EPA Review of
USDA-ARS-CMAVE Protocol

Proposal for a Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military

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Overview

- Protocol for a laboratory study of the repellent efficacy of military uniforms containing 1% etofenprox
- Submitted by Dr. Ulrich Bernier, United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology
- Protocol is a special study, non-EPA guideline, that is not similar to previous mosquito field studies reviewed by the HSRB

Overview 2

- Research is proposed to satisfy EPA registration requirements
- This protocol and subsequent study may be used to standardize the experimental approach to evaluating the efficacy of repellent treated textiles

Overview 3

- Sponsor will test the hypothesis that etofenprox treatment provides bite protection when mosquitoes are exposed to treated fabric compared to an untreated control
- Etofenprox is recommended by the World Health Organization for use in public health vector control programs as a direct spray to infested areas or indirectly by treating fabrics, such as mosquito nets

Comparisons to Skin-Applied Repellent Studies Reviewed by the HSRB

- Laboratory vs. Field
- Different repellent effect
- Different efficacy measures
- Subjects will receive mosquito bites

Science Assessment: USDA-ARS Protocol for a Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military

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Study Objectives

- This study is designed to determine the bite protection level of etofenprox-treated U.S. Military Fire Resistant Army Combat Uniforms (FRACUs) treated initially at an application rate of 1% wt/wt, and to assess the bite protection performance after 0x, 20x, and 50x washes.

Study Objectives 2

- The results of this research will allow for determination of whether etofenprox-treated FRACUs meet the Department of Defense's specifications for minimum bite protection level.
- The research has societal value because U.S. military personnel serving domestically and abroad are at risk of contracting insect-transmitted diseases.

Study Objectives 3

	Bite Protection Specifications (%)		
Uniform	0x wash cycle	20x wash cycle	50x wash cycle
Army FRACUs (test material in the proposed protocol)	85%	80%	70%

Acute Toxicity of the Test Material

- Acute Dermal = LD_{50} >2,100 mg/kg body weight
- Acute oral = LD_{50} >5,000 mg/kg body weight
- Minimally irritating to the skin and eyes
- Not a skin sensitizer

MOE Estimate

- Estimated maximum dose = based on the assumption of 100% etofenprox absorption from 6 treated sleeves = 635.4 mg/subject
- Assuming 70 kg subject, equivalent dose rate is 635.4/70 = 9.08 mg/kg
- Margin of Exposure (MOE) = 231
- EPA's Level of Concern $\geq$ 100

Evaluating Skin Irritation

- Dermal observations resembling skin irritation were observed in a previously conducted 28-day dermal toxicity study with **technical etofenprox** on rabbits

- The etofenprox registrant, Mitsui Chemicals, will soon be conducting a product-specific 28-day dermal toxicity study in rabbits with **etofenprox-treated fabric**

Experimental Design
Testing Paradigm for each Mosquito Species

	Subject Right Arm		Subject Left Arm	
Test Set	**Treatment Condition**	**Specimen**	**Treatment Condition**	**Specimen**
1	Coat Untreated Unwashed Control	Sleeve 1	Trouser Untreated Unwashed Control	Sleeve 2
2	Coat Treated Washed 50x	Sleeve 3	Trouser Treated Washed 50x	Sleeve 4
3	Coat Treated Washed 20x	Sleeve 5	Trouser Treated Washed 20x	Sleeve 6
4	Coat Treated Unwashed (0x)	Sleeve 7	Trouser Treated Unwashed (0x)	Sleeve 8

Experimental Design 2

Fabric and Treatment Condition	Number of Fabric Specimens	Number of Subjects	Number of Species	Total Replicates per Fabric Type
Coat Untreated Unwashed Control	1	8	2	16
Coat Treated Washed 50x	1	8	2	16
Coat Treated Washed 20x	1	8	2	16
Coat Treated Unwashed (0x)	1	8	2	16
Trouser Untreated Unwashed Control	1	8	2	16
Trouser Treated Washed 50x	1	8	2	16
Trouser Treated Washed 20x	1	8	2	16
Trouser Treated Unwashed (0x)	1	8	2	16

