Designation: E 2275 – 03$^{\epsilon 1}$

Standard Practice for Evaluating Water-Miscible Metalworking Fluid Bioresistance and Antimicrobial Pesticide Performance[1]

This standard is issued under the fixed designation E 2275; 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.

ε[1] NOTE—Footnote 1 was editorially revised in November 2003.

1. Scope

1.1 This practice addresses the evaluation of the relative inherent bioresistance of water-miscible metalworking fluids, the bioresistance attributable to augmentation with antimicrobial pesticides or both. It replaces Methods D 3946 and E 686.

1.2 In this practice relative bioresistance is determined by challenging metalworking fluids with a biological inoculum that may either be characterized (comprised of one or more known biological cultures) or uncharacterized (comprised of biologically contaminated metalworking fluid or one or more unidentified isolates from deteriorated metalworking fluid). Challenged fluid bioresistance is defined in terms of resistance to biomass increase, viable cell recovery increase, chemical property change, physical property change or some combination thereof.

1.3 This practice is applicable to antimicrobial agents that are incorporated into either the metalworking fluid concentrate or end-use dilution. It is also applicable to metalworking fluids that are formulated using non-microbicidal, inherently bioresistant components.

1.4 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 888 Test Methods for Dissolved Oxygen in Water

D 1067 Test Methods for Acidity or Alkalinity of Water

D 1193 Specification for Reagent Grade Water

D 3342 Method for Dispersion Stability of New (Unused) Rolling Oil Dispersions in Water

D 3519 Test Method for Foam in Aqueous Media (Blender Test)

D 3601 Test Method for Foam in Aqueous Media (Bottle Test)

D 4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water

D 4627 Method for Iron Chip Corrosion for Water-Dilutable Metalworking Fluids

D 5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E 70 Test Method for pH of Aqueous Solutions with the Glass Electrode

E 1326 Guide for Evaluating Nonconventional Microbiological tests Used for Enumerating Bacteria

E 2169 Practice for Selecting Antimicrobial Pesticides for use in Water-miscible Metalworking Fluids

2.2 *Other Standards:*

4.027 Synthetic Hard Water[3]

9215A.6a Heterotrophic Plate Count Media, Plate Count Agar[4]

9216 Direct Total Microbial Count[4]

Microbiological Test <71> [5]

2.3 *Government Standard:*

40 CFR 156 Labeling Requirements for Pesticides and Devices

3. Terminology

3.1 *Definitions:*

3.1.1 *active ingredient*, *n*—the chemical component or components of an antimicrobial pesticide that provides its microbicidal performance.

3.1.2 *antimicrobial pesticide*, *n*—chemical additive registered under 40 CFR 152, for use to inhibit growth, proliferation or both of microorganisms.

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides 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] *AOAC International Methods of Analysis*, AOAC International, Gaithersburg, MD.

[4] Available from American Public Health Association (APHA) *Standard Methods for the Examination of Water and Wastewater* 800 I Street, NW Washington, DC 20001.

[5] Available from U.S. Pharmacopeia/National Formulary (USP/NF), 12601 Twinbrook Parkway Rockville, MD 20852.

3.1.3 *as supplied*, *adj*—antimicrobial pesticide finished product including the active ingredient(s), solvent and any additional inactive ingredients.

3.1.4 *biocide*, *n*—any chemical intended for use to kill organisms.

3.1.5 *bioresistant*, *adj*—ability to withstand biological attack.

3.1.5.1 *Discussion*—Bioresistant, or recalcitrant, chemicals are not readily metabolized by microorganisms.

3.1.6 *biostatic*, *adj*—able to prevent existing microbial contaminants from growing or proliferating, but unable to kill them.

3.1.6.1 *Discussion*—Biostatic additives may be registered antimicrobial pesticides or unregistered chemicals with other performance properties. The difference between biocidal and biostatic performance may be attributed to dose, chemistry or both.

3.1.7 *dose*, *n*—concentration of antimicrobial pesticide added to treated solution.

3.1.7.1 *Discussion*—Dose is generally expressed as either ppm active ingredient (a.i.) or ppm as supplied (a.s.).

3.1.8 *inactive ingredient*, *n*—component of antimicrobial pesticide that is not directly responsible for the pesticide's antimicrobial performance.

3.1.8.1 *Discussion*—Inactive ingredients may include, but are not limited to solvents and chemicals that improve the pesticide's non-biocidal performance properties, such as miscibility and reactivity with non-target molecules in the treated material.

3.1.9 *minimum inhibitory concentration (MIC)*, *n*—lowest treatment-dose that will prevent test population from growing, proliferating or otherwise contributing to biodeterioration.

