Designation: E2144 – 21

An American National Standard

Standard Practice for Personal Sampling and Analysis of Endotoxin in Metalworking Fluid Aerosols in Workplace Atmospheres[1]

This standard is issued under the fixed designation E2144; 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*

1.1 This practice covers quantitative methods for the personal sampling and determination of bacterial endotoxin concentrations in poly-disperse metalworking fluid aerosols in workplace atmospheres. Users should have fundamental knowledge of microbiological techniques and endotoxin testing.

1.2 Users of this practice may obtain personal or area exposure data of endotoxin in metalworking fluid aerosols, either on a short-term or full-shift basis in workplace atmospheres.

1.3 This practice gives an estimate of the endotoxin concentration of the sampled atmosphere.

1.4 This practice seeks to minimize interlaboratory variation but does not ensure uniformity of results.

1.5 It is anticipated that this practice will facilitate interlaboratory comparisons of airborne endotoxin data from metalworking fluid atmospheres, particularly metal removal fluid atmospheres, by providing a basis for endotoxin sampling, extraction, and analytical methods.

1.6 In 1997, the Occupational Safety and Health Administration (OSHA) empanelled a Standards Advisory Committee to make recommendations to the Administration regarding measures that the Administration could take to improve the health of workers exposed to metalworking fluids. A report to the Assistant Secretary of Labor for OSHA was submitted in July 1999. Subcommittee E34.50 believes that the user community would benefit significantly if a standard method was developed to give the community guidance on a methodology for the sampling and analysis of personal airborne endotoxin exposure assessments in facilities using water-miscible metal removal fluids, based on the LAL assay or other endotoxin detection technologies as they become available.

1.7 This practice does not attempt to set or imply limits for personal exposure to endotoxin in metalworking fluid aerosols in workplace environments.

1.8 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.9 *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.*

1.10 *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]

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

D2881 Classification for Metalworking Fluids and Related Materials

D4840 Guide for Sample Chain-of-Custody Procedures

D5337 Practice for Flow Rate Adjustment of Personal Sampling Pumps

E1370 Guide for Air Sampling Strategies for Worker and Workplace Protection

E1497 Practice for Selection and Safe Use of Water-Miscible and Straight Oil Metal Removal Fluids

E1542 Terminology Relating to Occupational Health and Safety

E2523 Terminology for Metalworking Fluids and Operations

[1] This practice is under the jurisdiction of ASTM Committee E34 on Occupational Health and Safety and is the direct responsibility of Subcommittee E34.50 on Health and Safety Standards for Metal Working Fluids.

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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.

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

2.2 *Government Standards:*[3]

29 CFR 1910.1000 Air Contaminants

29 CFR 1910.1450 Occupational Exposure to Hazardous Chemicals in Laboratories

2.3 *Other Documents:*

Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids[4]

NIOSH Manual of Analytical Methods (NMAM), 5th Edition[5]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms relating to this practice, refer to Terminologies D1356, E1542, and E2523.

3.1.2 *control standard endotoxin (CSE), n*—a purified preparation of endotoxin based on the USP Reference Standard Endotoxin (RSE); used in laboratories to prepare standard solutions.

3.1.3 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of Gram-negative bacteria.

3.1.3.1 *Discussion*—Though endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amoebocyte Lysate (LAL) test.

3.1.4 *endotoxin unit (EU), n*—a biological potency unit equivalent to the FDA Reference Standard Endotoxin (RSE).

3.1.4.1 *Discussion*—The current RSE (EC-6) is equivalent to 1 ng = 10 EU.

3.1.5 *Gram-negative bacteria, n*—prokaryotic cells that have a complex cell wall structure that stains characteristically when subjected to the differential Gram staining procedure.

3.1.6 *Limulus amebocyte lysate (LAL) assay, n*—a biological assay dependent on a series of cascading enzyme reactions that occur when Limulus blood cell (amebocyte) lysate combines with endotoxin.

