Designation: ~~E645 – 13~~ E645 – 18

Standard Practice for Evaluation of Microbicides Used in Cooling Water Systems[1]

This standard is issued under the fixed designation E645; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. ~~Scope~~ Scope*

1.1 This practice outlines a procedure for evaluating the efficacy of microbicides (algicides, bactericides, and fungicides) that will be used for controlling microbial growth in cooling water systems. The microbicides will be evaluated using simulated or real cooling tower water against (*1*) microbes from cooling water, (*2*) microbes in microbiological deposits (biofilms) from operating cooling systems, or (*3*) microorganisms known to contaminate cooling water systems, or a combination thereof. This practice should be performed by individuals familiar with microbiological techniques.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate ~~safety~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D3731 Practices for Measurement of Chlorophyll Content of Algae in Surface Waters
D4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water
D4412 Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits
E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents
E1326 Guide for Evaluating Non-culture Microbiological Tests
E1427 Guide for Selecting Test Methods to Determine the Effectiveness of Antimicrobial Agents and Other Chemicals for the Prevention, Inactivation and Removal of Biofilm (Withdrawn 2009)[3]
E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 For definitions of terms used in this practice, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *algicide, n*—a chemical agent that kills algae; unicellular or filamentous chlorophyll-containing plants.

3.2.2 *bactericide, n*—a physical or chemical agent that kills bacteria, but not necessarily bacterial spores.

3.2.3 *biofilm, n*—a dynamic, self-organized accumulation of microorganisms and environmental by-products immobilized on a substrate and embedded in an organic polymer matrix.

3.2.4 *cooling system, n*—equipment and coolant used for the removal of heat from processes, equipment, or both.

3.2.4.1 *Discussion*—

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

***A Summary of Changes section appears at the end of this standard**

The most common medium used for removal or transfer of heat is water. The heated water then can be discharged into a receiving body (once through cooling system) or it can be cooled and reused (recirculating cooling system).

3.2.5 *cooling tower, n*—a structure used to dissipate heat in open recirculating cooling systems.

3.2.6 *cooling water, n*—any water-based solution that absorbs and transfers heat in a heat exchange system.

3.2.7 *fungicides, n*—a physical or chemical agent that kills fungi; that is, vegetative mycelia and/or budding yeasts including spores and/or conidia.

3.2.8 *microbial biofouling, n*—the unwanted accumulation of bacterial, fungal, or algal cells, or any combination thereof and their products on surfaces.

3.2.8.1 *Discussion*—

Often this accumulation is accompanied by deposition of organic and inorganic material.

3.2.9 *microbicides, n*—a physical or chemical agent that kills microorganisms.

4. Summary of Practice

4.1 Microbicides are evaluated against microbes under conditions simulating a cooling water system. Microbicides at concentrations that are expected to control the microbes are added to cooling water. At selected time periods, the number of microbes or measurable component of the microbes are determined and compared to values at the start of the experiment. Bacteria (aerobic and anaerobic), fungi, and algae may be detected by a number of methods, such as plate counting, Most Probable Number (MPN), chlorophyll content, adenosine-5′-triphosphate (ATP). The investigator will determine the range of microbicide concentration for acceptable efficacy based upon laboratory testing that may be used to satisfy registration or customer needs.

5. Significance and Use

5.1 This practice determines potentially effective microbicides for use in cooling water systems using cooling water and deposits/biofilm obtained from the field. The addition of deposits/biofilms addresses the need to include the major source of microorganisms in cooling water systems. Even with this addition, laboratory results may not be totally predictive of microbicidal effectiveness in the field. This is because conditions in the field affecting microbicide effectiveness are difficult to mimic in the laboratory. These conditions that affect microbicide efficacy include blow-down rate, addition of makeup water, water hardness, hydrocarbon leaks, pH, sediment loading, dissolved solids, microbes in slime (biofilms), and deposits (salts, iron minerals, organics, and so forth) on surfaces. An additional factor is the difficulty in enumerating all microbes in the water due to the lack of adequate recovery media. Guidelines that address formation of and testing for surface-attached microbes (biofilms) may be found in Guide E1427, while a guideline for unconventional measurement of microbes is found in Guide E1326.

6. Apparatus

6.1 *Balance*—a calibrated analytical balance sensitive to 0.1 mg to weigh the candidate microbicide for preparation of stock solutions.

6.2 *Containers*—flasks, bottles, or test tubes suitable for shaking shall be sterile for use.

6.3 *Colony Counters*—manual, such as Quebec, Buck, or Wolffhuegel, or a proven colony image analyzer (electronic/scanner type) are suitable for counting plates after incubation.

6.4 *Spiral Plater (alternative).*

6.5 *Constant Temperature Shaker*—a reliable constant-temperature shaker ~~±2°C~~±2 °C (water bath or incubator shaker) to provide mixing and aeration and to maintain temperature during the contact period at a setting within the temperature range selected in 10.2.

6.6 *Petri Dishes,* sterile, 100 by 15-mm plastic or borosilicate glass.

6.7 *Pipettes*—standard pipettes, sterile, with appropriate calibrations, or other suitable delivery systems, such micropipetters.

6.8 *Sterilizers*—pressurized steam sterilizer (for media, containers, and so forth), hot air oven for containers, and filter apparatus for filter sterilization (disposable filter units, 250 mL, 0.22-µm pore size).

6.9 *Stirrer*—required to mix the cooling water sample while it is being dispensed into test containers. This can be a magnetic stirrer, a propeller-type stirrer, or any other suitable device.

