METRIC
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DETAIL PERFORMANCE SPECIFICATION

TEXTILES: VECTOR PROTECTION TREATMENT OF

This document is approved for use by all Departments and Agencies of the Department of Defense (DoD).

1. SCOPE

1.1 Scope. This specification covers the treatment of textiles for vector protection performance on fabrics and garments.

2. APPLICABLE DOCUMENTS

2.1 General. The documents listed in this section are specified in sections 3 and 4 of this specification. This section does not include documents cited in other sections of this specification or recommended for additional information or as examples. While every effort has been made to ensure the completeness of this list, document users are cautioned that they must meet all specified requirements of documents cited in sections 3 and 4 of this specification, whether or not they are listed.

2.2 Government documents.

2.2.1 Specifications, standards, and handbooks.

DEPARTMENT OF DEFENSE SPECIFICATIONS

MIL-DTL-32075 - Label: For Clothing, Equipage, and Tentage (General Use)

(Copies of these documents are available online at https://quicksearch.dla.mil.)

Comments, suggestions, or questions on this document should be addressed: Attn: DLA Troop Support, 700 Robbins Street, Philadelphia, PA 19111-5096. Since contact information can change, verify the currency of the address information using Acquisition Streamlining and Standardization Information System (ASSIST) online database https://assist.dla.mil.

AMSC N/A FSC 8305

2.2.2 Other Government documents, drawings, and publications. The following other Government documents form a part of this document to the extent specified herein. Unless otherwise specified, the issues are those cited in the solicitation or contract.

ENVIRONMENTAL PROTECTION AGENCY (EPA)

EPA Product Performance Test Guidelines

OPPTS 810.370 Insect Repellents For Human Skin and Outdoor Premises

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

FIFRA as amended by the Food Quality Protection Act of 1996 and the Pesticide Registration Improvement Act of 2003

Title 40, Chapter I, Subchapter E in the Code of Federal Regulations (CFR) governing the use of pesitcides

(Copies of these documents are available online at https://www.epa.gov/pesticideregistration/pesticide-registration-manual-introduction).

2.3 Non-Government publications. The following documents form a part of this document to the extent specified herein. Unless otherwise specified, the issues of these documents are those cited in the solicitation or contract.

AMERICAN ASSOCIATION OF TEXTILE CHEMISTS AND COLORISTS (AATCC)

AATCC TM135 - Test Method for Dimensional Changes of Fabrics after Home Laundering

(Copies of these documents are available online at https://www.aatcc.org.)

AMERICAN SOCIETY FOR QUALITY (ASQ)

ASQ/ANSI Z1.4 - Sampling Procedures and Tables for Inspection by Attributes

(Copies of this document are available online at https://asq.org.)

INFORMA HEALTHCARE

Repeat Insult Patch Test – Modified Draize Procedure – Principles and Methods of Toxicology, A Wallace Hayes (editor).

(Copies of this document are available online at https://www.crcpress.com.)

2.4 Order of precedence. Unless otherwise noted herein or in the contract, in the event of a conflict between the text of this document and the references cited herein, the text of this document takes precedence. Nothing Unless a specific exemption has been obtained, nothing in this document, supersedes applicable laws and regulations.

3. REQUIREMENTS

3.1 First article. When specified (see 6.2), a sample shall be subjected to first article inspection in accordance with 4.2.

3.2 Conformance inspection. When specified, a sample shall be subjected to inspection in accordance with 4.3.

3.3 Recycled, recovered, environmentally preferable, or biobased materials. Recycled, recovered, environmentally preferable, or biobased materials should be used to the maximum extent possible, provided that the material meets or exceeds the operational and maintenance requirements, and promotes economically advantageous life cycle costs.

3.4 Figures. Figures 1 through 3 are furnished for informational purposes only. To the extent of any inconsistencies between the written document and the figure, the written document shall govern.

3.5 Insect protection. The fabric/garment shall be treated to provide insect protection as specified in 3.5.1 and 3.5.2.

3.5.1 Insect repellent treatment.

3.5.1.1 Regulatory compliance. The insect repellent treatment process and treated items shall comply with Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as amended (see 2.2.2). The treatment concentration shall comply with EPA Toxicity Category IV. The treated item shall be labeled in accordance with registrants EPA registration and 3.6.1, 3.6.2.

3.5.1.2 Insect repellent treatment application. The treatment shall be uniformly applied across the fabric/garment and strictly controlled to ensure that the Active Ingredient (AI) concentration level meets the requirements specified in Table I or as specified in the contract or purchase order (see 6.2) when tested in accordance with 4.7.1. The treatment shall be durable to laundering (see 3.5.2) and not degrade any performance characteristics of the fabric or garment or present any latent defects to the fabric or garment (see 6.5).

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TABLE I. Insect repellent AI treatment level requirements.

Repellent active ingredient (AI) 1/	Condition	AI level (w/w)% 2/
Etofenprox	Initial	0.90% ± 10%
Permethrin	Initial	0.52% ± 10%
Other Active Ingredients (A.I.) 3/	Initial	See 6.3

1/ Only one (1) AI shall be selected for use.
2/ Individual dose rate requirements are material specific and shall be as specified in the contract or purchase order.
3/ Additional AIs to be added as approved.

