopy.

The data in Table 8 are plotted in Figure 2 and Figure 3, which were prepared for this Review. Figure 2 plots the mean cell concentration for the Required and Alternative Methods. Linear regression analysis was used to measure the strength of the relationship between the two approaches, and the best-fit line was plotted. In this case, there was a significant, positive, linear relationship between the two methods ($R^2 = 0.98$, $p < 0.05$). In Figure 3, the coefficients of variation (CV) of both methods were plotted. Here, no relationship was evident, although the CV for the Required Method was lower (<30%) than that of the Alternative Method (approximately 20-65%). In both figures, the statistical analyses were conducted using SigmaPlot. In sum, there was good agreement between the methods, but the precision of the Alternative Method was greater than that of the Required Method. Note that the term “Alternative Method” is not used in either figure, as the experiment’s results (from Table 8,

describing measurements of one cultured phytoplankter) were reported using the Autotroph Method only (not a combination of the Autotroph and Heterotroph Method).

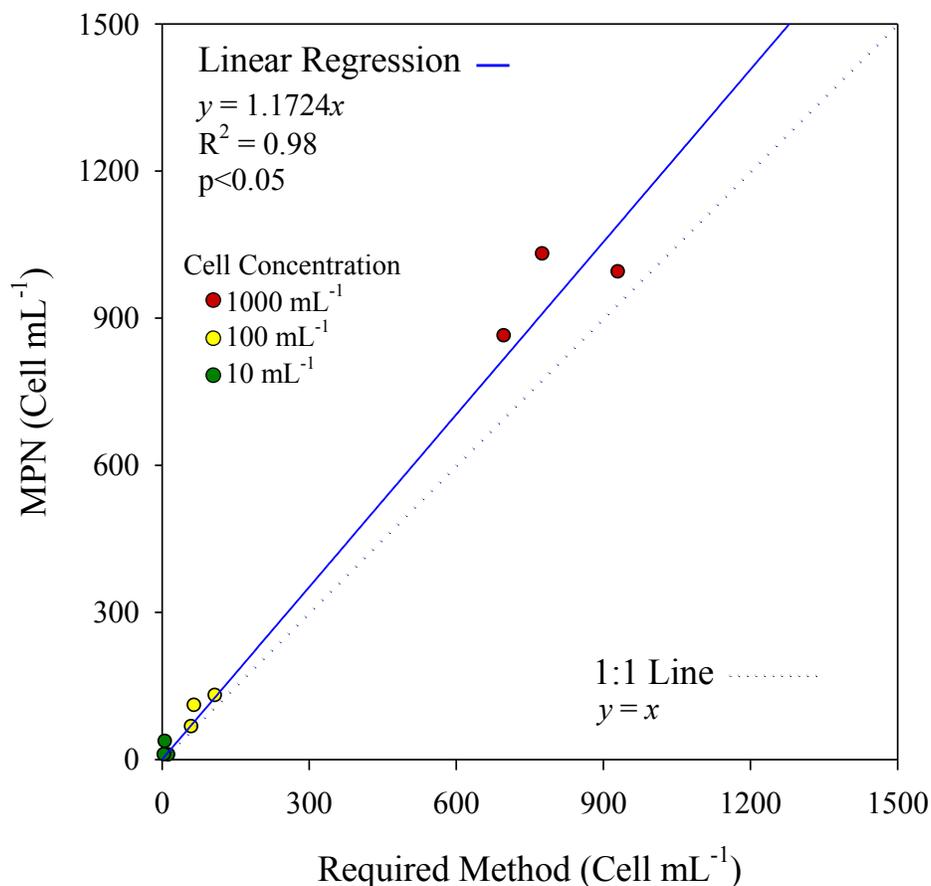


Figure 2. Comparison of the measurements of cell concentration of cultured algae performed using the Required Method and the Autotroph (Most Probable Number [MPN]) Method. Data are from Miller and Petri (2015 [Exp. 6]). The dotted line indicates a 1:1 (perfect) relationship between the methods.

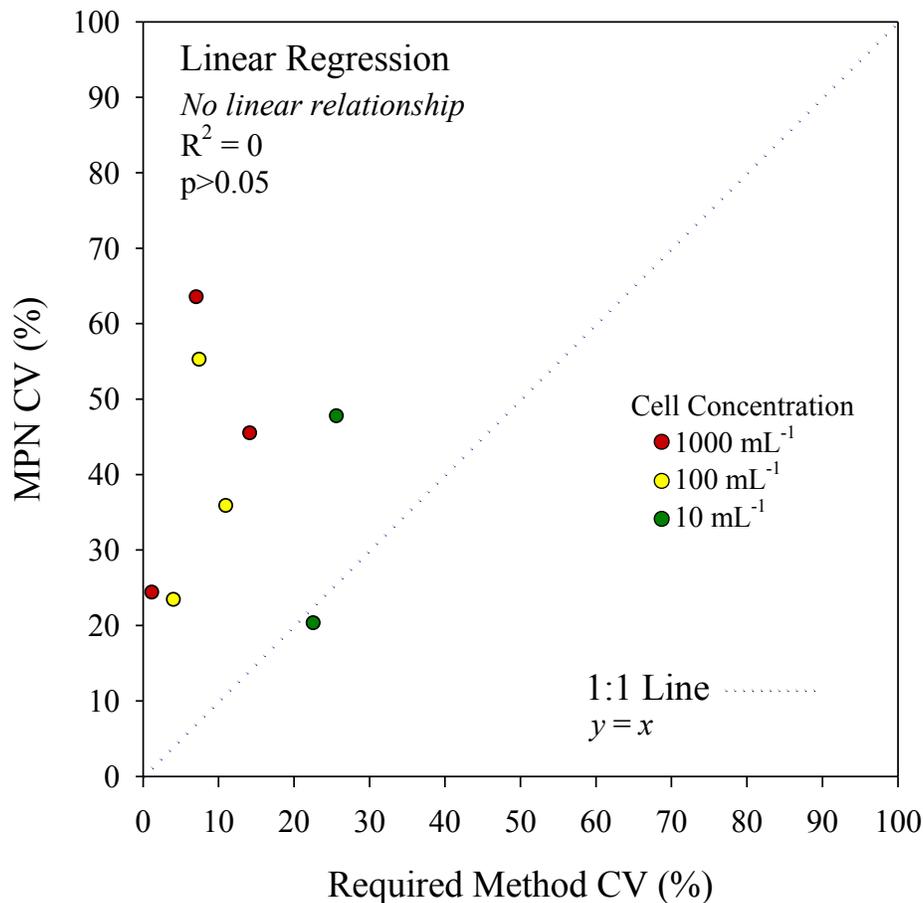


Figure 3. Comparison of the coefficient of variation (CV) of cell concentrations of cultured algae performed using the Required Method and the Autotroph (Most Probable Number [MPN]) Method. The dotted line indicates a 1:1 (perfect) relationship between the methods.

3.5.3.4 Method Reproducibility and Sensitivity

Validation tests were performed at two locations, and in the analysis of cultured organisms (Miller et al. 2015b [Exp. 5]) yielded results that were comparable with a FOA of approximately 0.5 (see Table 7 in this Review). However, in other experiments, different outcomes—in one case, with a FOA of >30—were realized based upon the culturing temperature and growth media. This result points to the need for site-specific validation.