3.1.7 *metal removal fluid (MRF), n*—any fluid in the subclass of metalworking fluids used to cut or otherwise take away material or piece of stock.

3.1.7.1 *Discussion*—Metal removal fluids include straight or neat oils (Classification D2881) not intended for further dilution with water, and water-miscible soluble oils, semisynthetics, and synthetics, which are intended to be diluted with water before use.

3.1.7.2 *Discussion*—Metal removal fluids become contaminated during use in the workplace with a variety of workplace substances including, but not limited to: abrasive particles, tramp oils, cleaners, dirt, metal fines and shavings, dissolved metal and hard water salts, bacteria, fungi, microbiological decay products, and waste. These contaminants can cause changes in the lubricity and cooling ability of the metal removal fluid as well as have the potential to adversely affect the health and welfare of employees in with the contaminated metal removal fluid.

3.1.8 *personal sampler, n*—a portable sampling instrument that is attached to a person to ascertain the concentration of specific constituents in the air in the person's breathing zone.

3.1.9 *pyrogen-free, adj*—material(s) devoid of measurable endotoxin activity.

3.1.10 *pyrogen-free water (PFW), n*—processed water that is devoid of measurable endotoxin activity.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *field blank, n*—filter/cassette unit prepared for sampling that is taken to the sampling site and handled in the same manner as the analytical filter/cassette unit, but that is not a part of the sampling process.

3.2.2 *onset time, n*—time required for a change of 200 mOD (optical density) units relative to the initial OD value.

4. Summary of Practice

4.1 A known volume of workplace air in a facility utilizing metalworking fluids is drawn through a sample filter cassette unit.

4.2 The sample filter is extracted into a pyrogen-free solution to quantitatively release endotoxin absorbed from collected metalworking fluid aerosol.

4.3 The extract solution is subjected to quantitative endotoxin analysis techniques. The measured endotoxin concentration is reported in terms of endotoxin potency units per unit volume of air sampled.

5. Significance and Use

5.1 Endotoxins in metalworking fluid aerosols present potential respiratory health hazards to workers who inhale them. Therefore, a consensus standard is needed to provide reliable data on workplace airborne endotoxin concentrations where metalworking fluids are used.

5.2 This practice for measuring airborne endotoxin concentrations in metalworking fluid atmospheres will help to foster a better understanding of endotoxin exposure-response relationships.

5.3 This practice facilitates comparisons of interlaboratory data from methods and field investigative studies.

6. Interferences

6.1 Airborne endotoxin measurements resulting from use of LAL reagents are subject to inhibition/enhancement effects from a variety of biomolecular species and physicochemical phenomena, such as pH, temperature, filter matrix effects, cationic concentrations, LAL-reactive materials (LRM), enzyme influences, and lysate composition variability and sensitivity (a function of different lysate processing methodologies).

[3] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

[4] Available from U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Pkwy., Cincinnati, OH 45226, https://www.cdc.gov/niosh/docs/98-102/default.html.

[5] Available from U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Pkwy., Cincinnati, OH 45226, https://www.cdc.gov/niosh/nmam/default.html.

7. Apparatus

7.1 *Sampling:*

7.1.1 *Sampling Unit*—An apparatus consisting of a personal sampling pump, a 37-mm glass fiber filter, a two-piece, closed-face plastic cassette, and flexible connecting tubing between the personal sampling pump and the attached cassette/filter unit.

7.1.1.1 *Pump*—A constant-flow personal sampling pump with an on-board battery power source and a flow rate of 2.0 L/min (± 5 %).

7.1.1.2 *Filter Cassette,* pyrogen-free, closed-faced, two-piece polystyrene filter holder with 4-mm inlet and outlet, with caps.

7.1.1.3 *Filter (Membrane),* pyrogen-free, glass fiber, 37-mm diameter with a cellulose support pad.

7.1.1.4 *Connective Tubing,* flexible, appropriate inside diameter.