3.5.2 Percent (%) mosquito bite protection. The insect repellent treated items shall provide bite protection as specified in Table II, or as specified in the contract or purchase order (see 6.2) when assessed by the bite protection testing specified in 4.7.2. The treatment level shall provide the percent (%) mosquito bite protection specified in Table II. The treatment shall be durable to repeated laundering to maintain the efficacy requirement as specified in Table II. The contractor shall notify and request Government approval any time there is a change in the treatment formulation or processing conditions.

TABLE II. Efficacy requirements.

		Efficacy Requirements 1/		
		Initial (1 wash)	20 washes	50 washes
Woven	Objective	95%	95%	90%
	Minimum	85%	80%	75%
Knit	Objective	95%	95%	90%
	Minimum	75%	70%	50%

1/ Individual efficacy requirements are material specific and shall be as specified in the fabric/garment contract or purchase order.

3.6 Insect repellent labeling. Labels for insect repellent treated garments shall be in accordance with the following. Labels for treated fabric shall be in accordance with 5.2.

3.6.1 Insect protection label. The insect protection label shall include both permanent insect protection and brand labeling information and shall comply with approved EPA registration (see 6.4). The insect protection label shall be the approved EPA label and include the information as specified below (see example on Figure 1). Label shall comprise of ONLY the active ingredient that was cited in the contract or purchase order.

INSECT REPELLENT APPAREL Refer to hangtag for more information EPA REG. NO.: EPA EST. NO.:
- Do Not Dry Clean - Dry Cleaning removes active ingredient - Wash separate from other clothing - In military field operations, garment may be washed with other garments. - Do Not Re-treat - Dispose of garment in trash in accordance with Service regulations Repels mosquitoes Repellency remains effective for 50 washings **ACTIVE INGREDIENT (W/W)%** [Active Ingredient Name]…….……...AI % OTHER INGREDIENTS: (Fabric/Garment)…Other Ingredients % TOTAL……..100.00% It is a violation of Federal Law to use this product in a manner inconsistent with its labeling **DO NOT REMOVE THIS LABEL**

FIGURE 1. Insect repellent label (example).

3.6.2 Hang tag, insect protection. Each treated garment item shall be labeled with a individual paper tag attached to the fabric/garment conforming to Type VIII, Class 15 of MIL-DTL-32075. The paper tag shall be Swift tacked to the item. The color for the hang tag shall be white. The tag shall provide additional insect protection information in accordance with and as required by EPA registration and labeling. The hang tag shall be the approved EPA hang tag (see example on Figure 2). Hang tag shall comprise of ONLY the active ingredient that was cited in the contract or purchase order.

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INSECT REPELLENT APPAREL
EPA REG. NO.:
EPA EST. NO.:

- Do Not Dry Clean
- Dry Cleaning removes active ingredient
- Wash separate from other clothing
- In military field operations, garment may be washed with other garments.
- Do Not Re-treat
- Dispose of garment in trash in accordance with Service regulations
- For protection of exposed skin, use in conjunction with a repellent registered for direct application to the skin.

Repels mosquitoes
Repellency remains effective for 50 washings

ACTIVE INGREDIENT **(W/W)%**
[Active Ingredient Name]……..……...AI %
OTHER INGREDIENTS: (Fabric/Garment)…Other Ingredients %
TOTAL……. 100.00%

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

Retain hangtag for future reference on proper handling of this garment.

THIS TAG NOT TO BE REMOVED EXCEPT BY CUSTOMER

FIGURE 2. Insect repellent hang tag (example).

3.7 Toxicity. The finished fabric/garment shall not present a health hazard and shall show compatibility with prolonged, direct skin contact when tested as specified in 4.8. Chemicals recognized by the Environmental Protection Agency (EPA) as human carcinogens shall not be used.

4. VERIFICATION

4.1 Classification of inspections. The inspection requirements specified herein are classified as follows:

a. First article inspection (see 4.2)
b. Conformance inspection (see 4.3)

4.2 First article inspection. First article, submitted in accordance with 3.1 shall be tested to ensure that vector protection requirements (Table I and II) are being met when tested in accordance with 4.6, 4.7 and Table IV. The sample unit for treated fabric shall be three (3) random 1 yard-full width swatches of the finished fabric from a minimum lot size of 2,500 yards not to exceed 5,000 yards. The sample size for treated garments shall be three (3) full garments in size large or bigger from a minimum lot size of 1,500 garments not to exceed 2,500 garments. First Article Testing shall be unacceptable if one (1) or more sample units fails to meet AI content or if the lot average for %Bite Protection Testing fails to meet any requirement specified.

4.3 Conformance inspection. Conformance inspection shall include the visual examination of 4.5 (Table III) and the tests of 4.6 and 4.7 as applicable. Sampling for visual inspection shall be performed in accordance with ASQ/ANSI Z1.4 and with acceptance quality limits (AQLs) as specified in the contract and/or order, except where otherwise indicated (see 6.2). Sampling for end item testing shall be as specified in 4.6.

4.4 Inspection conditions. Unless otherwise specified in this specification or applicable procurement documents (see 6.2), all inspections shall be performed in accordance with this specification and all the requirements of referenced documents.

4.5 Visual examination. The treated fabric/garment shall be examined for the defects listed in Table III.

TABLE III. Defects.