3.6 Validation of the Required (FDA/CMFDA) Method

During the initial development of the Required Method at NRLKW, the Required Method was used to quantify samples of natural assemblages of protists collected from ambient seawater, as well as cultures of protists purchased from commercial vendors (Reed Mariculture; Campbell, California and the Provasoli-Guillard National Center for Culture of Marine Phytoplankton; West Boothbay Harbor, Maine). Cultures purchased from vendors and maintained in the NRLKW laboratory until use included the motile green flagellate *Tetraselmis* sp. (strain PLY

429); the non-motile pennate diatoms *Melosira octogona* (strain CCMP483) and *Amphora* sp.; the motile pennate diatom *Cylindrotheca closterium* (strain CCMP340); and the motile dinoflagellate *Prorocentrum hoffmannianum* (strain CCM2804). This variety of phytoplankton was chosen, as they represent a range in cell size (very close to the 10 µm threshold and larger), motility (some algae had that capability, some did not), and morphology (some were single-celled, and others were chain-formers). Various combinations of stain concentration and staining time were used, and heat killing was used to generate negative controls.

After studies at NRLKW showed good results (low false positive and false negative errors), the ETV Technical Panel decided to incorporate the method into the draft ETV Protocol. As the ETV Protocol was being reviewed and finalized, the Required Method was additionally validated in three locations: Baltimore, MD; Sequim, WA; and Boothbay Harbor, ME (Steinberg et al. 2011). The validation exercise was conducted by NRLKW researchers, who also developed the method (NRLKW will not benefit financially if the Required Method is widely used). Using this information, the Technical Panel revised this section of the Protocol, and the method was incorporated into the final draft (U.S. EPA 2010).

Among all four study sites, the results showed low false negatives on the basis of cell motility and the markers' presence (moving but non-fluorescent cells, 0-2%). It is possible, however, that non-moving, non-fluorescent cells represented false negatives. The false positive errors (heat-killed and fluorescent) were low in Florida and Maryland (5% and 3%, respectively), but high at the Washington and Maine sites (36% and 19%, with faint, but visible fluorescence from heat-killed cells, typically from heterotrophic or mixotrophic dinoflagellates). Steinberg et al. (2011) recommended that the method should be validated at each site where it was used, and at sites with high false positives, it was recommended that carefully selected fluorescence thresholds would be necessary.

Upon subsequent examination, the rate of false negatives in the study by Steinberg et al. (2011) has been questioned. While moving but non-fluorescing cells were considered false negatives, it is possible that non-moving, non-fluorescing cells (~10-25% of cells, depending on the site) could be living but non-fluorescing (i.e., false negatives). Initial trials at NRLKW with cultured organisms (some of which were non-motile) showed that cultures (i.e., cells in conditions under optimum light and nutrient conditions) did fluoresce green (i.e., were living) but did not fluoresce after heat killing (i.e., were dead). Nonetheless, the point is valid. As stated in the ETV Protocol, methods should be validated at facilities to ensure their appropriateness. Non-moving, non-fluorescing cells could be interrogated to determine if they were, indeed, dead or if they represented false negatives (for example, using the uptake of radiolabeled macromolecules).

Unpublished data by MacIntyre and Cullen (described in Miller 2015a and Miller et al. 2015e) showed 50% of 24 species of phytoplankton cultures yielded false negatives after heat killing, and 7 of 24 species of phytoplankton cultures were classified accurately (live/dead) using FDA and CMFDA markers. Notably, these laboratory experiments used flow cytometry to count living and dead cells, which is in contrast to the Required Method, which employs microscopy. This distinction is important because the microscopy allows an analyst to score an unstained but moving cell as living; a flow cytometer cannot measure movement; thus, it potentially

overestimates false negatives. On the other hand, data from a flow cytometer can be analyzed using a specific threshold (and the set point of the threshold is critical to the measurement), and thus, samples are quantified in an objective manner. These issues aside, the Required Method has been used successfully by laboratories conducting BWMS verification testing (largely using ambient communities, not cultured organisms, another difference with the MacIntyre and Cullen paper); that is, there has been good agreement with the Required Method and other methods (e.g., chl *a* fluorescence), when used. Nonetheless, this work illustrates the need for validation studies on ambient communities at each test facility and for additional work to identify ways to reduce the potential for false negatives when using the Required Method to quantify the number of live organisms. To determine the rate of false negatives for non-motile, non-fluorescing cells, a biochemical assay would be in order, perhaps using cellular uptake of radiolabelled substrates.

3.7 Statistical Review of the Alternative Method

Given the importance of statistics in the Alternative Method, it is prudent to consider the method itself and underlying assumptions of the MPN analysis. The potential biases were addressed above (Section 3.3 Potential Biases and Drawbacks of the Alternative Method). Below, the statistical grounds for the MPN Method are presented, followed by passages about the method's assumptions and calculations of its CIs.

3.7.1 Statistical Justification for the MPN Method

The MPN method is an inverse approach for estimating concentrations of living organisms; that is, observations (here, the number of MPN tubes that show positive growth after a 14-d incubation) are used to hindcast the initial conditions (here, the number of viable organisms in the original sample). First described a century ago (McCrary 1915), the method is a common microbiological assay for calculating concentrations based on observations of population growth in replicate serial dilution cultures (APPENDIX 1—MPN Statistical Theory). The MPN method has been particularly valuable for estimating concentrations of monocultures (single species or strains) of single-celled microorganisms; indeed, it was originally designed for use in this manner with bacteria. In the context of ballast water, then, the method has been proposed for use with ambient aquatic organisms, which comprise a mixed assemblage of species rather than a monoculture. In a given water body, the number of species varies geographically and seasonally, but it is on the order of dozens, if not hundreds, of species. For example, Marshal et al. (2005) identified 1400 phytoplankton species in the Chesapeake Bay, its subestuaries, and tidal tributaries.

The optimal MPN tube (usually containing a volume of 5 or 10 mL) will, as a result of serial dilutions from the original sample, contain either 0 or 1 organism, but detecting a single microorganism in the sample using traditional microscopy would not be feasible. However, since many microorganisms, such as bacteria, fungi, and algae, can reproduce via asexual cell division, and under the appropriate conditions for growth, a single cell can reproduce to concentrations that are easily detectable. For example, a bacterial colony that forms on the surface of a nutrient agar plate represents the reproduction by a single microorganism, a colony-forming unit (cfu), resulting in a large number of cells (i.e., a colony, which is visible to the

naked eye). Similarly, a suspension containing a single organism, given ideal conditions (e.g., nutrients, substrate, temperature, and light), will undergo exponential population growth. After sufficient time has occurred to undergo exponential growth and to populate a sample, bulk metrics can be used to indicate the presence of that colonizing organism. Changes in common bulk metrics, including chl *a* fluorescence for algae and turbidity for bacteria, between initial measurements and measurements after a period of culturing, denote the presence of at least one organism in the initial diluted sample capable of undergoing reproduction. Importantly, the MPN assay was developed for monocultures of bacteria (*Bacillus coli*, McCrady 1915). This foundation complicates the use of the MPN method for use with ambient, mixed assemblages of phytoplankton, because applying the method to such diverse communities may violate the assumptions of the method (as discussed in the next section).