3.2 *Abbreviations*:

3.2.1 *a.i.*—active ingredient

3.2.2 *a.s.*—as supplied

3.2.3 *ATCC*—american type culture collection

3.2.4 *CFU*—colony forming unit

4. Summary of Practice

4.1 End-use dilutions of one or more water-miscible metalworking fluids are dispensed into microcosms. The fluids may be fresh or aged, dosed with one or more antimicrobial pesticides or undosed. Microcosms are challenged with either uncharacterized or characterized biological inocula. After inoculation, microcosms are aerated either continuously or periodically to simulate recirculation conditions in coolant systems. Chips may also be added to microcosms to simulate chip accumulation in coolant systems.

4.2 After inoculation, fluid samples are drawn from each microcosm periodically and tested for the parameters of interest, including but not limited to microbial viable counts. Depending on the test objectives, the test duration may range from 24 h to three months.

4.2.1 Shorter test periods are used to evaluate microbicide speed of kill and metalworking formulation initial bioresistance.

4.2.2 Longer test periods are used to evaluate metalworking fluid formulation resistance to repeated challenges. For tests lasting longer than one-week, 10 to 80 % of the fluid is exchanged weekly with fresh fluid before the additional challenge. The percentage of fluid exchange should reflect anticipated fluid turnover rates in fluid's end-use application.

4.3 Bioresistance is determined as the test fluid's relative ability to prevent the proliferation of challenge microbes, retain its original chemical or physical properties of some combination of the above. The bioresistance of test formulations is defined relative to that of a benchmark or control formulation.

5. Significance and Use

5.1 This practice provides laboratory procedures for rating the relative bioresistance of metalworking fluid formulations, for determining the need for microbicide addition prior to or during fluid use in metalworking systems and for evaluating microbicide performance. General considerations for microbicide selection are provided in Practice E 2169.

5.2 The factors affecting challenge population numbers, taxonomic diversity, physiological state, inoculation frequency and biodeterioration effects in recirculating metalworking fluid systems are varied and only partially understood. Consequently, the results of tests completed in accordance with this practice should be used only to compare the relative performance of products or microbicide treatments included in a test series. Results should not be construed as predicting actual field performance.

6. Apparatus

6.1 *Air Supply*, air provided at no more than 110 kPa.

NOTE 1—Any air source that is free of organic vapors, organic matter or other objectionable material may be used. Sterile air need not be used for the uncharacterized inoculum, but shall be used for the characterized inoculum. If necessary, air may be sterilized either by inserting, in series, two commercially available in-line sterile filters designed for this purpose. Alternatively an in-line filter may be prepared as follows: Pack two 150 mm long drying tubes (bulb-type) loosely with borosilicate glass wool in series with neoprene stoppers, glass tubing and neoprene tubing. Wrap loosely in aluminum foil and steam sterilize at 103 to 138 kPa (15 to 20 psi) for 30 min or dry heat sterilize at 160°C for 2 h. Cool to room temperature while wrapped. Insert into air line with bulbs on upstream side. Whether using a commercial or fabricated filter, average lifetime in continuous use is two weeks. Discard sooner if upstream filter becomes wet or contaminated with oil.

6.2 *Aquarium Tubing*, 6.35 mm (0.25 in.) diameter, silicone or vinyl.

6.3 *Autoclave*, with both steam cycle (80 to 100°C) and sterilization cycle (15 min at ≥ 121°C) capability.

6.4 *Adjustable Volume Pipetters*, with sterile disposable tips. Pipetters will be used to deliver 1.0 µL to 2 mL volumes.

6.5 *Glassware*:

NOTE 2—Sterile laboratory ware or sterile disposable laboratory ware should be used according to standard microbiological practice.

6.5.1 *Glass Tubing*, 6.35 mm (0.25 in.) i.d., cut into 15 cm lengths with ends fire-polished.

6.5.2 *French Square Bottles*, 960 mL, with metal cap.

NOTE 3—Alternatively, 1 L capacity canning jars may be used.

6.5.3 *Pipetes, Bacteriological*, 10 and 2.2 mL.

6.6 *Incubator*, capable of maintaining a temperature of 25 ± 2°C.

NOTE 4—Although an incubator is preferred, incubation may be performed at ambient room temperature.

6.7 *Manifold*, aquarium style, multi-valve.

NOTE 5—The number of manifolds and valves per manifold will depend on the number of microcosms in the test array. Air for each microcosm shall be supplied through a single air valve. Where used, air sterilization filters shall be placed between the air valve and microcosm aeration tube.

6.8 *Metal Punch*, 1 cm diameter.

7. Reagents and Materials

7.1 *Reagents*:

7.1.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[6]

7.1.2 *Water Purity*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type III of Specification D 1193.

7.1.3 *Antimicrobial Pesticide(s)*:

NOTE 6—The measurement of antimicrobial pesticide (microbicide) efficacy in a medium as complex as metalworking fluid is relative, not absolute. Consequently, when this method is used to evaluate microbicide performance (8.3 or 8.4), it is prudent to always evaluate at least two antimicrobial treatments. Preferably one treatment should serve as a positive control; its efficacy in the test system having been established previously.