Examination	Defect	Classification	
		Major	Minor
Labels, hangtags	- Any EPA label or tag having incorrect printed content		201
Packaging	- Any item not packaged in accordance with contract or purchase order		202
	- Any package (box,roll,pallet,etc) not labeled in accordance with EPA requirements as stated in Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)	101	

4.6 End item testing. The fabric/garment shall be tested for the characteristics listed in Table IV. The methods of testing as specified wherever applicable and as listed in Table IV shall be followed. All test reports shall contain the individual values utilized in expressing the final results. The sample unit for treated fabric shall be four (4) random full width swatches of 1/4 yard minimum each, lot size shall not exceed 12,500 yards. The sample unit for treated garments shall be four (4) full garments, lot size shall not exceed 5,000 garments. The lot shall be unacceptable if one (1) or more sample units fails to meet AI content.

TABLE IV. Fabric/garment testing.

Characteristic	Requirement paragraph	Test method
Insect repellent content 1/ Initial	Table I	4.7.1
Mosquito bite protection 1/ Initial After 20 launderings After 50 launderings	Table II Table II Table II	4.7.2 AATCC TM135, 3, V, Aiii and 4.7.2 2/ AATCC TM135, 3, V, Aiii and 4.7.2 3/
Toxicity	3.7	4.8

1/ If fabric is pre-treated, this testing can be done prior to cut and sew.
2/ Laundering cycles 5, 10, 15, 19 and 20 shall be performed without adding any detergent to minimize detergent accumulation in specimen.
3/ Laundering cycles 5, 10, 15, 19, 20, 25, 30, 35, 40, 45, 49 and 50 shall be performed without any detergent.

4.7 Methods of testing and inspection.

4.7.1 Insect repellent active ingredient content analysis. The insect repellent content of treated fabric or garments shall be determined by solvent extraction followed by analysis using a Gas Chromoatograph-Mass Spectrometry (GC/MS). Testing for permethrin/etofenprox shall be conducted according to the following test method.

Evaluation of insect repellent treated fabric materials: extraction and analysis by GC/MS

NOTE: The conditions described in this method are optimum for the accelerated solvent extractor and the gas chromatograph employed and may vary based on the gas chromatograph used. The carrier gas flow rate shall be adjusted so the elution of the first insect repellent isomer is greater than five (5) minutes. Alternate methods of extraction and analysis are subject to government approval and laboratory cross correlation prior to implementation.

A. Apparatus.

A.1 Analytical balance. 0.0001g sensitivity, Mettler Toledo, or equal

A.2 Analytical balance. 0.000001g sensitivity, Mettler Toledo, or equal

A.3 Glassware.

A.3.1 10-100mL volumetric flasks
A.3.2 Funnel
A.3.3 Pipettes

A.4 Automatic die cutter. Freeman Atom, or equal

A.4.1 Cutting die. 7.62 cm (3-inch) diameter circular steel die cutter

A.5 Extraction apparatus.

A.5.1 Accelerated Solvent Extractor (ASE). Thermo Fisher or equal

A.5.1.1 Liquid Nitrogen Cylinder or Compressed Nitrogen Cylinder to Deliver High Pressure Gas, 230psi
A.5.1.2 Complete Extraction Cells
A.5.1.3 Cellulose filters, to fit extraction cells if needed
A.5.1.4 60mL Amber Glass Collection Vials
A.5.1.5 Solvent Resistant Teflon-Silicone Coated Septa
A.5.1.6 3mm-4mm borosilicate glass beads

A.5.2 Soxhlet.

A.5.2.1 Electric heater with variable control
A.5.2.2 Heat resistant glass flask when using Soxhlet extractor. The flask shall be a 250mL, flat or round bottom, and single neck.
A.5.2.3 Extractor condenser
A.5.2.4 Boiling condenser
A.5.2.5 Cellulose extraction thimbles

A.6 Agilent 6890N (G1530N) Series Gas Chromatograph (GC). Gas Chromatograph equipped with ChemStation software, or equal

A.6.1 Carrier Gas Cylinder, Appropriate Regulator Set at 80psi
A.6.2 Capillary Column, 5% Phenyl Methyl Siloxane/30.0m x 250µm x 0.25µm nominal, 325ºC Max, or equal.
A.6.3 Split Inlet Liner, Packed with Silanized Glass Wool/5mm
A.6.4 Injector Microliter Syringe, Capable of Delivering 1µL
A.6.5 Amber Injection Vials
A.6.6 Rinse Vials

A.7 Agilent series 5973N (G2579A) Mass Spectrometer(MS) or equal.

A.7.1 Performance Turbo Pump MSD (EI Mode), or equal

A.8 Ultrasonic cleaner. Cole-Palmer, or equal

A.9 High temperature convection oven. 500ºC Max

A.10 Refrigerator storage. 4ºC

B. Reagents.

B.1a Permethrin analytical standard (for items treated with permethrin). Permethrin standard shall be ≥ 97%, purity/specified technical, or equal, mixture of Cis/Trans Isomers.

B.1b Etofenprox analytical standard (for items treated with Etofenprox). Etofenprox standard shall be ≥ 97% purity/specified technical, or equal.