3.7.1.1 Key Assumptions of the MPN Method

The following assumptions, which were written in reference to bacteria but are also applicable for phytoplankton, are “necessary to support the MPN method” (U.S. FDA 2010; the assumptions are also presented below in Table 9, prepared for this Review):

- “The bacteria are distributed randomly within the sample.
- The bacteria are separate, not clustered together, and they do not repel each other.
- Every tube (or plate, etc.) whose inoculum contains even one viable organism will produce detectable population growth or change.
- The individual tubes of the sample are independent.”

Table 9. Summary of the key assumptions of the Most Probable Number (MPN) method.

Assumption	Relevance	Examples of Violations
All cells must be capable of growth	Every viable organism must be capable of reproducing	Viable cells that cannot be induced to reproduce in an MPN culture*
Random distribution	Organisms must be randomly distributed in the sample, so that the probability that an organism occurs in a subsample is a function of subsample volume and its concentration	Chain- or colony-forming species
Separate cells	Organisms must be separate, un-clustered, and not repelling each other	Chain- or colony-forming species
Independence	Individual tubes are independent of each other	Samples that are not independent

*These cells do not have to be cultured in perpetuity, but they must have the capacity to show population growth over the 14-d incubation period.

The critical assumption of the MPN method is that each viable cell is capable of reproducing, so its original presence in the diluted subsample is detectable through population growth and an increase in the measured parameter over the course of the MPN incubation. The ability of an organism to reproduce must be independent of other organisms, so individuals that require conjugation for reproduction and population growth (Coats and Heinbokel 1982) or organisms that only reproduce in the presence of a symbiont (e.g., *Dinophysis sp.*, a phytoplankter that ingests and harbors algae; Myung et al. 2006) may violate the assumption of independent reproduction. Also, inhibitory factors, such as toxicity due to the presence of trace metals (e.g., Paytan et al. 2009) and viruses (e.g., Fuhrman 1999) in the cultures, may negatively affect the population growth rates of marine algae.

If every cell may not be capable of being cultured in MPN tubes, and in turn, may not be detected, then the mathematical underpinnings of the MPN calculations are not applicable. In fact, the Proposal includes data showing not all species of phytoplankton are capable of being cultured—at least under the conditions that were used—at all times (see Section 3.5.3.2 Method Specificity). This point is not addressed in the application other than to indicate it will present a small bias, approaching 0% when the historical record is considered (but that percentage is greater when the ability to grow in each test is considered). The ability of every cell to be cultured, however, is a fundamental tenant of the MPN Method, and not meeting that criterion may invalidate the use of the Alternative Method. Thus, this critical shortfall needs to be resolved. Indeed, the ETV Technical Panel has formed a task group to address this issue, and the data presented in the Proposal have been the basis for many of the task group's meetings to assess the MPN Method. The assumption that tubes are independent can be met if initial samples are independently collected (i.e., not pseudoreplicates, that is, collected from separate samples). As far as the assumptions about clumping, they can be ameliorated by mixing, given that the TF can verify that mixing is sufficient to disperse colonial organisms at that location and that the specified procedure for mixing does not lead to mortality.

Regarding the assumptions of randomness and individual cells, these are partly addressed in the discussion of biases in Table 3. The issue of positive or negative interactions within species within MPN tubes, however, is not addressed. Positive interactions (in which one species spurs the growth of one or more other species) would result in an overestimation of the number of viable cells (the MPN) in the original sample, and while that is an error, it is conservative. The error of greater concern would be an underestimation from negative interactions among species. It would be impracticable to test all of the potential interactions at a given location. Regardless, assuming the tubes in an MPN matrix with the highest dilution would contain only one cell—not interacting with other cells—the problem is largely ameliorated. Negative interactions among species could occur in the tubes at lower dilutions (and containing multiple species), but presumably, some cells would survive. Even if none survived the incubation period, the most dilute tubes would show growth (assuming they contained culturable cells), so the density of cells determined by the MPN assay would be >0 .

3.7.1.2 Confidence Intervals in the Autotroph Method

A description of the equation used to calculate MPN is found in APPENDIX 1—MPN Statistical Theory. The calculations of uncertainty for the approximation of the true density of organisms (δ) are derived elsewhere (Hurley and Roscoe 1983). When δ is defined, that is, when δ is not equal to 0 or infinity, the standard error (SE, which is calculated to show the reliability of the mean) is a function of δ , v (subsample volume) and n (number of subsamples), which is summated for the number of dilutions (m):

$$\text{Eq. 1} \quad SE_{\log\delta} = \left(\delta^2 \sum_{i=1}^m \frac{v_i^2 n_i}{e^{(v_i\delta)} - 1} \right)^{-1/2}$$

The 95% CIs are calculated below using the δ and the SE (a CI of 95% is commonly used in statistical analyses). The CI indicates the probability (in this case, 95% probability) that the *actual* δ , if known, would fall within the range of values between the upper and lower CI. The upper and lower CI (CI_{Upper} and CI_{Lower} , respectively) are derived using the following equations:

$$\text{Eq. 2} \quad CI_{Upper} = e^{(\log(\delta) + 1.96 \cdot SE_{\log\delta})}$$

$$\text{Eq. 3} \quad CI_{Lower} = e^{(\log(\delta) - 1.96 \cdot SE_{\log\delta})}$$

Typically, the upper and lower CIs are reported with MPN estimates. As an aside, other metrics of uncertainty have also been described and may also be reported. For example, the rarity index identifies unexpected outcomes—such as more dilute samples with higher growth than less dilute samples (Jarvis et al. 2010). Standard methods for MPN analyses, including methods published by the EPA (U.S. EPA 1978), the U.S. Food and Drug Administration (U.S. FDA 2010), and other scientific authorities (APHA et al. 1999), include tables that list the corresponding 95% CIs. Inclusion of these values when reporting the outcome of an MPN analysis, while not stated explicitly by the EPA or FDA, is an appropriate practice for assuring data quality (e.g., U.S. EPA 1984). Likewise, the calculators evaluated in the submitted documents all report CIs (Trojan Marinex 2015b), even though the Proposal does not advocate reporting the CIs.

As for all SE calculations, larger sample sizes (n) generally result in smaller SE. To demonstrate this, a single dilution MPN was simulated for this Review by assuming a sample volume of 0.1 mL and various numbers of tubes (Table 10). In each case, 20% of the sample tubes were positive for growth, and in all cases, the MPN (cells mL⁻¹) in the original undiluted sample was set at 2.23 viable organisms mL⁻¹. The precision of the estimate of a 5-tube MPN was limited: the range of CI spanned two orders of magnitude (from 0.31-15.9 organisms mL⁻¹). Higher numbers of sample tubes yield narrower CI ranges. The Proposal stipulates an MPN matrix should consist of 3 dilutions x 5 tubes per dilution. From Table 10, it is recommended that 10

tubes are used per dilution to ensure that an MPN value generated from an MPN table does not have a large CI, e.g., a value of 2.23 with 5 tubes had an upper CI of 15.9 (over the discharge standard) whereas a value of 2.23 with 10 tubes had an upper CI of 8.95 (below the discharge standard) (Table 10).