Experimental Design 3

- The test cages are approximately 59,000 cm^3 in volume and each will contain 175 to 225 female mosquitoes (density of ~1 mosquito/300 cm^3)
- Female mosquitoes will be preselected from stock cages by using a specially designed draw box that uses odors from the hand of a laboratory staff person to attract mosquitoes upwind in to a trap

Endpoints and Measures

- Unit of measure for determination of the repellent effects is percent bite protection
- Presence of blood in the mosquito's abdomen will confirm a 'mosquito bite'
- For each test set, the treatment % bite values will be corrected to account for the bite through values in the untreated control using Abbott's Formula

Endpoints and Measures 2

- Percent bloodfed in untreated control treatment after test interval
- Percent bloodfed in etofenprox treatment after the test interval

Endpoints and Measures 3

- Percent Bite Protection =

  [1 – (treatment rate) / (control rate)] × 100%

- Treatment rate (or proportion) =
  (# bloodfed female mosquitoes after test interval) /
  (total # of female mosquitoes after test interval)
  ~~when subject used treated fabric~~

- Control rate (or proportion) =
  (# bloodfed female mosquitoes after test interval) /
  (total # of female mosquitoes after test interval)
  ~~when subject used untreated fabric~~

Statistical Analysis Plan

The objective is to estimate the mean level of bite protection and associated 95% confidence intervals for different 'treatments' [i.e. different combinations of fabric types (coats and trousers), number of washes, and mosquito species].

Statistical Analysis Plan 2

Table 3.5.1 Impact of the Number of Replications on the Number of Subjects

True bite-through rate for control fabric (θ_C)	*50%*		*20%*	
True bite protection for treated fabric (β_T)	*80%*	*95%*	*80%*	*95%*
Number of Subjects	Expected half-width of a 95% confidence interval for % bite protection			
3	5.2%	2.7%	8.8%	4.5%
4	4.5%	2.3%	7.5%	3.8%
5	4.0%	2.0%	6.7%	3.4%
6	3.7%	1.9%	6.0%	3.0%
7	3.4%	1.7%	5.6%	2.8%
8	3.2%	1.6%	5.2%	2.6%
9	3.0%	1.5%	4.9%	2.4%
10	2.8%	1.4%	4.7%	2.3%
15	2.3%	1.2%	3.8%	1.9%
20	2.0%	1.0%	3.3%	1.3%

Statistical Analysis Plan 3

- The proposed sample size of 8 subjects represents a reasonable compromise between decreasing confidence interval width and limiting unnecessary human experimentation.

Statistical Analysis Plan 4

- Data Analysis
  - The numbers of bloodfed and total female mosquitoes found with treated and control fabric for each subject will be analyzed as binomial distributed data in a generalized linear model (GLiM) using a log link.

Measures to Ensure Reliability

- Standard Operating Procedures (SOPs) will be in place that must meet Good Laboratory Practices requirements.
- Subjects' attractiveness to mosquitoes will be determined prior to testing
- Laboratory technicians will assist subjects with placing the test sleeves on their arms and excluding all exposed skin from mosquito exposure. Laboratory technicians will assist subjects with insertion and removal of their arms in/from the cages.
- Counts of bloodfed mosquitoes and the total number of mosquitoes in the cage will be determined by a research technician.

Compliance with Scientific Standards

The following elements are adequately addressed:

- Available toxicity studies with etofenprox
  - Adequately characterize toxicological profile of the formulation except for dermal irritation from intermediate exposures to treated fabric.
  - Support estimate of acceptable Margin of Exposure (MOE)

Compliance with Scientific Standards

- The following elements are generally acceptable but require refinement and clarification:
  - Experimental design
  - Statistical analysis

Science Comments and Recommendations

- Conduct a product-specific 28-day dermal toxicity study in rabbits with etofenprox-treated fabric
- EPA recommends that the proposed efficacy study not be conducted until the results of the product specific dermal toxicity study have been submitted to and reviewed by EPA

Science Comments and Recommendations 2

- Provide justification for testing two vector mosquito species instead of three
- Consider recruiting more than two alternates
- The statistical plan for analyzing the data will need to take into account how alternate subjects will be handled

Science Comments and Recommendations 3

- Please add more details to the protocol about what will happen if a subject withdraws midway through the study and an alternate is brought into the study as a replacement.
- Will an alternate who replaces an original subject complete all eight pairs of sleeves, or only the pairs of sleeves that were not completed by the original subject?

