UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

March 20, 2014

MEMORANDUM

SUBJECT: Science and Ethics Review of USDA Protocol for Laboratory Evaluation of Bite Protection from Repellent-treated Clothing for the United States Military

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REF: Bernier, U. (2014) Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military. Unpublished document prepared by United States Department of Agriculture – Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology. March 7, 2014. 210 p.

We have reviewed the referenced protocol for a laboratory test of mosquito repellent-impregnated clothing for the United States Military from both scientific and ethics perspectives. This review assesses the scientific aspects of the proposed research for a special efficacy study to assess etofenprox-treated U.S. Military uniforms in terms of the recommendations of the EPA and of the EPA Human Studies Review Board. Ethical aspects of the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L and the recommendations of the EPA Human Studies Review Board.

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA's checklist is appended to this review as Attachment 5. All elements of required documentation are provided in the submitted protocol package.

Volume 1 of USDA's submitted package includes the following documents—all considered in this review:

- WIRB-approved protocol dated 2/28/14 (pp. 5-192)
- WIRB-approved informed consent form (approved 3/04/14) (pp. 193-203)
- WIRB-approved recruiting advertisement (p. 204)
- WIRB-approved telephone script for receiving phone calls in response to advertisement (p. 205)

Volume 2 of USDA's submitted package includes documentation of communications with WIRB, as well as copies of CVs and ethics training certifications for the principal investigator and staff.

Volume 3 of USDA's submitted package provides statistics analysis for the proposed study.

B. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of our observations about the ethical aspects of the proposed protocol. Supporting details are in the attachment.

1. **Societal Value of Proposed Research:** 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. 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. Currently, permethrin is the only pesticide that is EPA registered for treatment of military uniforms. Due to global issues regarding disease vector resistance to pyrethroids such as permethrin, other insecticide candidates must be considered. This protocol provides a method for evaluating the bite protection of fabrics that are treated or impregnated with substances that repel or reduce arthropod bites.

2. **Subject Selection:** Ten adult subjects will be recruited from the general population in Gainesville, Florida (8 initially assigned for participation, plus two alternates). Participants will self-identify in response to a printed advertisement in a local newspaper (the Gainesville Sun) or posted on bulletin boards in University of Florida buildings. Callers responding to the advertisement will be screened, scheduled for informed consent

meetings, and enrolled. While it is possible that people who respond to the advertisement are different in some unknowable ways from those who do not respond, there is no reason to think that respondents in the Gainesville, Florida area are not typical of people who would respond to these types of advertisements in other areas of the United States.

3. **Risks to Subjects:** Risks to subjects include the risk of exposure to biting mosquitoes; the risk of exposure to disease vectors; the risk of exposure to the test material; risks related to receiving an unexpected result on a pregnancy test; and the risk of a loss of confidentiality related to the pregnancy test and subjects' participation in the study. Risks are minimized in the protocol by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to the test material; applying clear stopping rules; and by incorporating procedures to keep the subjects' identities and results of pregnancy testing private, and to permit discrete withdrawal. All practical steps to minimize subject risks have been taken, and the remaining risks have a low probability of occurrence.

4. **Benefits:** This research offers no direct benefits to subjects, but may provide indirect benefits to subjects and society by providing data that could be used by EPA to register a novel repellent treatment for military clothing, thereby allowing for better protection of American military forces from nuisance bites and bites that lead to arthropod-borne diseases. The results may also lead to better protection of U.S. civilians both domestically and while they travel abroad because novel repellents may be incorporated into civilian clothing as a potentially better alternative to the repellent-treated clothing already available on the market.

5. **Risk/Benefit Balance:** No practical opportunities to further reduce risk to subjects while maintaining the robustness of the scientific design have been overlooked. The residual risk to subjects is very low, and reasonable in light of the potential benefits of the data to society.

6. **Independent Ethics Review:** The Western Institutional Review Board (WIRB) has reviewed and approved the protocol, informed consent form, and recruitment materials. WIRB is independent of the investigators and sponsors. Satisfactory documentation of WIRB procedures and membership is on file with the Agency and has been provided to the HSRB members with the background materials for this protocol.

7. **Informed Consent:** The protocol contains a complete and satisfactory description of the process by which potential subjects will be recruited and informed, and the process for seeking their consent to participate. A copy of the WIRB-approved consent documents meeting all requirements of 40 CFR §§26.1116 and 26.1117 is included in the proposal.

8. **Respect for Subjects**: Subject-identifying information will be recorded only once; all subsequent data records and reports will refer to individual subjects only by an arbitrary code. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing. Candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason. Subjects who

withdraw will be compensated for time spent up to the point of withdrawal. Medical care for research-related injuries will be provided by the sponsor Landis International at no cost to the subjects.

C. Compliance with Applicable Ethical Standards

This is a protocol for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to EPA under the pesticide laws. Thus the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply. A point-by-point evaluation of how this protocol addresses the requirements of 40 CFR 26 Subparts K and L and the criteria recommended by the HSRB is appended as Attachment 1.

EPA Ethics Comments

Before the research is conducted, the documents should be revised as follows and resubmitted for review by the approving IRB:

- In section 8.6 of the protocol (V1:33), titled "Detailed Stepwise Test Procedure," insert an explanation of how, when, and by whom subjects' hands and arms will be inspected for cuts or other skin conditions. Language similar to what appears on page 4 of the informed consent (V1:196) form would suffice.

- In section 8.6 of the protocol (V1:33), titled "Detailed Stepwise Test Procedure," insert an explanation of how the pregnancy testing for female subjects will be handled, clarifying where in the sequence of stepwise procedures the pregnancy testing will occur. Language similar to what appears on page 3 of the informed consent form (V1:195) or in section 8.1.4 of the protocol (V1:24) would suffice.

- Please clarify which member of the research team will verify pregnancy test results of female subjects who desire to remain in the study after taking the pregnancy test. The protocol and consent form are inconsistent on this point. Section 8.1.3 of the protocol says that the result must be presented "to a staff member" (V1:24), and section 8.1.4 of the protocol states that the result must be shown to "the study director or an appropriate designee for verification of negative results" (V1:24). The consent form states that if, after taking the test, a subject still wishes to participate, she must show the result to a female laboratory technician. (V1:195) A female member of the research team would be the best person to verify negative pregnancy test results.

- Please revise the first sentence in section 11.0 of the protocol, titled "Benefits and to Whom Benefits Accrue," as follows: *"While there are no direct benefits to the subjects participating in this research study ~~beyond a small compensation for their time~~, there are…"* The proposed payment to subjects is considered compensation for lost time and inconvenience,

not a benefit of participating in the research. This study provides no direct benefits to subjects. (V1:45)

- In section 8.1.2 of the protocol, titled "Inclusion/Exclusion Criteria," consider expanding exclusion #6 as follows: *"Exclusion of people known to be sensitive to the test material, pesticides, or other chemical products"* (V1:23)

- In section 8.1.2 of the protocol, titled "Inclusion/Exclusion Criteria" (V1:23), add an exclusion for people with open cuts or scrapes or skin conditions such as psoriasis or eczema on their hands or forearms. Cut, scrapes or other skin conditions might increase the risk of skin reactions or sensitivity during the testing. Also, add this exclusion to the "Restrictions" section of the consent form (V1:195).

- In the "Study Procedures" section of the consent form (V1:196), insert text similar to what appears below as #2, and adjust the numbering of the subsequent procedures accordingly:

  "2. It is important that you NOT be in this study if you are pregnant. So, before the testing begins, each female volunteer will be asked to go to a private area and will be given a home pregnancy test kit. A female researcher will be able to explain how to use it and answer questions. If you are a female, you will be asked to take the test in a private restroom. If, after taking the test, you still wish to participate in the study, you will be asked to show the result of the test to a female laboratory technician so that she can verify that you are not pregnant. If you withdraw from the study after taking the pregnancy test, you will not be asked to share the result of the test with anyone."