B.2 Solvent mixture. Solvent mixture shall be 80% Acetonitrile/Analytical Grade and 20% Methanol/Analytical Grade

B.3 High purity helium carrier gas. Carrier gas shall be ≥ 99.999%

B.4 Cleaning solutions. Cleaning solutions shall be as follows:

B.4.1 Micro-90 Ultra Cleaning Solution, or equal
B.4.2 Reversed Osmosis Water, 98% Rejection Rate

C. Calibration of apparatus.

C.1 Analytical balance.

C.1.1 Pre-Weighing procedures. Prior to weighing, initiate the internal weight calibration function or use an external certified weight set to verify that the balance is operating properly.

C.1.2 Manufacturer calibrations. Obtain manufacturer certifications within 12 months prior to taking measurement.

C.2 Gas Chromatograph equipped with Mass Spectrometer (see A.6, A.7).

C.2.1 Perform the manufacturer's recommended calibration procedures prior to analyses.

C.2.2 Before samples or required blanks can be analyzed, the instrument must meet the initial calibration acceptance criteria (see G).

C.3 Cleaning techniques. Establish cleaning techniques to ensure that no insect repellent carries over from experiment to experiment. The techniques listed below have been determined to be suitable:

C.3.1 Evaporate excess solvent from extraction glassware and wash using conventional methods (see B.4).

C.3.2 Bake off residual organic substances from glassware in high temperature convection oven, 500°C, for three (3) to six (6) hours (see A.9).

C.3.3 Sonicate ASE Cells using the same solvent mixture used for the extraction process (see A.8).

D. Sampling and test specimens.

D.1 Sample size. The sample size to be tested shall be selected in accordance with ASQ/ANSI Z1.4, unless otherwise noted in the contract or purchase order.

D.2 Test specimens.

D.2.1 From each sample being evaluated, use a 7.62-cm cutting die to provide a 7.62-cm (3-inch) diameter specimen. Three specimen shall be cut and tested for each test condition (i.e. each required laundering increment). Cut specimen from single ply areas so that no two (2) specimen shall contain the same warp/filling or course/wale yarns (for example, for the coat areas-front left, front right, back, right sleeve, left sleeve; and for the trouser areas-front left leg, back left leg, right front leg, back front leg, and front left or right fly

D.2.2 Weigh each specimen to the nearest milligram (see A.1).

E. Standard preparation. (Follow E.a for Permethrin and E.b for Etofenprox)

E.1.a Prepare six (6) concentrations of Permethrin standards which are nominally 20, 50, 75, 100, 150, and 200 ng/µL, (1 ng/µL is equal to 1 part per million [ppm]).

E.1.1.a Using the balance specified in A.2, weigh 10 (± 1 mg) of Permethrin analytical standard (B.1a) crystals and place into a 50 mL volumetric flask and fill to mark with 80% acetonitrile/20% methanol solvent to obtain the standard of 200 ng/µL. Make all appropriate dilutions from this flask using appropriate measurments based on volumentric flask size selected to obtain the additional standards.

E.1.2.a Calculate the actual concentrations of the standards based on the weight of the permethrin.

E.1.b Prepare six concentrations of Etofenprox standards which are nominally 40, 100, 150, 200, 300, and 400ng/µL, (1 ng/µL is equal to 1 part per million [ppm]).

E.1.1.b Using the balance specified in A.2, weigh 20 (± 1 mg) of Etofenprox analyitical standard (B.1b) crystals and place into a 50mL volumetric flask and fill to mark with 80% acetonitrile/20% methanol solvent to obtain the standard of 400ng/µL. Make all appropriate dilutions from this flask using appropriate measurments based on volumentric flask size selected to obtain the additional standards.

E.1.2.b Calculate the actual concentrations of the standards based on the weight of the etofenprox.

F. Extraction procedure (see A.5). Specimen shall undergo solvent extraction using one of the below extraction procedures.

F.1 ASE.

F.1.1 Preparing specimens. Fold and roll each specimen and place into an ASE cell. Fill the void with glass beads to conserve solvent. Place all cells onto ASE cell tray.

F.1.2 Quality control. Extract a specimen blank for every run to detect if any carryover of insect repellent is significant.

F.1.3 ASE procedures.

F.1.3.1 Parameters.

Cell Size	Machine type dependent
Collection vials	60 mL, light blocking/amber
Solvent	80% Acetonitrile, 20% Methanol

Approximate Gas Pressures:

System	50 psi
System Solvent	10 psi
Oven Compression	130 psi

Extraction Settings:

Preheat	0 min
Heat	5 min @ 100ºC
Static w/Solvent	10 min @ 1500 psi
Flush Volume	90%
Purge	90 sec
Cycles	2

F.1.3.2 Preparation for analyses. Dilute or concentrate each vial to 40 mL and prepare a 1 mL aliquot from every specimen extraction into an amber injection vial for GC/MS analysis. Recovery of active ingredient must be 95% or greater (see F.4).

F.2 Soxhlet. Place each specimen into the cellulose Soxhlet extraction thimble. Add 160 mL of the acetonitrile/methanol mixture and boiling chips into a 250 mL flask. Assemble the Soxhlet apparatus and extract the treated specimens for six (6) hours or until an extraction recovery of 95% or greater has been achieved (see F.4). Concentrate the extract by rotoevaporation, or equal, at 35ºC to a final volume of 40 mL.

