Designation: E979 – 20

Standard Practice for Evaluation of Antimicrobial Agents as Preservatives for Invert Emulsion and Other Water Containing Hydraulic Fluids[1]

This standard is issued under the fixed designation E979; 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.

INTRODUCTION

Invert emulsion hydraulic fluids typically contain 60 % mineral oil and 40 % water (by volume). These fluids routinely are prepared using proprietary, oil-soluble, emulsifying agents, as well as other emulsifiable constituents. They are recommended for use where conditions indicate a low-cost, fire retardant product, compatible with water-based metal working fluids.

The high water content of these hydraulic fluids makes them susceptible to microbial attack. Uncontrolled microbial growth in these fluids can cause cartridge filter unit plugging, maladorous conditions, or general biodeterioration. Problem microorganisms associated with these fluids include bacteria and fungi.

The hydraulic system is essentially a closed one in which water of evaporation is added to maintain a fixed volume. The inclusion of an efficacious preservative in the water containing hydraulic fluids can prevent microbial growth and the resulting problems that follow.

1. Scope*

1.1 This laboratory practice is designed to evaluate the utility and effectiveness of antimicrobial agents intended to control microbial growth in invert emulsions and other water containing hydraulic fluids.

NOTE 1—Procedures for preparation of water soluble hydraulic fluids and recovery of organisms appear in Practice E2169.

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, health, and 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]

D1129 Terminology Relating to Water

D4454 Test Method for Simultaneous Enumeration of Total and Respiring Bacteria in Aquatic Systems by Microscopy (Withdrawn 2015)[3]

D5465 Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E1326 Guide for Evaluating Non-culture Microbiological Tests

E2169 Practice for Selecting Antimicrobial Pesticides for Use in Water-Miscible Metalworking Fluids

E2523 Terminology for Metalworking Fluids and Operations

E2694 Test Method for Measurement of Adenosine Triphosphate in Water-Miscible Metalworking Fluids

[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

3. Terminology

3.1 Terms used in this practice are defined in Terminologies D1129 and E2523.

4. Summary of Test Method

4.1 The antimicrobial agent to be evaluated is incorporated into an emulsion system by (*a*) addition to the aqueous phase employed in the preparation of the emulsion, (*b*) in doses to the formulated system, or (*c*) by other methods suitable for the test compound.

4.2 A heavy bacterial or fungal inoculum, or both, is then added.

4.3 The resulting mixture is aerated and passed over the surface of a simulated filter system for a minimum period of eight weeks either continuously or with shutdowns to simulate actual operations conditions.

4.4 The degree of microbial control is determined by periodically quantifying the bioburden in the emulsion by direct microscopic count (Test Method D4454), plate count (Practice D5465), or other appropriate method (Guide E1326) and visual observations for microbial fouling of the simulated filter surface.

NOTE 2—A knowledge of standard microbiological techniques is required for this procedure. It is also required that good laboratory practices be followed throughout these tests. This means appropriate containment for the microbiological systems being evaluated. The systems should be maintained in an enclosure so that during the aeration process the mists and aerosols generated do not contaminate the laboratory environment.

5. Significance and Use

5.1 This procedure is designed to determine the effectiveness of antimicrobial agents intended for microbial control in invert emulsions and other water containing hydraulic fluids.

6. Apparatus

6.1 *Air Supply*—Any air source which is free from organic vapors, organic matter, or other objectionable material may be used.

NOTE 3—If desired, air may be sterilized as follows:

Pack two 150-mm long drying tubes (bulb type) loosely with glass wool in a series with neoprene stoppers, glass tubing, and neoprene tubing. Wrap loosely in aluminum foil and steam sterilize at 15 to 20 psi for 30 minutes. Cool to room temperature while still wrapped. In-line pre-sterilization air filters are available from most local laboratory supply houses.

Insert into air line with bulbs on upstream side. Average lifetime in continuous use is two weeks. Discard sooner if upstream filter becomes wet or contaminated with oil.

6.2 *Colony Counter*—Any one of several types may be used.

6.3 *Incubator*—Any cabinet capable of maintaining a temperature of 35 ± 1 °C may be used.

6.4 *Test Cabinet*—A large cabinet capable of maintaining a temperature of 35 ± 1 °C, able to house several two litre beakers, and into which an air line can be introduced.

6.5 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

6.6 *Simulated Filters:*

6.6.1 *Strainer,* 3-in. epoxy coated, ¼-in. mesh gutter strainer.[4]

6.6.2 *Screen,* 16 by 18 in. fiberglass screening material.

NOTE 4—Fiberglass mesh screening material (16 by 18 in.) is available from any local hardware dealer.

6.6.3 *Wire,* 20-gauge, galvanized or stainless steel.

6.7 *Tubing,* ¼-in. ID Tygon.

NOTE 5—Tygon is available from most local laboratory supply houses.

6.8 *T-Connectors,* ¼-in. polypropylene.

6.9 *Laboratory Blender*—Any standard adjustable speed laboratory blender having a 2-L capacity glass or metal container is satisfactory.

6.10 *Hypodermic Needle,* 16-gauge needle.

6.11 *Microscope,* Brightfield microscope equipped with 40× and 100× objectives.

6.12 *Labware:*

6.12.1 *Culture Dishes*—100 by 15 mm sterile culture dishes made of glass or plastic are required for making standard plate counts.

NOTE 6—Presterilized and disposable plastic petri dishes are available from most local laboratory supply houses.

6.12.2 *Bacteriological Pipettes of 1.1 or 2.2-mL capacity.*

NOTE 7—Presterilized and disposable 1.1-mL bacteriological pipettes are available from most local laboratory supply houses.

6.12.3 *Water Dilution Bottles*—Any sterilizable glass containers having a 150 to 200-mL capacity and tight closures may be used.

NOTE 8—Milk dilution bottles of 160-mL capacity having screw-cap closures are available from most local laboratory supply houses.

6.12.4 *Two-Liter Borosilicate Glass Beakers.*

6.12.5 *Bent Glass Rod.*

6.12.6 *Screw Cap Culture Tubes,* autoclavable, 15 by 150 mm.

6.13 *Water Bath*—Maintain at 46 ± 2 °C to anneal agar based microbiological media.

6.14 *Aluminum Foil.*

7. Reagents and Materials

7.1 *Invert Emulsion Emulsifier.*[5]

7.2 *Paraffinic Mineral Oil.*

7.3 *Deionized or Distilled Water* (>2 MOHM quality)

[4] The sole source of supply of the apparatus known to the committee at this time is Billy Penn Corp., Philadelphia, PA 19122. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, [1] which you may attend.

[5] The sole source of supply of a satisfactory emulsifier for the preparation of invert emulsion hydraulic fluids (Compound #5162) known to the committee at this time is the Lubrizol Co., Wickliffe, OH. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.