40 CFR 26 Subpart L, at §26.1703, as amended effective August 22, 2006, provides in pertinent part:

> EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

This protocol requires that subjects be at least 18 years old and excludes female subjects who are pregnant or lactating. Thus §26.1703 would not forbid EPA to rely on a study executed according to this protocol.

D. Summary Assessment of Scientific Aspects of the Proposed Research

"*The objective of this proposed study is 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% etofenprox (weight/weight), and to assess the bite protection performance after 0x, 20x, and 50x washes. This is a non-guideline study; therefore, it is not designed to fulfill the requirements of a specific OCSPP (formerly OPPTS) Guideline. This is considered a special study. This study will be conducted in accordance with EPA, FIFRA (Federal Insecticide,*

Fungicide and Rodenticide Act), Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 (October 1989)." (V1:16).

The basic experimental unit in this study is a sleeve test. Each test involves a subject exposing a (unwashed treated, untreated, washed treated) fabric-sleeved arm into a cage of one species of mosquito for 15 minutes per hour for up to eight hours (See Figure 8.2.1, V1:26). The data obtained from each 15 minutes test with each experimental unit will be counts of the number of bloodfed female mosquitoes and the total number of female mosquitoes in each test cage. The observed bite-through proportion (or 'rate') is the proportion of bloodfed female mosquitoes to the total number of mosquitoes in each test cage, which will be expressed as percent bite protection. Etofenprox treatment bite through rates will be corrected using Abbott's formula for 'background' bite through rates in the control (untreated fabric sleeve). To increase testing precision, each subject will serve as their own treatment and control. Therefore, the experimental design consists of 4 groups tested in the following order per mosquito species. The test groups are:

- 1 test with an untreated FRACU fabric-sleeve, which serves as the control.
- 1 test with treated unwashed (0x) FRACU fabric.
- 1 test with treated washed (20x) FRACU fabric.
- 1 test with treated washed (50x) FRACU fabric.

FRACU fabric from coats (shirts/blouses) and trousers will be tested as described in the tables below (3.5.3, and 3.5.4). Each subject (8 subjects) will test each group once per species for a total 8 replicates per group per species, resulting in 16 replicates per fabric group for this experiment as shown in Table 3.5.2.

The widely accepted method of evaluating the efficacy of insecticide treated clothing includes laboratory aging of this treated clothing by laundering through standardized wash cycles per the American Association of Textile Chemists and Colorists (AATC) laundering protocol (See Appendix E on V1:171). Testing will be conducted with treated and untreated clothing prior to laundering (0x wash cycle) and at the 20x and 50x wash cycles. This protocol is similar to the protocol accepted as Standard Operating Procedure by the U.S. Military to evaluate their repellent treated combat uniforms.

The unit of measure for determination of the repellent effects in this proposed experiment (% bite protection based on the proportion of bloodfed to total mosquitoes in a cage) differs from skin applied repellent evaluations where the "Landing with Intent to Bite" measure is used and efficacy is measured as Complete Protection Time. A detailed justification for the proposed test system is presented in the protocol (V1:16). In brief, the repellent effect created by skin applied repellents is instantaneous and non-toxic, whereas mosquitoes exposed to the treated clothing must remain in contact with the treated cloth for a longer time period to illicit a repellent effect. The resulting repellent effect is usually a toxic effect that results in 'excito-repellency' or incapacitation due to exposure to the fast acting insecticide. The proposed specifications for testing success are shown in the table below, which is taken from the table on p.12 of 210 in Volume 1 and page 3 of 21 in Volume 3. These specifications are in-line with current U.S. Military specifications for treated uniforms.

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

This protocol also proposes to evaluate the repellent effect (% bite protection) of treated clothing using only two mosquito species - unlike skin applied repellent studies conducted under field conditions where three species are evaluated. In the proposed study representative species from the genus *Anopheles* (malaria vector) and genus *Aedes* (vector of dengue and yellow fever) will be evaluated. A mosquito species from the genus *Culex* (vector of West Nile virus or St. Louis encephalitis) will not be tested. Justification for exclusion of the third species is not mentioned with referral to current military test specifications instead.

The study director included a justification for sample size in Table 3.5.1 (V1:19). These tables show that the overall bite protection value for a treated fabric is dependent on control bite through rates and the percentile of bite protection. Precision improves with the number of subjects based on the width of the 95% confidence interval for the 80^{th} and 95^{th} percentiles with control bite through of 50% and 20%. However, this precision does not improve very much beyond 8 test subjects. Precision can decline if percent control bite through rates decline or are low, therefore, as proposed by the study director, mosquitoes will be screened for their 'aggressiveness' to ensure that control bite through rates are substantial. The testing paradigm including replication is described in Tables 3.5.2, 3.5.3, and 3.5.4 (V1:20).

The objective of the data analysis 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]. The numbers of bloodfed and total female mosquitoes found with treated and control fabric (clothing) for each subject will be analyzed as binomial distributed data in a generalized linear model (GLiM) using a log link, generalized estimating equations or a mixed effect GLiM. This is largely dependent on the 'subject term', which may be treated as a fixed or random effect to adjust for within-subject differences (V1:22). Volume 3 includes a very detailed explanation of the analysis and justification for the study design. Alternate subject data appear to be treated together with existing subject data in the event of a dropout but the protocol is silent on this aspect of data analysis. The analysis does not take into account what happens if less than 8 subjects complete the study.

1. **Study design:**

   Replicate subjects will be used in this study to evaluate bite protection for a U.S. military clothing fabric (FRACU) treated with insecticide/repellent (etofenprox). A fabric's "bite protection" is a measure of the relative level to which a treated fabric prevents bites compared to the untreated control. As described in §8.5 (V1:30) of the protocol the observed bite protection for a subject is calculated using the subject's bite-through rates for the treated fabric and for a corresponding untreated/unwashed control fabric. (Each subject serves as their own control.) The purpose of the control is to compensate for influences related to the subject's individual attraction level, the general host-seeking

response of the test mosquito population, and to correct for bite-through rate of the untreated fabric. The treatment and control values for a subject are then used in Abbott's formula to calculate the observed bite protection level of the sleeve for that subject.

Treated clothing sets will be evaluated at the specific standardized wash intervals: unwashed (0x), 20x washes, and 50x washes. Separate fabric specimens for each wash interval are tested, similar to that described in U.S. military GL/PD specifications (Appendix C, pg. 34 and Appendix D, pg. 36). Two species of mosquitoes, *Aedes aegypti* and *Anopheles albimanus***,** will be tested separately. Eight subjects (preferably the same eight subjects) will be used to test each fabric and mosquito species combination. Two alternate subjects will also be recruited. The exposure time to mosquitoes at each test interval (once per hour) for control and treated replicates is 15 minutes per arm. A summary of the experimental design and testing paradigm for each mosquito species are shown below (Vol 1:18-20):

Table 3.5.2 Experimental Design

Fabric and Treatment Condition[1]	Number of Fabric Specimens	Number of Subjects	Number of Species[2]	Total Replicates per Fabric Type
Coat Untreated Unwashed Control[3]	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[3]	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

[1] Fabric treatment conditions are either untreated and unwashed (Control) or treated and unwashed (0x), treated and washed 20 times (20x) or treated and washed 50 times (50x).
[2]The test species are *Aedes aegypti* or *Anopheles albimanus.*
[3]Each subject serves as their own control for the bite protection calculation.