6.10 *Volumetric Flasks,* 100 mL, are convenient for preparing microbicide stock solutions. Smaller volume flasks may be used where appropriate.

6.11 *Blender*—a blender, stomacher, sonic bath, or vortex mixer to homogenize the microbial deposit before mixing it with the cooling water.

6.12 *Microscope,* providing a magnification range of 400 to 1000× with a suitable light source. Phase contrast or dark-field capability may be necessary.

6.13 *Filter apparatus,* with 0.2 µm filter.

7. Reagents and Materials

7.1 *Purity of Reagents*—The principal reagent used is water, but other solvents may be necessary in preparing the microbicide stock solutions. Reagent grade organic solvents are normally used if water is not a suitable diluent for dissolving a microbicide. If a solvent is used, an additional control must be performed that has solvent without any microbicide added to the cooling water sample. This is used to demonstrate that the solvent has no appreciable effect on the test results.

7.2 *Purity of Water*—All reference to water as a diluent or reagent shall mean distilled water or water of equal purity, unless otherwise noted.

7.3 *Culture Media:*

7.3.1 A general bacterial agar medium, such as glucose extract agar, tryptic soy agar, R2A agar, or dry film is used for conducting bacterial counts on test samples. Other media, such as selective or differential types (that is, for the quantification of sulfate-reducing bacteria, Test Methods D4412) may be used for detecting desired bacteria. MPN or ATP (Test Method D4012) measurement may also be used to quantify the ~~bacteria~~bioburdens (Guide E1326). Once a specific agar medium or other method of measurement is chosen, it must be used throughout this procedure.

7.3.2 A general fungal medium, such as an inhibitory mold agar or Sabouraud dextrose agar, is used for conducting fungal counts on the samples. This medium must be able to inhibit the growth of bacteria.

7.3.3 Bristol's medium,[4] or a suitable equivalent, is the recommended medium for the growth of algae.

7.4 *Dilution Water Blanks*—Sterile, 99 or 9-mL phosphate buffered saline or phosphate buffered magnesium chloride dilution blanks are convenient for diluting test samples for viable counts. Buffer strength and salinity can be adjusted to mimic experimental or field conditions.

7.4.1 *Phosphate Buffered Dilution Water Blanks.*

7.4.1.1 *Phosphate Buffer Solution, Stock*—Dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of water. Adjust pH to 7.2 ± 0.2 with NaOH solution (40 g/L) and bring to 1000 mL with water. Sterilize by filtration or autoclave.

7.4.1.2 *Phosphate Buffered Saline Dilution Water*—Add 1.25 mL of stock phosphate buffer solution and 8.75 g of NaCl to a volumetric flask, fill with reagent water to the 1000-mL mark, and mix. Final pH should be 7.2 ± 0.2. Dispense in amount that will provide 99 ± 2 mL or 9 ± 1 mL after sterilization into screw-cap dilution bottles or tubes. Sterilize immediately.

7.4.2 *Phosphate Buffered Magnesium Chloride Dilution Water*—Add 1.25 mL of stock phosphate buffer solution and 5.0 mL of magnesium chloride solution (81.1 g $MgCl_2 \cdot 6\ H_2O$/L, reagent grade water) to 1000 mL of water. Adjust pH to 7.2 ± 0.2. Dispense in amounts that will provide 99 ± 2 mL or 9 ± 1 mL after sterilization into screw-cap dilution bottles or tubes. Sterilize immediately.

7.5 *Cooling Water Sample:*

7.5.1 The cooling water sample will be collected in a sterile container (1-gal or 2.2-L plastic bottles are convenient). The temperature and pH should be determined at the time of sample collection. The presence of additives in the cooling tower water may affect the effectiveness of the microbicides, therefore, a history of the samples should be obtained or analysis of the water for additives should be conducted. Stop biocide addition at least 4 h before collection of samples, or an appropriate biocide inactivator must be added to the sample. Do not expose samples to temperature extremes during transit. If a variation of 1.0 pH unit exists between the time of sampling and testing, the sample should be discarded. The test procedure should be initiated within 24 h after collection. Samples received from the field must be refrigerated ~~(4 ± 2°C).~~(4 ± 2 °C).

7.5.2 Collect deposits of microbial composition in sterile containers from any affected areas of the cooling tower, such as the distribution deck, slats, or sump area. Transport the deposit samples with the water sample following the same precautions. Upon receipt at the laboratory, conduct microscopic examination of the deposits to confirm that they are microbiological in nature. If testing for algicidal or fungicidal activity, or both, the sample must contain algae or fungi, or both.

8. Preparation of the Test Samples

8.1 The cooling water sample may be used as received or inoculated with known microorganisms. If the water is used only as a substrate and known microorganisms[5] will be added as inoculum, the water should be filter-sterilized (using a 0.2 µm filter system) prior to the addition of microorganisms. If a biofilm sample or microbiological deposit is available, it may be used as the inoculum in either filtered or non-filtered sterilized cooling water. The biolfilm or slime must be homogenized/disaggregated so that no clumps are present. This can be accomplished by vortexing, sonicating, or any other method that disperses the clumps. No more than 10 % of the total weight (w/v) of the samples should be biofilm or deposit. A synthetic cooling water may also be used as the sample water.

[4] Starr, R. C., and Zeikus, J. A., "The Culture Collection of Algae at the University of Texas at Austin," *Journal of Psychology*, Vol 23, No. 5, 1987, pp. 1–47.

[5] Pesticide Assessment Guidelines, Subdivision G, Product Performance, U.S. Environmental Protection Agency, November 1982, Section 92.4, or most current edition.