F.3 Storage. After the AI is extracted from the specimen, store the extract in light blocking amber vials in refrigerator until ready to inject (see A.10). Specimen extractions shall be stored in a refrigerator for no longer than three (3) months. When ready to analyze, bring the vials to room temperature in the area of evaluation before injection into GC/MS.

F.4 Extraction efficiency.

F.4.1 Select three (3) random specimen from any treated fabric sample and perform three (3) consecutive extractions.

F.4.2 Quantify the level of insect repellent recovered from each specimen for each consecutive extraction, through GC/MS analysis.

F.4.3 Verify that the percent recovery of insect repellent for any specimen size and composition, is 95% or greater by comparing the recovery level from the first extraction, to that of subsequent extractions. Combine the insect repellent concentrations obtained from each of the three (3) extractions, if the initial extraction yields insect repellent levels 95% or greater than the total percent of insect repellent extracted three (3) sequential times, then the extraction efficiency is 95% or greater.

NOTE. To ensure that the extraction efficiency is being accurately calculated, the insect repellent levels in the second extraction should be minimal, and the level by the third extraction should be trace or zero.

NOTE. Initial verification of extraction efficiency of this test method must be performed. Once an extraction efficiency of 95% or greater is established, no further demonstration of the extraction efficiency is needed.

G. Analytical procedure.

G.1 Quality control. Laboratory blanks that contain no analyte are used to ensure specimens are free of contaminants or to ensure there is no cross contamination during a run. Inject a blank containing 80% acetonitrile/20% methanol before every set of standards and before and after every ten (10) specimens. If any blank, after multiplying concentration by five (5), is greater than any specimen result, the specimen data points are invalid and a system check must be run to identify the source of the carryover. After system maintenance has been performed, repeat injections of the standards for the calibration curve, new blanks, and new aliquots of the specimens affected by the previous carryover.

G.2 Standard injection.

G.2.1 All six (6) standards will be injected into the GC inlet at the beginning and at the end of each series of specimen to "bracket" the specimen injections. Check linearity of the standards for each set of injections by plotting the responses (area counts) on the x-axis vs. the calculated standard concentrations on the y-axis. A 3rd order polynomial regression line with R-squared value of 0.99 or greater is acceptable. If the R-squared value is less than 0.99, a new set of standards must be prepared and injected again until an R-squared value of 0.99 is obtained. Derive the equation of the 3rd order polynomial for sample calculations.

G.3 Specimen injection. Run specimen injections in duplicate. Sample extracts, standards, and blanks must be analyzed within an analytical sequence such as listed below:

Initial calibration (Standards)
Instrument blank at the end of the initial calibration
Specimen Series 1 (extracts 1-10, 1st quantitation)
Instrument blank
Standard Series 1
Instrument blank
Standard Series 2
Instrument blank
Subsequent specimen series, (ex. 11-20, including blanks, and standard series)
Final calibration (Standards)

NOTE. After the initial calibration, the analytical sequence may continue as long as acceptable instrument blanks and the standards are analyzed at the required frequency. If any specimen count does not fall on the standard calibration curve, the evaluator may dilute that specimen by 1:10 and re-run; calculations of the insect repellent level must be adjusted using the factor of 10.

G.4 GC/MS parameters (see A.6).

G.4.1 Injection procedures.

G.4.1.1 Place all injection vials into auto sampler tray. To avoid vapor pressure differences, all vials must be at room temperature and contain identical volumes.

G.4.1.2 Inject 1 µL into the GC/MS. Use high purity helium carrier gas (see B.3) and appropriate column.

G.4.1.3 Ensure that rinse vials in the injector port contain 80% acetonitrile/20% methanol above the minimum solvent line.

G.4.2 Instrument settings. The following parameters will be used in the analysis:

Oven Temperature	250 ºC
Injector Temperature	275 ºC
Detector Temperature	280 ºC
Injection volume	1 µL
Carrier Gas Flow Rate	1.3 mL/min
GC Run Time	10 min
Split Ratio	3:1

MS Single Ion Monitoring:	
Scan Parameters	EM Voltage Gain Factor of 1
Real Time Plot	10 min
Resolution	Low
Solvent Delay	4 min
Start Time	4 min, 4.26 Cycles/sec
Ions Monitored	183 (quantitation) for Permethrin 163 (quantitation) for Etofenprox 163 (confirmatory) for Permethrin 135 (confirmatory) for Etofenprox

G.4.3 Evaluation procedures.

G.4.3.1a. Quantify the Permethrin content detected by the mass spectrometer by extracting ion chromatograms 183 (quantitation ion) and 163 (confirmatory ion).

G.4.3.1b Quantify the Etofenprox content detected by the mass spectrometer by extracting ion chromatograms 163 (quantitation ion) and 135 (confirmatory ion).

G.4.3.2 Integrate peaks manually from baseline to baseline using the software, or generation of report.

H. Calculations.

H.1 Concentration. The insect repellent concentration will be calculated from the area counts of the chromatographic curve and expressed in terms of mass per surface area (mg/cm^2), with the option of expressing in terms of weight per weight percent of specimen (W/W)%:

H.1.1 Concentration calculation. The concentration of insect repellent in milligrams per square centimeter shall be calculated as follows:

Concentration (mg/cm^2) =

$40\ mL \times (ax^3 + bx^2 + cx + d) \times (1{,}000\ \mu L/1\ mL) \times 1\ mg/1{,}000{,}000\ ng) \times (1/45.6037\ cm^2)$

Where:

40 mL = Final Volume
a, b, c and d = numbers derived from 3rd degree polynomial equation from standard series following specimen series
x = area count of the specimen curve
45.6037 cm^2 = area of specimen

H.1.2 Conversion to weight percent content (W/W)%.