Table 3.5.3 Testing Paradigm using *Aedes aegypti*

	Subject Right Arm		Subject Left Arm	
Test Set[1]	Treatment Condition	Specimen Designation	Treatment Condition	Specimen Designation
1	Coat Untreated Unwashed Control[2]	Sleeve 1	Trouser Untreated Unwashed Control[2]	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

[*]Each subject will have both their right arm and left arm tested simultaneously and complete Test Set 1-4 for *Aedes aegypti*. Each subject will have a break between test sets when new cages are being filled with mosquitoes. All cages will be washed after all test sets for each participant are completed.
[1] Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation.

Table 3.5.4 Testing Paradigm using *Anopheles albimanus*

	Subject Right Arm		Subject Left Arm	
Test Set[1]	**Treatment Condition**	**Specimen Designation**	**Treatment Condition**	**Specimen Designation**
5	Coat Untreated Unwashed Control[2]	Sleeve 9	Trouser Untreated Unwashed Control[2]	Sleeve 10
6	Coat Treated Washed 50x	Sleeve 11	Trouser Treated Washed 50x	Sleeve 12
7	Coat Treated Washed 20x	Sleeve 13	Trouser Treated Washed 20x	Sleeve 14
8	Coat Treated Unwashed (0x)	Sleeve 15	Trouser Treated Unwashed (0x)	Sleeve 16

*Each subject will have both their right arm and left arm tested simultaneously and complete Test Set 5-8 for *Anopheles albimanus.* Each subject will have a break between test sets when new cages are being filled with mosquitoes. All cages will be washed after all test sets for each participant are completed.
[1] Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation.

Laboratory-reared 6-11 day old adult mosquitoes will be used for the bite protection assay. Mosquito species have differing behavior and levels of 'aggressiveness', therefore, adult female mosquitoes of two of the more aggressive and anthropophilic species will be tested. One of these selected species will be *Aedes aegypti*, a vector of yellow fever and dengue fever that is found heavily in Asia and South America. The second species will be *Anopheles albimanus*, a tropical mosquito that is a highly aggressive biter, one of the most tolerant species when tested with topical skin repellents, and is a competent vector for malaria transmission. The mosquito colonies are reared at the Center for Medical, Agricultural, and Veterinary Entomology in a USDA facility in Gainesville, Florida. The colonies used have been maintained since being established in 1952 while in Orlando for *Ae. aegypti*, and in El Salvador in 1974 for *An. albimanus*. There have been period introductions of wild type species. Mosquitoes from a colony will respond on the whole more aggressively to attractant stimuli than strains reared from freshly collected wild-types (V1:27).

"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 (Fig. 8.3.1). The trap containing the mosquitoes will then be transferred to the test cage for subsequent testing by subjects (Fig 8.3.2, V1:28)."

2. Statistical design:

The proposed research will include eight human subjects and two alternates. As described, the subjects will serve as their own treatments and controls. Each fabric type will be replicated 16 times, 8x for each species. An analysis of the impact of the number of replications on the number of subjects was conducted as described below in Table 3.5.1 (V1:19) from the protocol. The effects of control bite through rates (20% and 50%) as well as bite protection (80^{th} and 95^{th} percentile) were evaluated in this analysis using 1000 simulated datasets for each number of subjects. (Volume 3 includes the complete explanation of this approach, the simulations, and related justifications on pp. 5-12)

These simulated data show that reliable data sets can be collected with 8 subjects and that increasing the number of subjects slightly increases precision, but this increase in precision does not provide enough information to justify a larger experiment.

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)[1]	*80%*	*95%*	*80%*	*95%*
Number of Subjects	Expected half-width of a 95% confidence interval for % bite protection[2]			
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%

[1]Bite incidence for treated fabric is calculated from bite protection as $\theta_T = \theta_C(1 - \beta_T/100)$

[2]Average half-width from 1,000 simulated datasets. Each dataset consisted of S subjects testing a pair of fabrics (control and treated). For each pair the total number of mosquitoes (M) was a Poisson (200) random variable, and the number of bloodfed mosquitoes was simulated as a binomial (θ,M) random variable. Subject-subject differences were simulated by adding a subject-specific normal (0,0.3) random variable to the logit of the true incidence for both control and treatment fabrics. For each simulated dataset a binomial generalized linear model was fit to the data using the GENMOD procedure in SAS. The model specified fixed effects for both subject and test material and used a log link. Bite protection confidence intervals were then obtained by back-transforming the confidence intervals for the contrast $\log(\theta_T)$-$\log(\theta_C)$.

The primary objective of the data analysis is to estimate the overall (or 'mean') level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in §8.5. These values will be averaged over all subjects to obtain mean observed bite protection values that can be used as a check on any model-based bite protection estimates.

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. A subject term will be added as a fixed effect in the model to adjust for subject-subject differences. (Alternatively, subjects could be treated as a random effect and the within-subject correlation accommodated using either generalized estimating equations or a mixed effect GLiM. The decision on how to analyze the collected data needs to be finalized.) Use of the log link makes it possible to obtain an estimate and confidence

interval for the ratio of the treatment and control bite-through rates. The estimates and confidence intervals for percent bite protection are obtained from the relationship:

$$\text{Percent Bite Protection} = [1 - (\text{treatment rate}) / (\text{control rate})] \times 100\%$$

The GLiM model-based bite protection estimates could be obtained by analyzing multiple models each with just a single treatment group and the matched control group. However, it may also be of interest to compare the bite protections of different types of treated fabric, number of washes, or mosquito species. In this case, it would be necessary to include all of the treatments (and species) of interest in the same model. Because the GLiM uses a log link, hypothesis tests concerning ratios of bite protection can be formulated as linear contrasts in the GLiM (V1:21-22) (V3:3-5).

3. How and to what will human subjects be exposed?

Subjects will be exposed to test material and two species of caged mosquitoes in the laboratory. Each subject will have etofenprox treated sleeves placed on both forearms and both arms will be exposed to caged mosquitoes for 15 minutes per hour [The step-wise procedure is described in detail in §8.4 (V1:28-30) and §8.6 (V1:33-42)]. This period of time allows mosquitoes to land, probe, and bloodfeed. The greatest number of bites is expected to be received during the first set of tests with the untreated, unwashed control sleeves. Subsequent tests will involve treated sleeves and it is expected that far fewer bites will be received by the test subjects.

4. Endpoints and Measures:

Efficacy will be measured as percent bite protection. The proposed study will estimate the overall (or 'mean') level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in §8.5 based on exposure to mosquitoes during a 15 minutes bioassay every hour for up to 8 hours. These values will be averaged over all subjects to obtain mean observed bite protection values that can be used as a check on any model-based bite protection estimates.

E. Compliance with Applicable Scientific Standards

This protocol adequately addresses the following elements according to applicable scientific standards:

- Prerequisite acute toxicity research to characterize toxicological profile of the formulation and calculate margin of exposure (MOE).
- Experimental design
- Pre-training of subjects

EPA Science Comments

The following elements in the protocol require revision before the research goes forward:

- Provide justification for testing two vector mosquito species instead of three (V1: 27).

- The statistical analysis must be finalized. Two approaches to GLiM use are discussed in the present version (V1: 21-22). "Recommendations for Data Analysis" expands on this discussion in V3: 13. The protocol should be amended to include the selected analysis before the study is executed.

- The protocol proposes only two alternate subjects. Consider recruiting more than two alternates in case more than two subjects withdraw midway through the study**.**

- Describe how the data will be analyzed if the number of test subjects at the end of the test is less than eight.

- 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 data from the subject who withdraws be discarded, or used alone, or used in combination with data from the alternate subject? 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? If the latter, that person must also be tested with an untreated sleeve against each mosquito species to establish their baseline. The statistical plan for analyzing the data will need to take into account how alternate subjects will be handled. There may be statistical implications of combining partial data sets from different subjects.

- The subjects and alternates need to be randomly selected from a larger pool of qualified potential subjects. The protocol states that the first 20 respondents to the advertisement will undergo a preliminary telephone screening. 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. The random selection may be accomplished by drawing names from a hat or through the use of a randomizer program such as http://randomizer.org.