Science Comments and Recommendations 4

- The subjects and alternates need to be randomly selected from a larger pool of qualified potential subjects.
- Please continue screening respondents to the advertisement until you have at least 20 qualified potential subjects. Then, randomly select the 8 subjects and 2 or more alternates from the pool of qualified potential subjects.

Science Comments and Recommendations 5

- Please address the distribution of male and female subjects and discuss if this will impact the results due to differences, if any, in attractiveness to mosquitoes.
- Please revise the protocol to specify exactly what will happen if there is unequal distribution or if only one sex is represented.

Science Comments and Recommendations 6

- The statistical analysis used to analyze the study data should be justified in the final report.
- Describe how the data will be analyzed if the number of test subjects at the end of the test is less than eight.

Ethics Assessment

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Value to Society

- Proposed study would test the repellent efficacy of FRACU material that has been treated with etofenprox
- Would allow for a determination of whether etofenprox-treated FRACUs meet DoD's specifications for minimum bit protection level
- Could allow for better protection of US forces serving abroad

Subject Selection

- Subjects will be recruited through an advertisement placed in a newspaper and posted on university bulletin boards
- Callers will be informed about the study using an IRB-approved script
- Callers will be screened for eligibility and then scheduled for informed consent meetings

Subject Selection 2

- Inclusion/Exclusion criteria are complete and appropriate, except:
  - Add exclusion for individuals known to be sensitive to pesticides or other chemical products
  - Add exclusion for individuals with cuts, scrapes or skin conditions on their hands or forearms

Subject Selection 3

- No potential subjects will be from a vulnerable population
- Subjects will be recruited through an advertisement printed in a local newspaper and posted on university bulletin boards

Consent Process

- Study Director meets individually with each interested candidate
  - Confirms eligibility criteria
  - Provides detailed explanation of the procedures of the study
  - Shows DVD further explaining testing process
  - Informs candidate of how many mosquito bites they are likely to obtain
  - Reviews Informed Consent Document
  - Answers questions
- Study Director confirms understanding and solicits consent to participate

Risks and Risk Minimization

Four categories of risk; protocol provides appropriate measures to minimize each

- Exposure to biting mosquitoes
- Possible exposure to arthropod-borne disease
- Exposure to test material
- Breach of privacy (pregnancy testing)

Benefits

- No direct benefit to subjects
- Primary direct beneficiary is sponsor
- If the treated materials are proven effective and superior to existing materials, indirect beneficiaries will include US military personnel who wear this etofenprox-treated FRACUs

Risk:Benefit Balance

- Risks have been effectively minimized
- Risks are reasonable in light of the expected societal benefits of the knowledge likely to be gained

Respect for Subjects

- Effective methods for protecting subjects' privacy
- Proposed level of compensation is appropriate
- Subjects will be free to withdraw at any time
- Medical care for research-related injuries will be provided at no cost to subjects

Independent Ethics Review

- The Western Institutional Review Board (WIRB) reviewed and approved the protocol and informed consent materials

Revisions Requested by EPA Before Research Proceeds

- Minor clarifications to protocol and consent form as detailed on pg. 4-5 of EPA Review
  - Explain process for inspection of subjects hands and arms
  - Resolve inconsistency re what member of the research team will verify pregnancy test results
  - Clarify that there are no benefits to subjects
  - Add exclusion for people sensitive to pesticides or chemical products
  - Add exclusion for cuts, scrapes, skin conditions on hands or forearms

Compliance with Ethical Standards

- All requirements of §26.1111, §26.1116, and §26.1117 are met
- All requirements of §26.1125 are met
- Requirements of §26.1203 are met
- If EPA's and HSRB's requested corrections are made, research conducted according to this protocol will likely meet the applicable requirements of 40 CFR part 26, subparts K and L

Charge Questions

If the proposed protocol is revised as suggested in EPA's review and if the research is performed as described:

- Is the protocol likely to generate scientifically reliable data, useful for estimating the level of mosquito bite protection provided by two different textiles treated with etofenprox?
- Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?