Concentration (W/W)% = [Concentration (mg/cm^2) multiplied by surface area (cm^2) divided by weight of specimen (mg)] multiplied by 100.

I. Report. Report the following information:

I.1 Individual specimen concentration. The insect repellent concentrations in milligrams per square centimeter to the nearest 0.001 mg/cm^2 of the three (3) individual specimens per sample unit for all units tested. A specimen is considered passing when the insect repellent concentration falls within minimum to maximum range of the insect repellent dose rate as specified in the contract.

I.2 Sample unit concentration. The insect repellent concentration of each sample unit shall be reported as the average of the (3) individual specimens from that sample unit. A sample unit is considered passing when the average of the (3) individual specimens from that sample unit falls within minimum to maximum range of the insect repellent dose rate as specified in the contract and no more than (1) specimen from the sample unit fails. The failing specimen cannot fall more than 0.040 mg/cm^2 outside of the specified minimum or maximum dose rate.

I.2.1 Retest. A single retest shall be allowed; when a sample fails, a complete set of specimens shall be pulled from an additional sample unit and retested. The retest shall then be used to rate pass or fail. All tested samples shall be reported.

I.3 Number of sample units tested. Four (4) sample units shall be tested. All sample units tested shall be reported. Five (5) is the maximum allowable sample units to be tested as part of a lot.

I.3.1 Number of specimen tested. Three (3) specimens per sample unit shall be tested. Additional specimen should not be tested.

4.7.2 Percent (%) mosquito biting protection assay. Percent (%) mosquito bite protection shall be measured on a finished insect repellent treated item, under three (3) test conditions and using a control specimen (non treated item of the same fabric) against the two (2) selected species specified in 4.7.2.3. The three (3) test conditions shall be one (1) unlaundered, two (2) after 20 launderings, and three (3) after 50 launderings, from treated items in the same lot. Corresponding active ingredient content for each of these conditions will be measured as specified in 4.7.1 to correlate biological toxicity with the particular garment treatment used to meet requirements specified in 3.5.2.

4.7.2.1 Number of determinations. Three (3) determinations will be run for each of the two (2) mosquito species (see 4.7.2.3.3). Each determination for each mosquito species will be conducted with three (3) volunteers using three (3) different fabric conditions; after 1 laundering, after 20 launderings and after 50 launderings and compared to non-treated control. A single untreated unlaundered control sleeve can be used for the three (3) determinations for each volunteer provided that the control is run against the same mosquito population, on the same day

of the specimens being tested, and tested on an arm that has not been used for testing a treated sleeve (see 4.7.2.3.6). The total number of specimens for the three (3) determinations is outlined below. It is estimated that one (1) coat yields three (3) specimens and one (1) trouser yields three (3) specimens consisting of largely a single ply fabric area (see 4.7.2.2), (See 6.5.)

Number of Insect tests **X**	Number of Determinations **X**	Number of Fabric conditions =	Total Specimens per sample
Mosquitoes 1/	3 x	3 x =	9 2/
Control 2/	1 x	1 x =	1 2/

1/ One (1) set of treated specimens will be used twice to test each mosquito species
2/ Total garments estimated, required to conduct three (3) determinations are; Three (3) treated coats and one (1) untreated coat

4.7.2.2 Specimen size. Specimens will be cut to the shape and dimensions illustrated on Figure 3. Specimens shall be cut from single fabric ply areas. To minimize the number of garments needed for each determination, multiple ply areas such as seam areas or hems may occur limitedly in the perimeter areas provided multiple plies of fabric in these areas shall-not create a gap between subject's arm and fabric (see 4.7.2.3.5). Specimens will be cut with gloved hand and placed in a plastic bag and the glove disposed of to avoid residual contamination of controls. When failure point is being quantified, the laundered samples may be used to accomplish the additional launderings needed.

4.7.2.3 Procedure. The procedure for the biting protection assay is derived from the "EPA Product Performance Test Guidelines, OPPTS 810.3700, Insect Repellents For Human Skin and Outdoor Premises, December 1999 (see 2.2.2), and is described in part below, noting any exceptions to this procedure.

4.7.2.3.1 Applicable protocol. Section 3 of OPPTS 810.3700 addresses treated fabric material and section (3)(iii) specifies that laboratory studies are to be conducted as described in (d)(1) of the OPPTS 810.3700 guideline.

4.7.2.3.2 Fastening test specimen. Section (3) (iii) recommends "fastening a strip of the impregnated material to the test subject's forearm". This will be accomplished by utilizing specimen size and shape specified in 4.7.2.2 (see Figure 3) and by ensruing that it covers the entire forearm of the test subject without gaps for mosquito access. With the arm in the pronated position, the fastening seam that closes the specimen on the volunteer's arm shall be located on the top of the forearm. Attachment of the treated specimen will be done with gloved hands, which will be disposed of prior to attaching the control to the alternate arm.