- Section 8.1.5 of the protocol states that "it is desired that random selection will yield a nearly equal distribution of male and female candidates…" (V1:24) However, it is possible that random selection will yield an unequal distribution of males and females. Please revise the protocol to specify exactly what will happen if there is unequal distribution or if only one sex is represented. Will you continue to randomly select from the pool of eligible subjects until you have an equal number of males and females? Or until you have at least one subject of each sex?

- Due to dermal observations resembling skin irritation in a 28-day dermal toxicity study conducted 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. The new study will test the same fabric that subjects will be exposed to in the proposed efficacy study under review here. **Do not initiate this research until the results of the product specific fabric study have been submitted to and reviewed by EPA.**

- Present values for 70% true bite protection for treated fabric in the protocol in Table 3.5.1 to illustrate the change in the 95% confidence interval with the number of subjects as this value was selected as the performance standard for FRACU fabric washed 50x (V1: 12). The change in the width of the 95% confidence interval is addressed in Volume 3 for the 70% level of bite protection performance in Tables 4, 5 and 6 on pp. 10-12.

- Data compilation and processing. The protocol provides a sample data sheet that is to be used for every 15 minute sleeve test (V1:190 and 192). However, little detail is provided in the protocol on how the data from these sheets will be compiled and processed before entry into Excel, JMP, or SAS and how the resulting records will be handled and archived (§9.0, V1:43).

- Amend the protocol to identify the Quality Assurance Unit (V1:15).

Attachments:

1. Summary Review of Protocol (dated 2/28/14)
2. §26.1111 Criteria for IRB approval of research
3. §26.1116 General requirements for informed consent
4. §26.1117 Documentation of informed consent
5. §26.1125 Criteria for Completeness of Proposals for Human Research

EPA Protocol Review

Title: Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military

Date: February 28, 2014

Principal Investigator and any sub-investigators: Ulrich R. Bernier, Ph.D.

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1. Societal Value of Proposed Research

(a) What is the stated purpose of the proposed research?

In this study, military uniform fabric will be treated with a pesticide called etofenprox. Treated fabric will be compared to untreated fabric to determine if the treatment can decrease mosquito bites to human skin that is covered by the sleeve. Specifically, this study will 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 against two species mosquitoes (*Aedes aegypti* and *Anopheles albimanus*). (V1:16[1])

[1] This pagination convention is used throughout this review. "V1" refers USDA Volume 1, "V2" refers to USDA Volume 2, etc. Entries after the colon are page references; many page images bear more than one page number. In Volume 1, the cited page number is from the expression "Page n of 210" found at the bottom right-hand corner. Volume 2 page references are from the expression "Page n of 580" found at the bottom right-hand corner. Volume 3 page references are from the expression "Page n of 25" found at the bottom center of the page.

(b) What research question does it address? Why is this question important? Would the research fill an important gap in understanding?

The purpose of this study is to develop a standardized protocol that can be used to evaluate the bite protection of fabrics that are treated or impregnated with substances that repel or reduce arthropod bites to determine if they provide sufficient protection against mosquitoes. Due to global issues regarding mosquito resistance to ester-pyrethroids, other insecticide candidates must be considered. Thus, comparisons of new insecticide/repellent candidates should be evaluated in products where performance has already been determined with a known standard, *i.e.* permethrin.

A standardized protocol will enable the EPA to receive consistent and scientifically reliable data for new repellent clothing treatments. The bite protection data will provide information about: 1) the relative level to which bites are received through the fabric with the repellent treatment compared to bites received through the untreated control fabric; 2) whether the fabric is in the "plateau" region of bite protection for the repellent-treated surface, such that application of additional chemical does not improve bite protection performance; 3) the relative bite protection capability of one fabric construction and composition *vs*. another fabric construction and composition; and 4) the bite protection efficacy of a new product for EPA registration. Because these data are acquired in a laboratory setting, there are fewer associated risks than determining where the optima lie than using wild-type mosquitoes in a field setting. Bites are measured in these studies by the presence of a blood meal in the abdomen of the female mosquito (V1:9).

(c) How would the study be used by EPA?

EPA will consider the study to satisfy product specific efficacy data requirements and acceptable label claims for repellent efficacy for the test material.

(d) Could the research question be answered with existing data? If so, how? If not, why not?

EPA requires product-specific efficacy data to support its registration. No previous testing of this product against mosquitoes under the proposed use pattern has been conducted.

(e) Could the question be answered without newly exposing human subjects? If so, how? If not, why not?

Human subjects are required because they represent the target system for the test material, and sufficiently reliable non-human models for repellency testing have not been developed.

2. Study Design

(a) What is the scientific objective of the study? If there is an explicit hypothesis, what is it?

"The objective of this study is 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." (V1:16)

(b) Can the study as proposed achieve that objective or test this hypothesis?

The objective cited may be achieved by the study as proposed if the protocol is revised and amended to explain, in more detail, the following items noted on pages 12-13 of this review.

2.1 Statistical Design

(a) What is the rationale for the choice of sample size?

The rationale for the sample size appears on pp. 18-19 of 210 (§ 3.5) in Volume 1. The researcher's justification for sample size is based on the argument that the precision of the overall (mean) bite protection value for a treated fabric will depend on the true bite through rate for the control fabric and the true level of bite protection. This justification is illustrated in Table 3.5.1 on p. 19 of 210 in Volume 1. Based on the width of the 95% confidence intervals in this table: 1) the experimental variability decreases as control treatment bite through rates increases; 2) as true bite protection increase, the width of the 95% interval decreases; and 3) the precision of overall bite protection value does not appear to increase very much once the number of subjects in the experiment exceeds 8.

(b) What negative and positive controls are proposed? Are proposed controls appropriate for the study design and statistical analysis plan?

Each subject will serve as their own treatment and negative control for each test set as described on p. 20 of 210 in Volume 1 in Tables 3.5.2, 3.5.3, and 3.5.4. The controls are appropriate to calculate the overall bite protection because percent bite protection will be calculated by counting bloodfed female mosquitoes in the treatments and comparing them to the untreated control. Both arms will serve as a control treatment replicate, one for coat fabric and the other for trouser fabric.

(c) How is the study blinded?

The study is not blinded. Untreated sleeves will be tested first followed by 50x, 20x and 0x fabrics.

(d) What is the plan for allocating individuals to treatment or control groups?

All subjects will be assigned to control and treatment groups as described on p. 20 of 210 in Volume 1.

The subjects and alternates need to be randomly selected from a larger pool of qualified potential subjects. The protocol states that the first 20 respondents to the advertisement will undergo a preliminary telephone screening. Please continue screening respondents to the advertisement until you have at least 16 qualified potential subjects. Then, randomly select the 8 subjects and 2 or more alternates from the list of 16+ qualified potential subjects. The random selection can be accomplished by drawing names from a hat or through the use of a randomizer program such as http://randomizer.org.

(e) Can the data be statistically analyzed?

Yes. *"Treated clothing sets will be evaluated at the specific standardized wash intervals: unwashed (0x), 20x washes, and 50x washes. Separate fabric specimens for each wash interval are tested, similar to that described in U.S. military GL/PD specifications (Appendix C, pg. 34 and Appendix D, pg. 36). Two species of mosquitoes, Aedes aegypti and Anopheles albimanus, will be tested separately. Eight subjects will be tested using each fabric and mosquito species combination"* (V1:19). Each subject will serve as their own treatment and control. The experimental design together with the testing paradigms for *Ae. aegypti* and *An. albimanus*, respectively, are illustrated in tables 3.4.2, 3.5.3, and 3.5.4 on p. 20 of 210 in Volume 1.

(f) What is the plan for statistical analysis of the data?