7.1.1.5 *Soap Bubble Meter*—A primary standard used for sampler flow rate calibration.

NOTE 1—An alternative primary standard is acceptable.

7.2 *Extraction:*

7.2.1 *Sonicator Bath*—Ultrasonic/water bath apparatus with a minimum peak frequency of 40 kHz with cavitation adjustment and thermostat control.

7.2.2 *Vortex Mixer,* general purpose, with a minimum speed of 500 rpm.

8. Reagents and Materials

8.1 *Control Standard Endotoxin (CSE)*—Endotoxin preparations used for calibration standards shall be referenced to the Federal Drug Administration (FDA) Reference Standard Endotoxin (RSE), which is presently EC-6 RSE. Calibration standards data and corresponding regression data are expressed in EU.

8.2 *Endotoxin Detection Reagents,* utilized in accordance with manufacturer's directions.

9. Hazards

9.1 Aerosols of endotoxin preparations pose a potential respiratory hazard to susceptible laboratory personnel who are directly involved with an endotoxin assay.

9.2 Follow good laboratory procedures for worker protection and waste disposal, including 29 CFR 1910.1450.

9.3 Inhalation or dermal exposure to metalworking fluids may pose health problems for personnel involved in aerosol sampling. Provision of personal protective equipment (PPE) in the form of respirators or protective clothing, or both, may be indicated (see Practice E1497 and Criteria Document for a Recommended Standard: Occupational Exposure to Metalworking Fluids).

9.4 Review material safety data sheets (MSDS) for materials in use at a facility to identify potential hazards to determine appropriate personal protective equipment (see 29 CFR 1910.1000).

10. Pump Calibration and Standardization

10.1 Calibrate the airflow rate of the sampling pump onsite before each sampling period. The final flow rate shall be determined after sample collection is complete. Samples should be voided if flow rate changes significantly during the sample period.

10.2 Maintenance and repairs of the sampling and analytical equipment should be performed according to the recommendations of the manufacturer and should be documented in maintenance records.

10.3 Airflow rate procedures shall be performed in accordance with Practice D5337 or NMAM Calibration of Personal Sampling Pumps.

11. Sampling

11.1 Plan air-sampling strategies for metalworking fluid aerosol endotoxin analysis using Guide E1370.

11.2 *Filter/Cassette Unit Setup:*

11.2.1 Aseptically transfer a glass fiber filter and support pad to a closed-face, three-piece polystyrene cassette, and then assemble the cassette and seal the perimeter seams with PTFE tape.

11.2.2 Affix a label to the cassette with a unique sample identifier. The sample shall link to the following information: date of sample, location of work operation, sample volume, investigator/worker code, and any other pertinent information.

11.2.3 Store and transport at least one unused (blank) filter/cassette unit from the same lot as described in 11.2.1.

11.3 *Personal Sampler:*

11.3.1 Uncap filter/cassette unit and attach to calibrated personal sampler pump with flexible tubing.

11.3.2 Set the sampling rate of the personal sampling pump to 2.0 L/min (± 5 %) and record room temperature.

11.3.3 Attach the filter cassette in the breathing zone of the individual being tested, activate the personal sampling pump, and record the starting time. Total sampling duration shall be determined on the basis of partial or total workday shifts or discrete work activities.

11.3.4 Deactivate the sampling pump after the sampling period and record the stopping time, temperature, and any unusual conditions in the sampling area that could bias the outcome of the sampling procedure.

11.3.5 Remove the used filter/cassette unit and cap at each end.

12. Storage and Shipment

12.1 Store the used labeled filter/cassette unit(s) in a suitable container at 4 ± 2 °C until shipped or analyzed. Do not freeze sample at any time.

12.2 Samples should be shipped via overnight delivery. If the shipment will take more than 24 h to arrive at its destination, ship the samples in a suitable container at 4 ± 2 °C.

12.3 Maintain procedures for sample custody in accordance with accepted chain-of-custody procedures (see Guide D4840).