4.7.2.3.3 Test mosquitoes. OPPTS 810.3700 section (d) (1) addresses laboratory tests conducted with mosquitoes and stable flies but this test shall only utilize two species of mosquito. The results of this evaluation for the mosquitoes is a contractual requirement. Insect genus, species and subspecies, colony origin and approximate age shall be used as specified below and in 4.7.2.3.3.1.

Mosquitoes:
Aedes (Stegomyia) aegypti
Anopheles albimanus

4.7.2.3.3.1 Mosquito characteristics. Mosquito ages employed for these tests shall be 5 to 11 days old after emergence from the pupal stage. Mosquitoes shall be laboratory-reared and disease free, and have been kept in stock cages containing both males and females. The mosquitoes will be maintained on 10% sugar water and will have not been provided a blood meal. Methods should be used to preselect females for the studies. Either a hand draw box or suitable aspirating device will be used to collect host-seeking mosquitoes to generate the required cage density
(see 4.7.2.3.3.3).

4.7.2.3.3.2 Mosquito rearing. Mosquitoes for these tests shall be reared under optimal conditions for larvae, as described in OPPTS 810.3700, section (d)(1)(iii).

4.7.2.3.3.3 Cage conditions. A cage density of 200 (± 25) female mosquitoes per cage is required to meet the biting pressure density of at least one (1) female mosquito per 300 cm^3 cage volume. (Cages shall be 60,000 (± 6,000) cm^3, with a sleeved opening for the arm of the volunteer to be inserted.) Cages shall be constructed of a lightweight clear plastic on three (3) sides, or an aluminum bottom panel with light weight clear plastic on two (2) sides. The top of the cage and side opposite the cloth sleeve should consist of screen rather than plastic. Tests shall be conducted with fluorescent lights on and under room conditions (22-27°C, and 30-80% RH). Tests should not be conducted if the temperature or humidity is outside of the specified range.

4.7.2.3.4 Subjects. A minimum of three (3) test volunteers shall be used in each test for each mosquito species at each test facility. The same three (3) subjects can be used to evaluate different insect species done at the same facility. Due to the replication, the number of volunteers is now decreased from the five (5) or more recommended in OPPTS 810.3700, section (c)(3)(i). Collection of data from both females and males are preferred for the testing. Cosmetics and alcohol shall be avoided 12 hours prior to and during the test. Volunteers shall read and sign the appropriate Institutional Review Board (IRB)-Human Use protocol forms, required for their consent, prior to being used in the test. IRB protocols shall be approved through the appropriate agencies' IRB mechanisms.

4.7.2.3.5 Volunteer's test area. The test area shall consist of the region from the wrist to approximately 1/2-inch before the elbow. Fabric material shall be secured around the forearm to eliminate gaps between the arm and material and with the fastened seam positioned on the top of the forearm as specified in 4.7.2.3.2. The ends of the garment, near the wrist and elbow shall be sealed with protective tape of adequate thickness to prevent mosquitoes from biting through the tape. The hand shall be gloved with a glove of appropriate thickness to prevent biting through to the hand.

4.7.2.3.6 Controls. For each set of specimens, a control shall be conducted. The control shall consist of the same fabric type as the specimen, will not be laundered and will not contain the insect protection treatment. It will be identical in size to the test swatch (see 4.7.2.2). Controls will be cut in a clean area and stored in separate plastic bags to avoid residual repellent treatment contamination. The controls will be tested on the arm opposite the treated specimen, or on the same arm used for experiments provided that the control is tested prior to testing treated specimens.

4.7.2.3.7 Biting exposure. Arms covered by the controls and treated specimens shall be exposed to a cage of mosquitoes for 15 minutes. The order of testing specimens on the arm will be sequential and in order of the most laundered to least laundered. Therefore, if the same arm will be used to test the control and specimens, the order of testing shall be control, followed by the specimens laundered 50 cycles, followed by the specimens laundered 20 cycles, and then conclude with testing the specimens laundered once. Tests should be conducted with as little elapsed time as possible in between testing of a volunteer's arms. Note: The same cage can be utilized when testing specimens treated with non-volatile repellents such as permethrin or etofenprox.

4.7.2.3.8 Raw data. Raw data shall consist of the mosquito information as described in 4.7.2.3.3, the number of mosquitoes used per cage, and method of selection of these mosquitoes. The number of male and female mosquitoes shall be counted and only the number of females used for purposes of identifying mosquitoes that bite compared to non-biting mosquitoes. The number of bites received for each sample (treatment or control) shall be counted and recorded.

4.7.2.4 Report. Calculation of the reduction in bites for the treatment, compared to the control, shall be expressed as a percentage that represents the percentage mosquito bite protection as shown below. Individual subject results for each trial (three (3) for each treatment type or control), shall be averaged with all trials for the other volunteer subjects in the study. An overall average % mosquito bite protection shall be calculated by Abbott's equation below and reported in this manner for each insect and for all volunteers tested. Total % mosquito bite protection will be calculated by averaging the overall results for each species and meet the requirements set forth in paragraph 3.5.2.

$$\%\ \text{MosquitoBite Protection} = \frac{(B_{NC}/F_C) - (B_T/F_C)}{(B_{NC}/F_C)}$$

where:

B_{NC} = bites recorded on the arm covered by the control fabric

F_C = female mosquitoes in the cage that are capable of biting at the start of the 15-minute period

B_T = bites recorded on the arm that was covered by the treated fabric.