"*The primary objective of the data analysis is to estimate the overall (or 'mean') level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in Section 8.5. These values will be averaged over all subjects to obtain mean observed bite protection values that can be used as a check on any model-based bite protection estimates.*

"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. A subject term will be added as a fixed effect in the model to adjust for subject-subject differences. (Alternatively, subjects could be treated as a random effect and the within-subject correlation accommodated using either generalized estimating equations or a mixed effect GLiM.) Use of the log link makes it possible to obtain an estimate and confidence interval for the ratio of the treatment and control bite-through rates. The estimates and confidence intervals for percent bite protection are obtained from the relationship:

"Percent Bite Protection = [1 – (treatment rate) / (control rate)] × 100%

"The GLiM model-based bite protection estimates could be obtained by analyzing multiple models each with just a single treatment group and the matched control group. However, it may also be of interest to compare the bite protections of different types of treated fabric, number of washes, or mosquito species. In this case, it would be necessary to include all of the treatments (and species) of interest in the same model. Since the GLiM uses a log link, hypothesis tests concerning ratios of bite protection can be formulated as linear contrasts in the GLiM" (V1:21-22). A complete explanation and related justifications can be found in Volume 3.

(g) Are proposed statistical methods appropriate to answer the research question?

The analysis will provide the overall bite protection values for each treatment group and the controls. As proposed, the analysis addresses mean values and associated uncertainties.

(h) Does the proposed design have adequate statistical power to definitively answer the research question?

Statistical power is adequately described in §3.5 of Volume 1 and is illustrated in Table 3.5.1. An in-depth analysis is presented in Volume 3. As expected the width of the 95% confidence interval decreases as the number of subjects increases but the relative improvement or 'gain' from increasing the number of subjects decreases as the number of subjects increase. The proposed sample size of 8 subjects represents a reasonable compromise between decreasing confidence interval width and limiting unnecessary human experimentation.

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to test material and mosquitoes in the laboratory. The test material will be cut out of treated military trousers or coats and sewn into "sleeves" at the USDA-ARS testing facility. The test material's active ingredient, etofenprox, has a low acute and chronic risk profile (§3.3 and §3.4, V1:17-18), and the inert ingredients are classified by the Agency as safe for this use. The test material has been tested in animals for acute toxicity. Subjects with known allergic reactions (§8.1.2) are excluded from participation in the test.

Subjects will be exposed to laboratory reared populations of mosquitoes free of mosquito-borne pathogens in the laboratory (V1:27, 44). Subjects with known allergic reactions to mosquito bites will be excluded from research participation (V1:23).

(a) What is the rationale for the choice of test material and formulation?

Efficacy data to satisfy product performance requirements and to support label claims for this product are required by EPA for registration. EPA requires submission of

product performance data for all products claiming efficacy against public health pests.

(b) What is the rationale for the choice of dose/exposure levels and the staging of dose administration?

There is no dosimetry phase in this experiment. Test sample sleeves contain 1% etofenprox (wt/wt). As explained in Volume 1 on page 11 of 210 in the 'Background' section of this protocol, the registrant performs the dosimetry experiments before efficacy testing is conducted to determine: 1) the 'plateau region' at which no additional bite protection is gained from additional compound and 2) the point at which the bite protection begins to decrease substantially, typically this done by treating fabric with a range of concentrations to determine the dose-response curves. The range of concentrations for these preliminary tests is selected to be within an acceptable range based on the toxicity profile of the insecticide/repellent compound to minimize risk to the individual wearing the fabric.

(c) What duration of exposure is proposed?

The exposure period is eight 15-minute periods (2 hours total) for both arms of each subject.

2.3 Endpoints and Measures

(a) What endpoints will be measured? Are they appropriate to the question(s) being asked?

Endpoints/Measures for efficacy evaluation:

- Number of bloodfed and total number of females mosquitoes in each test. The proportion of bloodfed/total will be calculated and expressed as a percentage value. This calculation will be performed for untreated control sleeves and treated sleeves (0x, 20x, and 50x washes).
- 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.
- The overall % bite protection will be calculated and expressed as a mean value for each treatment: 0x, 20x, 50x washes for coats and trousers.

The endpoints are appropriate to the questions being asked and address uncertainty associated with the samples size, between subject variation, % bite values, and the overall bite protection value.

The data form for each 15 minutes sleeve test is presented in Appendix I on page 190 of 210. A completed sample data sheet is shown in Appendix J on page 192 of 210.

(b) What steps are proposed to ensure measurements are accurate and reliable?

- Standard Operating Procedures (SOPs) will be in place that must meet Good Laboratory Practices requirements.
- 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.
- Laboratory technicians and the study director will track test sleeve samples and closely monitor the testing.
- Alternate subjects will be enrolled to ensure adequate sample size.
- Counts of bloodfed mosquitoes and the total number of mosquitoes in the cage will be determined by a research technician.
- The test sleeve samples will be assayed by the Analytical Unit (p. 15 of 210) and the amount of etofenprox reported (pp. 31-32 of 210) as a surface concentration of etofenprox in units of mg/cm^2, which is commonly done for treated fabrics.

(c) What QA methods are proposed?

As explained in Volume 1, §7.0 on p. 22 of 210 a separate, professional Quality Assurance Unit (QAU) will inspect the study: "Quality assurance of this study will be carried out in accordance with Good Laboratory Practice (GLP) Standards 40 CFR 160. Written reports of all findings from the Quality Assurance Officer will be provided to the study director and management. Any part of the study found by the Quality Assurance Officer to be likely to affect the integrity of the study will be brought the attention of the study director. A statement signed by the Quality Assurance Officer listing the phases inspected, inspection dates, and dates reported to the study director and management will be included in the final report. All deviations and amendments will be recorded and reported as per GLP guidelines.

Additionally, fabric samples will be retained indefinitely for further analysis and verification as requested by NSRDEC for quality control.

The quality assurance unit of the analytical laboratory will provide the study director and the study director's management with relevant data, process, and report audits to meet Environmental Protection Agency GLP requirements."

(d) How will uncertainty be addressed? Will point estimates be accompanied by measures of uncertainty?

Uncertainty is addressed in the experimental design and selection of the number of subjects as described in §3.5. The statistical analysis also discusses uncertainty in §6.0. Volume 3 provides any in-depth discussion of uncertainty associated with the experimental design and data to be generated and analyzed. The objective of the data analysis 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]. The numbers of bloodfed and total female mosquitoes found with treated and control fabric (clothing) for each subject will be analyzed as binomial distributed data in a generalized linear model (GLiM) using a log link, generalized estimating equations or a mixed effect GLiM. This is largely dependent on the 'subject term', which may be treated as a fixed or random effect to adjust for within-subject differences (V1:22). Volume 3 includes a detailed explanation of the statistical analysis.

3. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern?

The population of concern is U.S. military personnel who would wear FRACUs treated with etofenprox.

(b) From what populations will subjects be recruited?

Subjects will be recruited from the general population in Gainesville, Florida through the use of an advertisement placed in a local newspaper and on bulletin boards in University of Florida campus buildings.

(c) Are expected participants representative of the population of concern? If not, why not?

Since Gainesville, Florida is a university community and given that the advertisements will be placed within campus buildings, the population from which subjects will be recruited is likely younger than the general U.S. population, but may mirror the population of concern – U.S. military personnel – in terms of over-representation of younger individuals.

(d) Can the findings from the proposed study be generalized beyond the study sample?

Yes.

3.2 Equitable Selection of Subjects

(a) What are the inclusion/exclusion criteria? Are they complete and appropriate?

The inclusion/exclusion criteria are complete and appropriate except that "sensitivities to pesticides or other chemical products" and "people with cuts, scrapes or skin conditions such as psoriasis or eczema on their arms or hands" should be added as additional exclusions.