Table 10. Results of a theoretical matrix of MPN tubes with standard error (*SE*) and 95% confidence intervals (*CI*). In all cases, 20% of the total number of tubes (*n*) show positive growth (*p*); calculations were performed in the most simple case where there was only one sample volume (*v* = 0.1 mL).

Number of tubes (<i>n</i>)	Number of tubes with growth (<i>p</i>)	MPN (cells mL ⁻¹)	<i>SE</i>	Lower <i>CI</i> (cells mL ⁻¹)	Upper <i>CI</i> (cells mL ⁻¹)
5	1	2.23	1.00	0.31	15.9
10	2	2.23	0.71	0.56	8.95
20	4	2.23	0.50	0.84	5.96
50	10	2.23	0.37	1.20	4.16
100	20	2.23	0.22	1.44	3.46

MPN = Most probable number.

Notably, the Alternative Method does not account for the CIs generated by MPN tables. This aspect is troubling, as the CI can be relatively large (as noted above), and excluding the CIs can potentially result in a BWMS being considered to meet the discharge standard on the basis of the MPN, whereas the BWMS may not meet the discharge standard if the upper CI was taken into consideration (as is done in the statistical example in the ETV Protocol [U.S. EPA 2010, Table 12 therein]). The lack of the CI is not explained in the Method, other than by a statement that they are not used. The CI should be explained in the Alternative Method with a note that it should be reported with all results; for that matter, all data using the Required Method should also include the CI around the reported value.

4 EQUIVALENCY OF THE PROPOSED METHOD WITH THE REQUIRED METHOD

This Review’s technical opinion of the equivalency of the Alternative Method with the Required Method is provided in this section first by considering whether they measure the same endpoint. Next, the equivalency of the measurements made by the Alternative Method to the Required Method is considered. This task is accomplished by reviewing the uncertainties inherent in both the Required and Alternative Methods, chronicling (for completeness) relevant portions of the validation of the Required Method, evaluating the equivalence in which organisms are quantified by the Alternative Method (considering the methods’ precision, etc.), and reviewing the validation of the Alternative Method.

4.1 Ability of the Alternative Method to Measure the Same Proximate Aspect or Function of the BWMS

The Alternative Method and the Required Method must evaluate the same function of the BWMS. In this instance, the proximate issue is the effectiveness of the BWMS in reducing the concentration of “living” organisms in treated water to less than the criteria established in the USCG’s ballast water discharge standard. However, as discussed above, conceptually, non-viable organisms *do*, in the authors’ opinion, meet the intent of the USCG’s ballast water discharge rule, although not the letter of the regulation. Notably, the Alternative Method does not exclusively measure viability, since it combines a viability measurement (the Autotroph [MPN] Method) with a live-dead measurement (Heterotroph Method).

Of course, the fundamental tenet in equating non-viable organisms to dead organisms is that organisms scored as “non-viable” truly do not have the means to reproduce following treatment. That point has not been demonstrated unequivocally in the Proposal. The Alternative Method presumes that a sufficiently high dose of UV is provided by the treatment system that it results in permanent effective damage to reproductive ability in all organisms in all ecosystems. While UV radiation can damage cellular membranes and cytoplasmic proteins (Schwartz 1998), its primary mode of sterilization is through damage to DNA. Specifically, UV radiation causes dimerization (coupling) between pyrimidine bases, which in turn, interferes with DNA transcription and replication (Goodsell 2001; Oguma et al. 2002).

We know, however, that organisms with sublethal damage may undergo repair (Sinha and Häder 2002). Because the repair mechanisms exist, it is possible that a UV-damaged organism may be able to resume its basic cellular functions, including DNA replication and reproduction. For example, in light-dependent repair, photo-activated enzymes cleave the UV-created double bond between the pyrimidine bases, whereas in light-independent repair, UV-damaged portions of the DNA strand are excised (Goosen and Moolenaar 2008, Lesser et al. 1994). Regardless of the mechanism, if repair occurs within the MPN incubation period, a damaged organism may reproduce to reach a population size above the limit of detection, but after the conclusion of the 14-d incubation period. In this instance, the Autotroph Method would not count organisms capable of DNA repair as living organisms. This capability would confound the use of the Alternative Method (specifically, the Autotroph Method, which employs an MPN method), as it is predicated on the assumption that treated cells will be *permanently* non-reproductive, posing no risk of invasion, and thus equivalent to dead cells. The Required Method, on the other hand, does not distinguish among organisms that are (1) capable of growth and detection, (2) organisms that are potentially capable of growth and detection after repair, and (3) organisms that are not capable of growth and detection.

While a 14-d grow-out period (as stipulated in the Proposal) addresses self-repair at a given location, organisms in one geographic location may require a higher dose to achieve non-repairable damage than organisms in another location. Exploring this idea, it is important to note that planktonic organisms are not exposed to the germicidal wavelengths of UV-C (i.e., UV that kills organisms, wavelength peak = 254 nm) in the environment, as UV-C is attenuated in the atmosphere. However, UV-B (290-320 nm) does penetrate into the surface waters. In tropical

waters, the daily maximum fluence (energy per unit area) of UV-B at the surface waters is 4 W m⁻² (equivalent to 0.4 mW cm⁻²), however, approximately 90% of this UV-B radiation is attenuated in the top 5 m of the water column—even in water with low turbidity (Dunne and Brown 1996).

The realized incident dose of an organism in the surface water would depend upon its exposure time. Empirical studies have estimated the repair rate from examinations of phytoplankton growth along a series of wavelengths at various light intensities, estimating the ability of a population to repair UV damage relative to the amount of UV damage (Xing et al. 2015). The studies reveal that repair rates are species-specific (Smith and Cullen 1995), that organisms acclimated to high-intensity light—such as in shallow waters—may be more likely to undergo repair (Neale et al. 1998), and that other factors (e.g., nutrient concentrations) may influence the repair rate (Heraud et al. 2005).

The specific response of phytoplankton to germicidal UV—because this process does not occur in the environment and because, until recently, sterilization of non-pathogenic phytoplankton was not an industrial process—is not widely investigated. It is possible to extrapolate from the results of studies on environmental organisms that are acclimated to small fluences of UV radiation. That said, this Review hypothesizes that the potential for repair and regrowth following organisms' exposure to germicidal doses of UV light is minor considering that cellular adaptations, such as interfering compounds (the “UV sunscreens”) produced to attenuate UV and to mitigate damage are not optimized for UV-C (e.g., Gao and Garcia-Pichel 2011). Organisms that *could* undergo repair within the incubation period would be included within the concentration estimate, as they would have ample time to reproduce to achieve detectable concentrations. Nonetheless, it is advisable that the potential effect of self-repair on method parameters such as accuracy and precision is assessed prior to the method being approved. Particularly, empirical estimates of the maximum length of time required for repair would be useful in assessing whether the 14-d incubation period is sufficient for repair and reproduction. Note that such an experiment could be done in a straightforward manner at a land-based TF for the species that occur in one location, but making such a determination for shipboard testing, in which species from potentially all ports in the world could be in the ballast water, would be impracticable. The same issues would apply to any approach used in shipboard testing, including the Required Method.