7.1.4 *Metalworking Fluid(s)*:

NOTE 7—The number of metalworking fluids available is almost limitless. Recommendations for the use of any particular fluid cannot be made. If the primary intent is to evaluate the general efficacy of the microbicide(s) being tested, then it/they should be tested in various types of formulations. If the primary intent is to protect a particular formulation, then a microbicide-free version of that formulation should be used as the control and base-fluid to which the treatments are added.

7.1.4.1 *End-use Dilution Metalworking Fluid*—Dilute metalworking fluid concentrate in synthetic hard water (AOAC 4.027) to achieve the concentration at which it is used typically in recirculating metalworking fluid systems.

NOTE 8—Depending on the metalworking process, metal alloy being worked and formulation chemistry, metalworking fluid end-use dilution may range from 2 % (⁄v) to > 15 % (⁄v). If the formulation(s) being tested is (are) likely to be used at a variety of end-use strengths, they should be tested minimally at the high and low ends of the anticipated end-use concentration range. If the test objective is to evaluate microbicide performance in multiple metalworking fluid formulations, a 5 % (⁄v) end-use dilution is appropriate.

7.2 *Materials*:

7.2.1 *Inoculum*—The microbial inoculum may vary according to the user's requirements. It may be either characterized or uncharacterized. The challenge population should be acclimated to the metalworking fluid before being used in this method. Acclimatization shall be achieved by growing the challenge in the end-use dilution, negative-control metalworking fluid formulation.

7.2.1.1 Prepare an uncharacterized inoculum by adding 50 mL of spoiled metalworking fluid to 850 mL of freshly prepared end-use dilution, negative-control metalworking fluid. Aerate at 25 ± 2°C or at ambient room temperature for 24 h or until the microbial viable count reaches 10^9 CFU · mL^{-1}. Replace 800 mL of this fluid with freshly prepared portion of the negative-control fluid. Repeat the aeration and metalworking fluid replacement procedure for a minimum of three cycles before using the preparation as an inoculum.

7.2.1.2 Prepare a characterized inoculum by using standard microbiological techniques to isolate, maintain and identify specific microbes from spoiled metalworking fluid. Alternatively, cultures of specific interest may be obtained from a commercial type culture collection. Examples of commercial cultures that may be used are: *Aeromonas hydrophila* (ATCC 13444), *Candida albicans* (ATCC 752), *Desulfovibrio desulfuricans* (ATCC 7757), *Escherichia coli* (ATCC 8739), *Flavobacterium ferrugineum* (ATCC 13524), *Fusarium oxysporum* (ATCC 7601), *Klebsiella pneumonia* (ATCC 13883), *Mycobacterium immunogenum* (Rossmoore strain), *Proteus mirabilis* (ATCC 4675), *Pseudomonas aeruginosa* (ATCC 8689), *Pseudomonas oleovorans* (ATCC 8062) and *Saccharomyces cerevisiae* (ATCC 2338). Before using a characterized inoculum for metalworking fluid bioresistance testing, acclimate the inoculum following the procedure described for an uncharacterized inoculum (7.2.1.1). **Warning—** microbes recovered from metalworking fluids may be pathogenic. Do not pipet by mouth.

NOTE 9—As more bioresistant metalworking fluid formulations are developed, microbicide-free control fluid may not support microbial growth at normal end-use dilutions. If microbial viable counts do not increase by at least three logs within 48 h (for example, 10^4 CFU · mL^{-1} at time 0; 10^7 CFU · mL^{-1} at time 48), then the coolant should be augmented with 1 part in 10 of soybean-casein digest (7.1.3).

7.2.2 *Metal Chips*:

NOTE 10—Although ferrous chips are suitable for most tests, alternative materials may be substituted if the fluid is to be used with specific materials such as non-ferrous metals or ceramics. Chips should be prewashed with toluene (or similar non-polar solvent), then methanol (or similar polar solvent) and dried before use.

7.2.3 *Microbiological Media*—General retrieval media consistent with good microbiological practices are acceptable. Examples are:

7.2.3.1 Plate count agar (APHA Standard Methods 9215A.6a)

7.2.3.2 Soybean-casein digest medium (USP/NF Microbiological Test <71>).

7.2.3.3 Yeast extract-malt extract-glucose agar (APHA Standard Methods 9610B.2c)

7.2.3.4 Commercially available dip-slides prepared with bacterial recovery medium on one side and fungal recovery medium on the other side.

8. Procedures

8.1 Completed microcosm is shown in Fig. 1. To prepare jar lids, use 1 cm diameter metal punch to create two holes. Aeration tube will be placed into one of the holes. The second

[6] "Reagent Chemicals, American Chemical Society Specifications," American Chemical Society, Washington DC.