"Inclusion/Exclusion Criteria

1. Must be between the ages of 18-62 years old.
2. Must be able to speak and read English.
3. Children (under the age of 18), and pregnant or lactating women will be excluded.
4. Exclusion of people in poor health or physical condition.
5. People who are hypersensitive to or phobic of mosquito bites will be excluded.
6. Exclusion of people known to be sensitive to the test material.
7. Exclusion of people with a relationship to the study director or sponsor (students or employees of the study director or sponsor)." (V1:23)

(b) What, if any, is the relationship between the investigator and the subjects?

None. People with a relationship to the study director or sponsor (students or employees of the study director or sponsor) are excluded from becoming subjects.

(c) Are any potential subjects from a vulnerable population?

No.

(d) What process is proposed for recruiting and informing potential subjects?

"Subjects will be recruited from the general population in Gainesville, Florida through a printed advertisement placed in the Gainesville Sun (local newspaper) and on bulletin boards in University of Florida buildings. The advertisement (Appendix F) will contain a brief description of the testing and financial compensation for participation. Subjects will be compensated $20 for participating in the initial research consent meeting and $25 for each set of sleeves in the testing paradigm. Subjects that are pregnant (see below) will not be allowed to participate but will be paid $25 for screening process. Subjects will receive $200 for completion of testing with the full set of 8 pairs of sleeves. The advertisement will provide a phone number where interested respondents can leave a message. The messages will be reviewed by the study director. Respondents will be called by the study director and undergo preliminary screening via telephone interview to determine if they meet the inclusion and exclusion criteria. Individuals will be recruited from the community and are not from any particular employer or agency, and will have no relationship to the study director." (V1:23)

(e) If any subjects are potentially subject to coercion or undue influence, what specific safeguards are proposed to protect their rights and welfare?

Subjects will be recruited through advertisements in local newspapers. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In

addition, students or employees of the study director or sponsor are excluded from participation.

3.3 Remuneration of Subjects

(a) What remuneration, if any, is proposed for the subjects?

"Subjects will be compensated $20 for participating in the initial research consent meeting and $25 for each set of sleeves in the testing paradigm. Subjects that are pregnant (see below) will not be allowed to participate but will be paid $25 for completing the screening process. Subjects will receive $200 for completion of testing with the full set of 8 pairs of sleeves." (V1:23) Subjects that begin the study but do not complete it will be paid for each pair of sleeves at a rate of $25 per pair. (V1:201)

(b) Is proposed remuneration so high as to be an undue inducement?

No.

(c) Is proposed remuneration so low that it will only be attractive to economically disadvantaged subjects?

No.

(d) How and when would subjects be paid?

Payment will be made in cash before subjects leave the test facility.

4. Risks to Subjects

4.1 Risk characterization

(a) Have all appropriate prerequisite studies been performed? What do they show about the hazards of the test material?

Etofenprox is an EPA-registered pesticide with an essentially complete supporting toxicity database. It has been tested extensively in animals and is of low toxicity by all routes of exposure. The acute dermal LD_{50} of etofenprox is greater than 2,100 mg/kg body weight. Etofenprox is not a skin sensitizer.

The treated fabric that will be placed on the forearm of subjects will contain at most 105.9 mg of etofenprox (for 0x washes), which results in a maximum dose of 211.8 mg/subject. Assuming an adult weighs 70kg, the maximum etofenprox dose from exposure to the treated fabric equals 3.02mg/kg per subject, assuming that there is 100% absorption through the skin, resulting in an estimated MOE = 695. This MOE does not exceed EPA's level of concern (MOE= 100) (V1:18).

Results from toxicity testing:

- A primary eye irritation study on rabbits showed that etofenprox is a minimal irritant to the eyes.
- A dermal sensitization study in Guinea pigs (Buehler method) showed that etofenprox is not a contact sensitizer.
- A primary skin irritation study in rabbits study showed that etofenprox is minimally irritating to the skin.
- The single dose acute dermal LD_{50} of the etofenprox is >2,100 mg/kg in rabbits.
- The acute oral LD_{50} of etofenprox is >5,000 mg/kg in dogs.

Due to dermal observations resembling skin irritation in a 28-day dermal toxicity study with rabbits, the etofenprox registrant, Mitsui Chemicals, will soon be conducting a 28-day dermal toxicity study in rabbits with etofenprox-treated fabric. Do not initiate this research until the results of that study have been submitted to and reviewed by EPA.

(b) What is the nature of the risks to subjects of the proposed research?

Risks to subjects include the risk of exposure to biting mosquitoes; the risk of exposure to disease vectors; the risk of exposure to the test material; risks related to receiving an unexpected result on a pregnancy test; and the risk of a loss of confidentiality.

(c) How do proposed dose/exposure levels compare to the established NOAELs for the test material?

A dose/endpoint was not chosen for a dermal risk assessment since no systemic toxicity was observed at the limit dose (1000 mg/kg/day) following repeated dermal exposure to rabbits for 28 days to etofenprox technical. However, in the same study a dermal LOAEL was identified that equaled 400mg/kg/day based on an increased incidence of dermal observations (scabbing, crusting, desquamation, and exfoliation) together with histopathological changes that included diffuse epidermal hyperplasia in both sexes of rabbits. Based on a dermal absorption study in rats with etofenprox technical, very little etofenprox is absorbed through skin (7% of the applied dose) and metabolism is rapid with nearly all etofenprox excreted in feces and urine within 24 hours after application to the skin of test animals. Taken together, subjects in this study will be exposed to very little etofenprox when the treated fabric is placed on the skin during testing with the maximum dose of 3.02 mg/kg per subject being much lower than the systemic NOAEL >1000 mg/kg/day for the dermal route of exposure and more than 100x less than the dermal LOAEL observed in rabbits.

Due to dermal observations resembling skin irritation in a 28-day dermal toxicity study conducted 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. The new study will test the same fabric that subjects will be exposed to in the proposed efficacy study under review

here. Do not initiate this research until the results of the product specific fabric study have been submitted to and reviewed by EPA.

(d) What is the probability of each risk associated with the research? How was this probability estimated?

No numerical probability is estimated, but risks have a low probability of occurrence. Risks are minimized in the protocol by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to the test material; applying clear stopping rules; and by incorporating procedures to keep the results of pregnancy testing private and permit discrete withdrawal.

4.2 Risk minimization

(a) What specific steps are proposed to minimize risks to subjects?

Risks from exposure to biting mosquitoes.

- Candidates who are hypersensitive to or phobic of mosquito bites are excluded (V1:23, 195)
- Subjects are alerted in the consent form to the possibility of experiencing a skin reaction to mosquito bites, and are advised to inform the study director, laboratory technician, or on-call nurse if they believe they are having a reaction (V1:199)
- Over-the-counter topical steroid cream to relieve itching will be available for use by subjects (V1:199)
- A nurse familiar with the protocol will be on-call to provide advice or assistance in the event that a subject experiences a reaction (V1:199)

Risks of exposure to disease vectors.

"Since these mosquitoes are colony reared and not exposed to disease agents, the risk of contracting an infectious disease from bites is negligible. Mosquitoes from colony have been reared through immature stages and delivered to the laboratory testing room just prior to pupal eclosion. Mosquito pupae are received from colony and allowed to eclose into cages in the laboratory. For those mosquitoes to transmit pathogens such as dengue fever or malaria, they would need to escape, bite an infected human and then return back to the cage for selection in tests. Even if a mosquito escapes, the probability that it could return into a cage and be used in testing is negligible. There is also an incubation period during which the disease must be in the mosquito in order to transmit to another organism. Additionally, all mosquitoes that are contained in the cages are not allowed to feed on any organisms and thus do not have the opportunity to be infected with pathogens prior to testing. The mosquito colonies are reared at the Center for Medical, Agricultural, and Veterinary Entomology in a USDA facility in Gainesville, Florida using a membrane feeding system and bovine blood. The citrated bovine blood is pathogen free (certified sterile by the supplier). The

mosquitoes will not have been fed on any humans prior to the study. The colonies used have been maintained in this manner since being established in 1952 while in Orlando for Ae. aegypti, and in El Salvador in 1974 for An. albimanus." (V1:27)

Risks from exposure to the test material.