An experiment should be conducted so the portion of the Alternative Method that measures viability (the Autotroph [MPN] Method) is compared to the Required Method using samples that are treated with a BWMS that results in immediate mortality without the possibility of repair, such as electrochlorination. Here, organisms would be treated with a method intended to kill organisms (e.g., electrochlorination) or treated with a method to render them unable to reproduce (here, irradiated with UV at doses bracketing those used in BWMS), and then all organisms would be evaluated using Required (FDA/CMFDA) and Autotroph (MPN) Methods, which would, ideally, yield the same results (no living cells and no viable cells, respectively) for organisms treated with electrochlorination and UV, respectively. Additionally, the UV samples should be re-evaluated at a later time to determine if photorepair occurred. For example, samples would be held in nutrient-rich, conditions with appropriate light for an additional incubation

period (for a total incubation of 28 d) and re-evaluated to determine if any tube previously scored as negative would show growth. While such an experiment could not capture all of the variability inherent with geography or temporal changes, results showing no growth would be a good indicator that the UV treatment rendered cells permanently non-viable.

4.2 Comparison of Uncertainties

Measurement uncertainty is inherent with any approach for quantifying living organisms. The uncertainty, however, can be estimated following standard approaches (ISO 2008). As such, all factors or variables used to calculate the concentration of living organisms ≥ 10 and $< 50 \mu\text{m}$ are considered below. Sources of uncertainty are classified as *random uncertainty*, where measurement values are normally distributed around the actual value, or as *systematic uncertainty*, where measurements diverge from the actual measurement in a direction (e.g., organisms resistant to fluorescence labeling lead to a systematic undercounting of living organisms).

Both of the approaches described in the Required Method and the Alternative (Heterotrophic) Method use manual microscopy to directly count organisms ≥ 10 and $< 50 \mu\text{m}$. The measurement variables associated with the calculation of the population concentration (P) of living organisms via direct microscope counts can be generalized in the following equation (with variables defined in Table 11):

Eq. 4
$$P = \frac{I \cdot C \cdot D}{A \cdot S}$$

Table 11. Variables used to calculate concentrations of living organisms using microscope counting. Typical values are from the Naval Research Laboratory, Key West (unpubl.).

Variable	Description	Typical Values	Uncertainty Type
I	Individual (Ind.) count	30 – 300 Ind.	Random and Systematic
C	Concentrated sample volume	50 mL	Random
D	Dilution factor	1.015*	Random
A	Aliquot volume	1 mL	Random
S	Sample volume	5 L	Random

* Dilution factor (no units) accounts for the addition of the fluorescent labels (15 μL of the dissolved fluorochrome mixture is added to 985 μL of sample water).

In a forthcoming study (First et al. in prep.), the uncertainties associated with all of these variables were estimated. In empirical studies, the accuracy and precision of volumetric measurements were estimated for graduated cylinders, flasks and beakers, and volume-displacement pipettors. The highest CV observed, 2.4%, was for a pipettor used to dispense volumes of 10 μL ; in general, CVs were $< 1\%$. Therefore, sources of uncertainty in volume measurements are minor and random, and they apply to both methods. For simplicity, they will

be omitted in the comparison between the Required and Alternative Methods. The major uncertainty is aggregated under the individual counts of organisms, which includes both random error associated with the variability of detection and systematic error. The latter includes organism loss due to mortality during the sample processing, inefficient labeling or fluorescence detection, lack of detection of motility or chl *a*, or incorrectly including or excluding organisms based upon size. These factors are addressed below.

4.2.1 Loss and Mortality

Within samples, changes in organism concentrations occur continuously due to growth, predation, and senescence; some of these dynamic processes are affected by physical conditions within the sample container in a laboratory environment that will inevitably differ from the *in situ* conditions. In either the Required or Alternative Method, analysis should occur as soon after sample collection and processing to minimize these effects. The Required Method dictates that samples are processed immediately, with a sample holding time (prior to analysis) no longer than a hold time of six hours, with the caveat that each TF should validate its holding times. The Heterotroph Method may be completed up to 48 h after collection, which is based upon a set of validation experiments reported in the Proposal (Miller and Petri 2015). This guidance should be validated at each test facility using the Alternative Method, as experiments have shown a six-hour hold time to be appropriate at another location (U.S. EPA 2010).

Sample processing, particularly filtering, can also contribute to loss and mortality. Organisms >10 µm may be forced through the mesh netting as organisms are concentrated on sieves, a process used in both methods. An additional source of loss, however, can occur during physical disruptions (due to shear caused by pressure gradients near the mesh during filtering or mechanical damage during agitation to disassociate colonial species into individual cells) and rapid changes in water characteristics (e.g., temperature, salinity, dissolved oxygen). Complete filtration—i.e., allowing the entire filtrate to drain through a sieve, leaving organisms in a thin film of water on the mesh surface—would be expected to introduce variability into the analysis. Changes in water characteristics and resulting stress to organisms would be rapid in a thin film of water with a relatively high surface area exposed to the atmosphere. Thus, the suitability of using a filtration step in the Alternative Method (and the Required Method, for that matter) should be determined.

4.2.2 Inefficient Labeling

As noted in the paper describing the Required Method (Steinberg et al. 2011), certain organisms are weakly labeled with the combination of markers that are transformed by cellular enzymes. Using motility as a definitive indicator of living organisms, the proportion of living organisms not detected by their fluorescence signal (i.e., false negatives) and heat treated organisms exhibiting weak autofluorescence (i.e., false positives) varied among locations. Rates of false negatives were <2%, but false positive rates were 3%, 5%, 15% and 36% for samples from Baltimore, MD; Key West, FL; West Boothbay Harbor, ME; and Sequim, WA, respectively (Steinberg et al. 2011). Thus, the ETV Protocol notes that before the Required Method or any alternative method is used, “it is necessary that it undergo on-site validation by preparing, examining, and analyzing ambient samples that are killed (i.e., negative controls)” (EPA 2010).

Within the Autotroph (MPN) Method, the inability of all phytoplankters to grow in MPN assays may be considered to have a similar effect as inefficient—or insufficient—labelling. This fraction of the community must be accounted for. It may be possible to accommodate these organisms in MPN calculations of the concentration of viable organisms, but there is not yet agreement on the statistical adjustments for doing so.

4.2.3 Detection of Motility

Living heterotrophs are identified in the Alternative Method by motility and the absence of chl *a* fluorescence. Because not all living heterotrophs may be visibly moving at the time of viewing, this approach would potentially lead to the exclusion of organisms, and the rate of false negatives would depend upon the relative abundance of slow moving or motionless heterotrophs. The filter set prescribed by the Alternative Method may lead to the opposite error, one of false positives (Section 3.3.3 Drawbacks—Heterotrophs).