4.8 Toxicity test. When required, (see 6.2), an acute dermal irritation study and a skin sensitization study shall be conducted. When the results of these studies indicate the coat is not a sensitizer or irritant, a Repeat Insult Patch Test shall be performed in accordance with the Modified Draize Procedure (see 2.3). If the toxicity requirement (see 3.7) can be demonstrated with historical use data, on the finishing treatments used, toxicity testing may not be required (see 6.2).

5. PACKAGING

5.1 Packaging. For acquisition purposes, the packaging requirements shall be as specified in the contract or order (see 6.2). When packaging of material is to be performed by DoD or in-house personnel, these personnel need to contact the responsible packaging activity to ascertain packaging requirements. Packaging requirements are maintained by the Inventory Control Point's packaging activities within the Military Service or Defense Agency, or within the military service's system command. Packaging data retrieval is available from the managing Military Department's or Defense Agency's automated packaging files, CD-ROM products, or by contacting the responsible packaging activity.

5.2 Insect repellent treated item packaging. Every container (box, roll, pallet, etc) of insect repellent treated items must be labeled according to EPA requirements as stated in Federal Insecticide, Fungicide And Rodentcide Act (FIFRA) and 40 CFR. (see 2.2.2).

6. NOTES

(This section contains information of a general or explanatory nature that may be helpful, but is not mandatory).

6.1 Intended use. This document is intended for use in treating fabrics/garments to offer vector protection.

6.2 Acquisition requirements. Acquisition documents should specify the following:

a. Title, number, and date of this specification.
b. The specific issue of individual documents referenced (see 2.2).
c. When first article is required (see 3.1, 4.2).
d. The specific dose rate requirements for insect repellent treatment (see 3.5.1).
e. The specific % mosquito bite protection requirements (see 3.5.2).
f. When toxicity testing is required (see 3.7, 4.8).
g. Conformance inspection acceptance quality limits (AQL) (see 4.3).
h. Inspection conditions (see 4.4).
i. Packaging (see 5.1, 5.2).

6.3 Active Ingredients (AI): Active ingredients other than permethrin or etofenprox are allowed and encouraged. All AIs must meet the specification efficacy requirments, meet all EPA regulatory requirments and must be approved prior to use. AI concentration levels for new AIs will be determined based on the EPA approved registration.

6.4 Approved EPA insect repellent treatment registrations (as applicable). Samples of approved treatment registrations and labels are shown at the EPA web site: http://oaspub.epa.gov/pestlabl/ppls.home

NOTE: EPA registration does not certify that the insect repellent treatment meets the specification requirements.

6.5 Insect repellent garment application. To assist in minimizing defects that may be caused during the insect repellent application process, all garment closures should be engaged prior to the application.

6.6 Percent mosquito bite protection. The following facilities are known to perform percent mosquito bite protection testing in conformance with 4.7.2.

Aedes aegypti and Anopheles albimanus mosquitoes:
USDA-Center for Medical, Agricultural & Veterinary Entomology
Agricultural Research Service
1600/1700 SW 23rd Drive
Gainesville, FL 32608
POC: Kenneth J. Linthicum, PhD, BCE Center Director
Tel: (352) 374-5700 / Fax : (352) 374-5850
Kenneth.Linthicum@ars.usda.gov

6.7 Subject term (key word) listing.

Bite
Etofenprox
Insect
Mosquito
Permethrin

6.8 Certificate of compliance. The contracting activity may select to accept a certificate of compliance for any stated requirement. The contracting activity may request a certificate of compliance ensuring that all EPA, FIFRA and CFR (see 2.2.2) requirments are being met.

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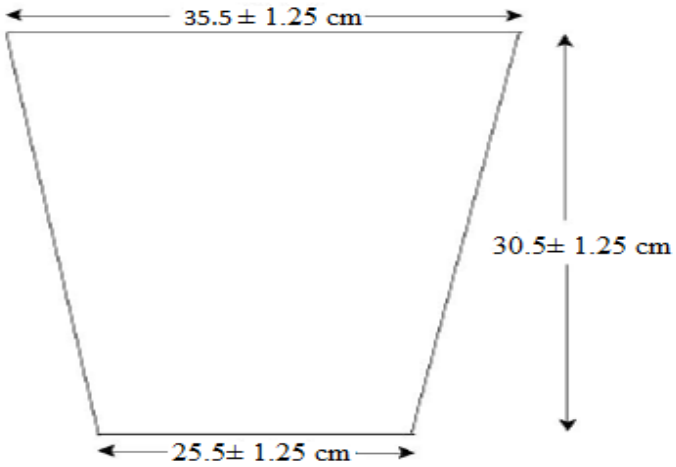

FIGURE 3. Test Specimen, % Bite Mosquito Protection Test.

Custodian:
Army - GL
Navy - NU
Air Force - 11

Preparing activity:
DLA-CT

Review activities
Army – MD
Navy - CG1, MC

(Project: 8305-2020-023)

NOTE: The activities listed above were interested in this document as of the date of this document. Since organizations and responsibilities can change, you should verify the currency of the information above using ASSIST Online database at https://assist.dla.mil.