- Candidates who are known to be sensitive to the test material are excluded. (V1:23) It is also recommended that the protocol exclude subjects who are sensitive to pesticides or other chemical products.
- It is recommended that the protocol exclude subjects with cuts, scrapes, or skin conditions such as psoriasis or eczema on their arms or hands. These conditions could increase the possibility of a reaction to test material.

Risks of stress and loss of confidentiality related to pregnancy testing

- The protocol provides for discrete handling of the pregnancy testing that is required of female subjects on the day of the study.
- Female subjects self-administer the pregnancy test in a private restroom. (V1:34, 195)
- After completing the test, each female subject is asked if she would like to continue in the study. If her answer is no, then no further questions are asked; she will not be asked to share the result with anyone. If her answer is yes, the result of pregnancy test will be verified by only one member of the research (EPA has recommended that the protocol specify that it be a female member of the research team). (V1:34, 195)
- For females who proceed with the testing, the result of the pregnancy test is recorded in the raw data and kept confidential. (V1:34)

(b) What stopping rules are proposed in the protocol?

"The study will be stopped if the test site becomes unsafe for any reason, biting pressure falls below threshold needed, biting pressure rises too high for subject comfort or safety, subject asks to withdraw, subject is unattractive to target species, subject exhibits hypersensitivity to insect bites during test, subject exhibits sensitivity to the test materials during the test." (V1:46)

(c) How does the protocol provide for medical management of potential illness or injury to subjects?

"On the day of the testing, there will be a nurse on call who has read the protocol and discussed the research with the study director to assist if needed. UF Health Shands Hospital is located 0.5 miles from the testing facility. Medical care for research-related injuries will be provided at no cost to the subjects." (V1:43)

"If you are injured or become ill during the study, tell the study director, laboratory technician, or on call nurse immediately. The study director will obtain emergency medical treatment for you, if necessary. If your illness or injury is a direct result of being in this study, the sponsor of this research will cover the costs of any necessary

medical treatment that is not covered by your insurance or the insurance of a third party under which you are covered.

"If, after participating in the study, you believe you have become ill as a direct result of your participation in the study, please call the study director, Ulrich Bernier Ph.D., at (352) 871-8469 (24 hours) or 352-374-5917 or Wendy L. Morrison, RN, BSN at 352-339-2179 (24 hours)." (V1:202)

(e) How does the protocol provide for safety monitoring?

Subjects are clearly and repeatedly informed that they may remove themselves for any reason from the study at any time. All subjects are asked to immediately tell the study director, laboratory technician, or on-call nurse if they believe they are experiencing a reaction or feel ill during the study. The consent form also states that if, after participating in the study, a subject believes he or she has become ill as a result of their participation in the study, they should contact the Study Director or the on-call nurse anytime, 24-hours a day. Their telephone numbers are provided.

On the day of testing, a nurse who has read the protocol and discussed the research with the study director will be on call. (V1:43)

(f) How does the protocol provide for post-exposure monitoring or follow-up? Is it of long enough duration to discover adverse events which might occur?

The consent form states: "If, after participating in the study, you believe you have become ill as a direct result of your participation in the study, please call the study director, Ulrich Bernier Ph.D., at (352) 871-8469 (24 hours) or 352-374-5917 or Wendy L. Morrison, RN, BSN at 352-339-2179 (24 hours)." There is no time limit given. *(V1:202)*

(g) How and by whom will medical care for research-related injuries to subjects be paid for?

"If your illness or injury is a direct result of being in this study, the sponsor of this research will cover the costs of any necessary medical treatment that is not covered by your insurance or the insurance of a third party under which you are covered." (V1:202)

5. Benefits

(a) What benefits of the proposed research, if any, would accrue to individual subjects?

There are no direct benefits to subjects.

(b) What benefits to society are anticipated from the information likely to be gained through the research?

"While there are no direct benefits to the subjects participating in this research study beyond a small compensation for their time, there are indirect benefits to both the subjects and society. First, the data collected in this study will be used establish the level at which insecticide/repellent treated uniforms prevent mosquito bites. Data generated from this study may be used for U.S. EPA registration of a novel insecticide/repellent treatment for military clothing.

"It is expected that this laboratory data can be translated to bite protection of covered areas of the body for individuals in the field. Because not all individual biting arthropods carry disease agents, a reduction (not necessarily complete elimination) of biting pressure is expected to also minimize the risk of disease transmission and subsequently reduce overall arthropod-borne disease rates. Reduction of disease rates is dependent upon the infectivity rates of the insects in a given area, the overall biting pressure, and use compliance. Through factory treatment, we can ensure a high level of compliance for use of treated military clothing which should significantly reduce overall arthropod-borne disease among military populations, particularly when used in conjunction with other prevention methods, such as topical repellents and mosquito netting.

"These studies are conducted to better protect American military forces from nuisance bites and from bites that lead to arthropod-borne diseases. The results of these studies may also lead to better protection of U.S. civilians both domestically and while they travel abroad because novel repellents can be incorporated into civilian clothing as a potentially better alternative to the permethrin-treated clothing already available on the market." (V1:45)

(c) How would societal benefits be distributed? Who would benefit from the proposed research?

One beneficiary will likely be the sponsor who is seeking EPA-registration for etofenprox-treated clothing. Indirect beneficiaries would include the U.S. military and civilians who may benefit from wearing etofenprox-treated clothing.

(d) What is the likelihood that each identified societal benefits would be realized?

The testing is likely to demonstrate that the formulation is effective at providing the level of bite protection sought by the U.S. military, which could result in better protection of the U.S. military from mosquito bites and perhaps better protection of U.S. civilians through the use of a new type of repellent-treated clothing.

6. Risk/Benefit Balance

(a) How do the risks to subjects weigh against the anticipated benefits of the research, to subjects or to society?

"Risks have been minimized in terms of disease (through use of colony mosquitoes in a laboratory setting only) and with respect to numbers of replicates that a subject will be testing (one control and 3 treated specimens). In this study, the biting of subjects will be incurred voluntarily to help develop a new product that will provide optimized protection against mosquito bites in a field setting where the risk of disease is a bona fide threat." (V1:45)

The protocol systematically reduces risks to subjects without reducing the robustness of the scientific design. No reasonable opportunities to further reduce subject risk have been overlooked. The resulting residual risk to subjects is very low. The potential benefits from availability of a wider variety of effective repellent-treated clothing are likely to be realized, and make the residual risks to subjects in this proposed research reasonable.

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

Western Institutional Review Board

(b) Is this IRB independent of the investigators and sponsors of the research? Yes

(c) Is this IRB registered with OHRP? Yes

(d) Is this IRB accredited? If so, by whom?

WIRB has full AAHRPP accredditation.

(e) Does this IRB hold a Federal-Wide Assurance from OHRP?

Yes.

(f) Are complete records of the IRB review as required by 40 CFR 26.1125 provided?

Yes.

(e) What standard(s) of ethical conduct would govern the work?

This is a protocol for third-party research involving what EPA has interpreted to be intentional exposure of human subjects to a pesticide. The study is being conducted with the intention of submitting the resulting data to EPA under the Federal Insecticide

Fungicide and Rodenticide Act (FIFRA). Thus, the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply.

8. Informed Consent

(a) Will informed consent be obtained from each prospective subject?

Yes.

(b) Will informed consent be appropriately documented, consistent with the requirements of 40 CFR 26.1117?

Yes.