4.2.4 Detection of Chlorophyll *a*

In the Heterotroph Method, motile organisms are viewed using epifluorescence microscopy to detect chl *a* fluorescence, and organisms with a visible fluorescence signal are excluded from the tally, since they would be autotrophs that are quantified using the Autotroph (MPN) method. When chl *a* is present but not detected, the organism is classified as a heterotroph and counted as living. This situation represents a false positive and increases the total count of organisms, which yields a conservative estimate of organism concentrations for judging whether the sample meets or exceeds the discharge standard. False negatives can occur when fluorescence of chl *a* in algae contained within the food vacuoles of heterotrophic organisms leads to the false categorizing of such organisms as autotrophs, and erroneous exclusion of the heterotrophic (algae-consuming) organisms from the total count. The likelihood of this occurrence would vary among locations and depend upon the relative abundance of herbivores, which tend not to dominate this size class. This idea is not considered in the Proposal, nor is the threshold for chl *a* fluorescence. Further, it should be restated that the filter set described in the Alternative Heterotroph Method is not optimized to detect chl *a*.

4.2.5 Size Classification

Both the Alternative and Required Methods stipulate that organisms $<10\ \mu\text{m}$ and $>50\ \mu\text{m}$ are excluded from tally. In the Required Method, microbeads with dimensions near these size thresholds may be used as visual references during microscopic examinations. The error associated with incorrect sizing of organisms is inherent to both methods, but the exact approach for categorizing organisms by size that is used in the Heterotroph Method (whether comparisons to microbeads or measuring with an eyepiece micrometer) should be detailed in the Proposal.

4.2.6 Uncertainty of the Alternative Autotroph (MPN) method

Although the MPN method is complex in design, there are actually only three values that are needed for calculating the concentration of organisms: sample volume per tube, the total number of tubes, and the number of tubes exhibiting positive (algal, in this case) growth. Measurements of volumes, as discussed above, are generally accurate and precise if calibrated pipettes are used. Similar to the random error associated with other volumetric measurements, the uncertainty of the measurement of volume is excluded, as it is small relative to other sources of error. The total number of tubes is known, so there is no uncertainty associated with this value. However, the uncertainty of the number of positive tubes results from the ability to detect reproduction within the tubes, including the degree to which the fundamental MPN assumptions (that organisms are randomly distributed and capable of growth independently to reach detectable concentrations [Cochran 1950]) are violated. These explicit assumptions and the implicit assumptions that follow are addressed Section 3.7 Statistical Review of the Alternative Method). The Proposal listed all of these considerations and provided arguments to address the major considerations. Although grazing was addressed, the possibility of viral lysis was not considered. If MPN tubes show an unusual lack of growth, tubes should be examined for the presence of virus-like-particles, which can be done relatively easily, using epifluorescence microscopy (e.g., Hennes and Suttle 1995), rather than electron microscopy. Since viruses could be numerically more abundant than algae, it is possible that multiple viruses could suppress the growth of a population. Of greater concern, however, are the inability of some phytoplankton cells to grow and the lack of standardization among calibration techniques among different models of fluorometers used at various laboratories, as discussed above.

Notably, the Alternative Method does not require that a measurement of uncertainty (e.g., CI) be reported. This omission is particularly important because the MPN is an approximation, and the uncertainty of the estimation varies greatly with the number of tubes and sample volume (e.g., Table 10 in this Review). For this reason, the uncertainty range should be reported and taken into consideration in the resultant conclusion.

To determine equivalency, the accuracy and precision of the two methods should be assessed. As reviewed above, there is no means to determine the “true” concentration of organisms, and the methods measure different endpoints (living or viable organisms), although the experiment described above using two BWMS approaches does provide insight into the relationship between living and viable cells. Additionally, precision can be used to evaluate the methods. From the data presented in the Proposal, the CV (a measure of precision) is greater (indicating lower precision) in the Alternative Method than the Required Method (Figure 3).

4.2.7 Propagation of Uncertainty

When multiple measurements are used for calculations, the uncertainty should be propagated using standard formulas (Sokal and Rohlf 1995). However, it is not possible to calculate CIs when growth is observed in all tubes (e.g., the resulting value is “>1600 cells mL⁻¹”; See Section 3.5.3.1 Method Accuracy and Precision). In this case, the inequality should be reported. This situation would most likely be a concern when the concentrations of organisms in the uptake water are calculated, presuming the Alternative Method is used for uptake samples so that they can be directly compared to measurements of the discharge samples. The reason why it would be more likely to exceed the limit of detection for uptake samples is that the ambient concentrations of phytoplankton may vary over short time periods. While discharge samples are expected to contain <10 organisms mL⁻¹, concentrations in uptake samples would be harder to predict, and it would not be evident that the predicted concentration—the basis for the dilutions used for the MPN method—was incorrect until 14 d after collection. One way to avoid this situation is to perform an initial microscope count to estimate microalgal concentrations and to then adjust the MPN dilution series accordingly; however, the Alternative Method does not recommend such a step. In the experimental data provided in the Proposal, the values listed as greater or less than a certain values were considered categorical values. Effectively, this removes the inequality sign before the value (e.g., <1600 mL⁻¹ becomes 1600 mL⁻¹), which allows for averaging and other calculations (see Section 3.5.3.1 Method Accuracy and Precision).

4.3 Summary of Equivalency Determination

Below, the results of the equivalency determination are summarized. They are informed by the various types of uncertainty, as characterized immediately above, as well as by the validation experiments outlined in the Proposal. Additionally, the statistical nuances of the MPN method as it applies to ambient phytoplankton communities are considered.

4.3.1 Equivalency—Dead to Non-Viable Organisms

The first question addressed in this investigation of equivalency is the top-order issue of equivalence of “non-viable” organisms and “dead” organisms. While this Review finds them to be conceptually equivalent, the Proposal did not demonstrate that UV-treated organisms (using doses currently employed in BWMS) would remain non-viable. A validation experiment should be conducted to compare the results of the Autotroph (MPN) Method and the Required Method using samples treated with a BWMS (or simulated BWMS) and with a treatment that results in immediate mortality without the possibility of repair, such as electrochlorination. Samples

should be evaluated in the Autotroph Approach after the 14-d incubation period and then again after another 14-d period. This approach would provide an estimate of the frequency of cells capable of repair. If the estimates from both methods are equivalent after the 28-d period, then it would support the assertion that “non-viable” (UV-treated) organisms will not undergo repair.

4.3.2 Equivalency—Precision of the Required and Alternative Methods

While the validation for the Alternative Method involved more experiments than were undertaken for the Required Method, laboratory experiments showed the precision was greater in the Required Method than the Alternative Method: the CVs were 1-26% (average = 13%) for the Required Method and 20-135% (average = 50%) for the Autotroph (MPN) method (Table 8). In all but one case, the CV of the Required Method was lower than the CV of the Autotroph (MPN) Method. Thus, using a metric typically used to compare methods, precision, the Alternative Method was shown—using the data provided in the Proposal—to be less precise than the Required Method.

4.3.1 Equivalency—Agreement of the Required and Alternative Methods

Calculations of the FOA between the two Methods showed the Alternative Method at times yielded a count at least five times greater than the Required Method. The water samples were not treated with UV radiation, and in this case, the Required and Alternative Methods would be expected to have similar results (FOA of ~1). This result could be due to the inclusion of organisms <10 µm in the samples that were not removed by the filtration through the 10-µm filter.