(c) Do the informed consent materials meet the requirements of 40 CFR 26.1116, including adequate characterization of the risks and discomforts to subjects from participation in the research, the potential benefits to the subject or others, and the right to withdraw from the research?

Yes.

(d) What is the literacy rate in English or other languages among the intended research subjects?

Ability to speak and read English is a requirement for participation.

(e) What measures are proposed to overcome language differences, if any, between investigators and subjects?

N/A

(f) What measures are proposed to ensure subject comprehension of risks and discomforts?

Frequent opportunities to ask questions during the consent process.

(g) What specific procedure will be followed to inform prospective subjects and to seek and obtain their consent?

"The consent meeting will be held privately between the respondent and the study director….During the consent meeting, respondents will be given a detailed explanation of the procedures of the study and be asked to watch a movie of the testing process. They will be allowed sufficient opportunity to consider whether or not to participate before signing the consent form. The participants will be informed of how many bites they are likely to obtain and what symptoms of arthropod-borne reactions they should be alert for

after participation in the study. Participants will be provided a detailed consent form (Appendix H) outlining the risks of participating in the study." (V1:25)

(h) What measures are proposed to ensure fully voluntary participation and to avoid coercion or undue influence?

Candidates are offered repeated opportunities to decide not to participate; participants are offered repeated opportunities to withdraw. Exclusion factors rule out participation by employees or students of the Study Director. Recruitment of alternate subjects reduces the likelihood that subjects might be reluctant to withdraw.

9. Respect for Subjects

(a) How will information about prospective and enrolled subjects be managed to ensure their privacy?

Subject-identifying information will be recorded only once; all subsequent data records and reports will refer to individual subjects only by an arbitrary code. *"To protect your privacy, we will give you an identification number for this study and we will record and report all data under that number. We will keep only one record linking your name to this number, and we will store it away from other data. We will not identify you by name or in any other way in the study report." (V1:201)*

Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing. *"You will be asked to take the test in a private restroom. If, after taking the test, you still wish to participate in the study, you will be asked to show the result of the test to a female laboratory technician so that she can verify that you are not pregnant. If you withdraw from the study after taking the pregnancy test, you will not be asked to share the result of the test with anyone."* (V1:195)

(b) How will subjects be informed of their freedom to withdraw from the research at any time without penalty?

The informed consent form states: *"Your participation in this study is voluntary. You may decide not to be in the study or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled." (V1:202)*

"If you cannot keep your arms in the cage for the entire 15 minutes, you may remove them at any time." (V1:199)

(c) How will subjects who decline to participate or who withdraw from the research be dealt with?

Subjects who decide not to participate after completing the informed consent meeting will be paid $20. Subjects who decide not to participate after taking a pregnancy test will

be paid $25. Subjects who withdraw from the research will be paid for the number of pairs of sleeves that they completed at the rate of $25 per pair. (V1:201)

Attachment 2

§ 26.1111 Criteria for IRB approval of research
USDA Protocol for Bite Protection of Etofenprox-treated Fabric

Criterion	Y/N	Comment/Page Reference
(a)(1)(i) Risks to subjects are minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk.	Y	
(a)(1)(ii) Risks to subjects are minimized, whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.	N/A	
(a)(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.	Y	
(a)(3) Selection of subjects is equitable, taking into account the purposes of the research and the setting in which it will be conducted, and being particularly cognizant of the special problems of research involving vulnerable populations, such as prisoners, mentally disabled persons, or economically or educationally disadvantaged persons.	Y	
(a)(4) Informed consent will be sought from each prospective subject or the subject's legally authorized representative, in accordance with, and to the extent required by §26.1116.	Y	
(a)(5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §26.1117.	Y	
(a)(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.	Y	
(a)(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.	Y	
(b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, additional safeguards have been included in the study to protect the rights and welfare of these subjects.	N/A	

§26.1116 General requirements for informed consent
USDA Protocol for Bite Protection of Etofenprox-treated Fabric

Criterion		Y/N	Comment/Page Reference
No investigator may involve a human being as a subject in research covered by this subpart unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative		Y	
An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence		Y	
The information that is given to the subject or the representative shall be in language understandable to the subject or the representative		Y	
No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence		Y	
(a) In seeking informed consent the following information shall be provided to each subject	(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental	Y	
	(2) A description of any reasonably foreseeable risks or discomforts to the subject	Y	
	(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	Y	
	(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	Y	
	(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained	Y	
	(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained	Y	
	(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject	Y	
	(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled	Y	
(b) When appropriate, one or more of the following elements of information shall also be provided to each subject	(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject may become pregnant) which are currently unforeseeable	Y	
	(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	Y	
	(3) Any additional costs to the subject that may result from participation in the research	Y	
	(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	Y	
	(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject	N/A	
	(6) The approximate number of subjects involved in the study	Y	
(e) If the research involves intentional exposure of subjects to a pesticide, the subjects of the research must be informed of the identity of the pesticide and the nature of its pesticidal function.		Y	

§26.1117 Documentation of informed consent
USDA Protocol for Bite Protection of Etofenprox-treated Fabric

Criterion	Y/N	Comment/Page Reference
(a) Informed consent shall be documented by the use of a written consent form approved by the IRB and signed by the subject or the subject's legally authorized representative. A copy shall be given to the person signing the form.	Y	Consent form pp. 193-203
(b)(1) The consent form may be a written consent document that embodies the elements of informed consent required by §26.1116. This form may be read to the subject or the subject's legally authorized representative, but in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed; or	Y	Consent form meets requirements of §26.1116; procedure described in protocol §8.1.6 provides adequate opportunity to read it before it is signed.
(b)(2) The consent form may be a short form written consent document stating that the elements of informed consent required by §26.1116 have been presented orally to the subject or the subject's legally authorized representative. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining consent shall sign a copy of the summary. A copy of the summary shall be given to the subject or the representative, in addition to a copy of the short form.	N/A	

Attachment 5

40 CFR 26.1125 Submission of proposed human research for EPA review
USDA Protocol for Bite Protection of Etofenprox-treated Fabric

Any person or institution who intends to conduct or sponsor human research covered by §26.1101(a) shall, after receiving approval from all appropriate IRBs, submit to EPA prior to initiating such research all information relevant to the proposed research specified by §26.1115(a), and the following additional information, to the extent not already included:

Requirement			Y/N	Comments/Page Refs
The following Information, to the Extent not already included:	§1125(a) a discussion of:	(1) The potential risks to human subjects	Y	V1:43-44
		(2) The measures proposed to minimize risks to the human subjects;	Y	V1:43-44
		(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	V1:45
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	none
		(5) The balance of risks and benefits of the proposed research.	Y	V1:45
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		Y	V1:25
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	V1:23, 204
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.		Y	V1:25
	§1125(e): All correspondence between the IRB and the investigators or sponsors.		Y	Volume 2
	§1125(f): Official notification to the sponsor or investigator. . . that research involving human subjects has been reviewed and approved by an IRB.		Y	V1:208-210
all information relevant to the proposed research specified by § 26.1115(a)	(1) Copies of • all research proposals reviewed by the IRB, • scientific evaluations, if any, that accompanied the proposals reviewed by the IRB, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.		Y n/a Y n/a	Volume 2 V1:193-203
	(2) Minutes of IRB meetings . . . in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; • a written summary of the discussion of controverted issues and their resolution.		Y Y Y n/a n/a	Separately provided to HSRB members
	(3) Records of continuing review activities.		n/a	
	(4) Copies of all correspondence between the IRB and the investigators.		Y	Volume 2
	(5) • A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations; • any employment or other relationship between each member and the institution, for example, full-time employee, a member of governing panel or board, stockholder, paid or unpaid consultant.		Y Y	V2:22
	(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).		N	Separately provided to HSRB members
	(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).		n/a	n/a for protocols