4.3.2 Equivalency—Applicability and Use of the Most Probable Number Method

Regarding the MPN portion of the Alternative Method, statistical questions remain unresolved. Most importantly, the number of viable but unculturable organisms is not accounted for in the calculations of the number of viable cells. This omission will require data of the percentage of non-culturable taxa from a variety of locations and the assistance of statisticians to determine if it is appropriate to develop MPN tables to account for the percentage of non-growing organisms. In fact, at this point, it is uncertain if the percentage of non-growing organisms should be those that cannot be cultured in a single test (a relatively high number) or the percentage that cannot be cultured over a series of historical tests (a relatively low number).

This Review contends that the percentage of non-culturable organisms in a *single* test is relevant, as each MPN trial is of interest, not the sum of all trials; in any given test, the percentage of non-culturable organisms, whether due to BWMS treatment or non-culturability of the organism, should be known. Additionally, the percentage of non-growers in a single test is more relevant than an historical record, considering that a given assemblage of organisms’ capacity to reproduce under the defined set of culture conditions is not predictable. If the percentage of non-growers was well constrained (i.e., predictable with a narrow confidence range), then the final estimate of cell concentration could be increased based upon the known percentage of growers, if such a practice would not violate the mathematical principles of the MPN Method. However,

this approach would require an extensive validation effort, considering that physical factors—such as tidal cycles, day and night variations in solar radiation, and seasonal trends—are superimposed upon complex, dynamic physiological and life history characteristics of the plankton community. It is likely that converging on a predictable and stable estimate of non-growers would be challenging at land-based test facilities. Determining the appropriate percentage of non-culturable organisms for shipboard testing would be even more challenging. Again, the concerns with this method in shipboard testing apply to any approach, including the Required Method.

The MPN estimates are provided in this Proposal without measures of uncertainty (CIs or SEs), which is anomalous to other uses of the MPN method. In addition, at times, MPN estimates are undefined, e.g., results were reported in the Proposal as > or < a value, and in these instances, it is unclear how these results would be applied, such as when calculating the sample mean. In addition, the process for combining the uncertainty of the MPN estimate and the heterotrophic concentration is unclear; indeed, it is unclear how the uncertainty around the heterotrophic concentration is expressed (e.g., 1 SD, 95% CI, etc.). These omissions should be rectified.

5 COMPATIBILITY BETWEEN TYPE APPROVAL TESTING AND COMPLIANCE ASSESSMENT

The Alternative Method is discussed in the Proposal in the context of its use at land-based test facilities during TA testing, and it is possible to imagine the method being used during shipboard TA testing, if the water in the ports where ballast water was taken onboard was well characterized. Its applicability in compliance testing, however, is more difficult to envision. In fact, the only time compliance testing is mentioned in any of the documents comprising the Proposal is in the research paper by Cullen and MacIntyre (2015). Here, the authors indicate the Alternative Method could be useful in shipboard and compliance testing following careful experimentation. Below, this Review considers the applicability of the Alternative Method, specifically, the Autotroph (MPN) portion, in compliance testing.

When any method is reviewed for its use in ballast water compliance testing, a number of factors must be considered. Paramount among them is the time to complete an analysis. The sampling time must also be brief, but because a sampling step is required prior to using any compliance method or tool, it will not be considered here. Since the Autotroph (MPN) Method entails a 14-d incubation period, it is uncertain how it could be used for compliance testing, which will, ideally, proceed quickly, preferably to provide an “answer” to the compliance officer while he or she is onboard a vessel.

Next (and in no particular order), compliance testing, preferably, will require minimal training and equipment. Again, it is unclear how the Autotroph (MPN) method would meet these criteria. Even if the culturing equipment were minimized, it still would require a skilled technician to properly prepare the media and evaluate the many MPN tubes.

Importantly, any reliable and robust compliance method must be able to accurately analyze ballast water taken up anywhere in the world. Thus, if the MPN method is used, the culturability

of the global community of photoautotrophs must somehow be assessed. Because not all photoautotrophs can be maintained in MPN tubes (e.g., Miller et al. 2015a), if the MPN method was to be applicable for compliance, the portion of the autotrophic community that does not grow would need to be known. Likewise, the percentage of organisms that undergo self-repair must be known, particularly, organisms that may be able to undergo repair but do not reproduce to detectable concentrations within the 14-d incubation period. These parameters would require enormous effort to determine accurately over broad geographic and spatial scales. Therefore, estimates of these parameters would need to be used, which would warrant an increase in the CI around the estimates of living organisms.

Given these hurdles, it is reasonable to assume that if the MPN method were used for BWMS TA testing, another method would be required for compliance testing. From the research completed and work underway sponsored by several Administrations, it appears that variable fluorescence of photoautotrophs in unfiltered (whole) water samples is the leading parameter being considered for use in compliance assessment (noting, of course, that other methods may be developed later). Regardless, once a method(s) for compliance testing is determined, the correspondence between the TA method (i.e., MPN) and the compliance method would need to be determined. This analysis is underway to compare fluorescence-based tools (to potentially be used in compliance testing) and the Required Method (Drake et al., 2015). Such an exercise would be warranted if the Autotroph (MPN) Method were to be used in TA testing. If the two methods did not yield the same “answer” when they were used in preliminary studies or in validation studies, it is unknown how the difference would be resolved.

6 SUMMARY

This Review provides a technical opinion regarding the Proposal submitted to USCG by Trojan Marinex to use an Alternative Method to quantify the number of living organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$. The Proposal’s practicability, applicability, clarity, equivalency with the currently Required Method, and its potential use in compliance testing were reviewed, and the findings are summarized below. Where additional information or revisions to the method are recommended, it is the opinion of the reviewers that the experimental work should not require an enormous effort. The major findings are:

6.1 Practicability and Applicability

The Proposal argued that the current method used to quantify living organisms is not practicable for UV-based BWMS intended to render organisms non-reproductive. It was discussed that it is infeasible to increase the UV dose currently used in BWMS so the Required Method would indicate cells were dead following UV treatment (thus obviating the need for the Alternative Method). Using a summary of laboratory data submitted (but as yet unpublished) to a peer-reviewed journal, the Proposal argued that in systems currently designed, a UV dose sufficient to damage cells’ enzyme systems (specifically, esterase systems)—the foundation of the Required Method—and reduce cell concentrations from $1000 \text{ cells mL}^{-1}$ to 10 cells mL^{-1} would necessitate a 10-fold increase in the UV dose. Some species of cultured algae could not be killed at the dose used in laboratory experiments, although the dose was not specified (it should be). The Proposal

goes on to state that a 10-fold increase in dose would incur a concomitant 10-fold increase in the footprint of the BWMS and its electrical power requirements, which would not be practicable to install aboard a ship. This argument, however, did not include the engineering or economic calculations to demonstrate this point. They should be provided.

6.2 Full Method Description

The method described in the Proposal was for the most part, clear and understandable. Field and laboratory experiments were summarized (and the raw data were provided) to support the assertions made in the Proposal. Further, the sequence of (the many) experiments was clear as the proposers gathered more information to refine the MPN method. Some of the documents provided were not referred to in the other documents, but this is a minor quibble, as they were nonetheless useful.

This Review identified several concerns in this portion of the evaluation; largely, they are a lack of data to validate steps in the method or a lack of standardization. The items and their potential outcomes are addressed above, so they are briefly listed below in decreasing order of importance.

- The threshold for determining growth in an MPN tube is quite low, and it will be dependent on the fluorometer's model and calibration. These items should be specified and standardized in the Proposal.
- The filter set described in the Heterotrophic Method is not optimized to detect chl *a*.
- Additional data are required to determine if filters should be removed from the MPN tubes.
- From calculations prepared for this Review, it appears the number of MPN tubes used in testing should be increased; this point should be investigated.
- Along those lines, MPN values may be inflated if organisms <10 µm are inadvertently added to the MPN tubes; this potential should be investigated.
- The CIs around the MPN results should be explained in the Alternative Method with a note that they should be reported with all results; for that matter, all data using the Required Method should also include CIs. Similarly, the means to combine the CIs for the Autotroph and Heterotroph Methods should be provided.
- The Alternative Method allows the TO to determine the approach in which samples are collected, and that should not be the case: the Alternative Method should stipulate how samples are collected.
- The means to categorize organisms by size (as part of the Heterotroph Method), whether by making comparisons to microbeads or measuring organisms with an eyepiece micrometer, should be stated explicitly in the Alternative Method.
- The number of samples analyzed for autotrophs and heterotrophs in tests of Initial and Ongoing Precision and Accuracy should be increased from one to at least three. Likewise, an upper limit of the FOA should be defined in the Proposal.

6.3 Justification of Equivalence

The Proposal did not provide adequate justification that the proposed Alternative Method is equivalent to the Required Method for three reasons:

- (1) The equivalence between dead and non-viable organisms was not demonstrated.
 - The equivalence between dead and non-viable organisms was not demonstrated and should be, e.g., in an experiment using phytoplankton that are treated to induce immediate mortality without the possibility of repair (such as electrochlorination) and treated by UV radiation, and comparing the outcomes using the Required and Autotroph (MPN) Methods over a period >14 d (to assess the capacity of cells to undergo repair). This longer assessment period is important for organisms that are capable of repair but do not reproduce to detectable levels within the span of the 14-d incubation identified in the Alternative Method. These results could be augmented or replaced with similar experiments already described in the peer-reviewed literature.
- (2) The equivalence between the Required and Alternative Methods was not demonstrated.
 - While the validation for the Alternative Method was more extensive than that undertaken for the Required Method, laboratory experiments showed the precision of the two methods was lower in the Required Method: the CVs were 1-26% (average = 13%) for the Required Method and 20-135% (average = 50%) for the Autotroph (MPN) method (Table 8). In all but one case, the CV of the Required Method was lower than the CV of the Autotroph (MPN) Method. Thus, using a metric typically used to compare methods, precision, the Alternative Method was shown—using the data provided in the Proposal—to be less precise than the Required Method. Further, empirical measurements of the FOA (using ambient communities) between two methods varied widely among different experiments. At times, the Alternative Method yielded a count at least five times greater than the Required Method. This result is surprising, since the water samples in these trials were not treated with UV radiation; in untreated samples, the Required and Alternative Methods would be expected to have similar results (FOA of ~1). This result could be due to the inclusion of organisms <10 µm in the samples that are not removed by the filtration through the 10-µm pre-filter prior to analysis with the Alternative Method.
 - In addition, in the Proposal, the Alternative Heterotrophic Method was not compared to the Required Method (it was compared to a microscopy-based method, but it was not the epifluorescence-microscopy method outlined in the Required Method). For a robust comparison, the Heterotroph and Required Methods should be compared.
- (3) Statistical questions remain unresolved.
 - Regarding the use of the Autotroph (MPN) Method, most critically, the percentage of non-culturable taxa is not accounted for in the calculations of the number of viable cells. This percentage should be known at each TF using the Alternative Method, and upon the advice of statisticians, somehow incorporated into the calculations of cell densities. It is unclear how this factor would be incorporated into tests for shipboard verification of BWMS.
 - Other statistical concerns are more easily addressed: the MPN estimates are provided without measures of uncertainty (e.g., CIs). Additionally, at times, MPN estimates are undefined, e.g., results are reported as > or < the

detection limit, and in these instances, it is unclear how these results should be applied, such as when calculating the sample mean. Finally, the process for combining the uncertainty of the MPN estimate and the uncertainty in the heterotrophic concentration is unclear (i.e., how the CIs for both numbers are used—are they combined in a straightforward fashion, by adding them together, or are additional calculations required?). These omissions should be rectified.

6.4 The Alternative Method’s Use in Compliance Testing

It seems impracticable to use the Alternative Method (specifically, the MPN portion of it) for compliance testing. If the Alternative Method was used for BWMS TA testing and another method was used for compliance testing, it would be necessary to determine the correspondence between the two methods. At this point, it appears that variable fluorescence of photoautotrophs in unfiltered (whole) water samples may be the method used to determine ships’ compliance with the discharge standard. Developing the compliance assessment method includes a careful comparison of results obtained using the compliance method and the Required Method that is used for TA testing. If the Alternative Method was considered for use in Type Approval, it would be prudent to begin a similar study to determine how the Alternative Method and a variable fluorescence-based compliance method relate to one another. If the two methods did not yield the same “answer”, it is unclear how the difference would be resolved, or how compliance by ships using UV-based systems would be assessed.

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APPENDIX 1—MPN STATISTICAL THEORY

If organisms in a sample are uniformly distributed, the number of organisms present in a subsample is a function of the volume of the subsample and the initial concentration of organisms (in the sample). When multiple subsamples (n) of a known volume (v) are drawn from a sample, the probability that a subsample has at least one organism (i.e., a positive outcome, p) can be calculated based upon the following formula (McCrary 1915, but see Cochran 1950 for the derivation):

Eq. 5
$$nv = \frac{pv}{1 - e^{(-v\delta)}}$$

Where δ is the concentration of organisms. In MPN methods, δ is unknown. However, using observations of certain sets of positive outcomes, the equation can be used to solve for the unknown δ . The instances where δ cannot be calculated are when no positive outcomes are observed (i.e., $p = 0$) and all positive outcomes are observed ($p = n$). In these cases, δ is either undefined or incalculable.

Ideally, the volume chosen for the sample concentration will have an equal probability of containing 1 or 0 organisms. As there is no way to know this *a priori*, multiple volumes are usually incorporated in an MPN method, and the volume typically represent a serial dilution of the initial sample (e.g., 10^{-1} , 10^{-2} , 10^{-3} mL). As this method was commonly applied to estimate bacterial concentrations, which can range over several orders of magnitude, the broad range of dilutions and the large differences between any two volumes were justified. A single trial with multiple dilutions—each with different values for v , n , and p —requires that all multiple equations are solved simultaneously using an iterative approach to converge on a value for δ :

Eq. 6
$$\sum_{i=1}^m n_i v_i = \sum_{i=1}^m \frac{p_i v_i}{1 - e^{(-v_i \delta)}}$$

The equation is the summation of any number of dilutions (m). The iterative approach finds the value of δ that satisfies the equation, the most probable number.