(a) Introduction.

(1) Scope and Purpose. This guideline provides recommendations for the design and execution of studies to evaluate the performance of pesticide products intended to repel insects and other arthropods in connection with the products' registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This guidance applies to products intended to be applied directly to human skin, which may be formulated in various way, such as lotions, liquids, or sprays. It does not apply to products applied to clothing or used to repel insects from indoor or outdoor spaces. This guidance recommends appropriate study designs, and methods for selecting subjects, statistical analysis, and reporting.

Any protocol developed using this guidance must adhere to the requirements set forth in several statutes and regulations, including, but not limited to, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) under which EPA regulates repellents, and EPA's rules for the protection of human subjects of research, 40 CFR Part 26, Subparts K through Q, whether specifically identified or described in this guidance or not. Because these studies would support a FIFRA registration and involve intentional exposure of human subjects to the test repellents, among the applicable requirements are that protocols must be reviewed by an Institutional Review Board (IRB), by EPA, and by EPA's Human Studies Review Board (HSRB) before the study is initiated. (40 CFR §26.1109, §26.1125, and §26.1601.)

Although this guideline provides many detailed recommendations, it is guidance and does not bind regulated parties or the public to specific actions. Other test procedures or variants on the suggested procedures may satisfy applicable FIFRA and regulatory requirements, but deviations from this guideline should be fully explained and justified. This guideline is also not binding on EPA. Although it reflects EPA's current views on evaluating the performance of skin-applied repellents, future research or discoveries may lead the Agency to new or different views and interpretations. Moreover, this guideline does not supersede or overrule in any way the regulations governing the research conducted on human subjects as contained in 40 CFR Part 26, subparts K through Q ("Human Research Rule" or "Rule") or any other Agency regulations. To the extent there are any unintended conflicts between this guideline and the Human Research Rule or any other regulation, the regulation at issue governs.

(2) Related Requirements.

(i) FIFRA Informed Consent Requirement. Any research conducted under this guideline is subject to §12(a)(2)(P) of FIFRA, which defines it as an unlawful act "for any person to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are

reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test." Regulations implementing this statutory provision and defining associated record-keeping requirements can be found at 40 CFR §169.2(j) and (k).

(ii) Human Research Rule. Any research conducted under this guideline is covered by the requirements of EPA regulations for the protection of human subjects of research as set out in the Human Research Rule at 40 CFR part 26, subparts K, L, and M. Persons conducting topical repellent efficacy studies need to ensure compliance with all applicable requirements of that rule; the following paragraphs highlight a few of them:

(A) Applicability. Subparts K, L, and M of 40 CFR Part 26 apply to regulated third parties who conduct or sponsor research involving intentional exposure of human subjects, which is intended for submission to EPA for consideration in its actions taken under the pesticide laws—FIFRA and §408 of FFDCA. (40 CFR §26.1101) Efficacy testing of topically applied repellents meets the regulatory definitions of "research" with "human subjects" involving "intentional exposure," and must therefore be conducted and submitted to EPA in compliance with the requirements of these subparts. (See definitions at 40 CFR §26.1102.)

(B) Prohibition of research involving pregnant or nursing women or children. 40 CFR §26.1203 as amended effective August 22, 2006, provides:

"Under no circumstances shall a person conduct or sponsor [covered] research that involves intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child."

(C) Required review by Institutional Review Board (IRB). 40 CFR §26.1109(a) provides:

"An IRB shall review and have authority to approve, require modifications in (to secure approval), or disapprove all research activities covered by this subpart."

(D) Required Pre-testing submissions to EPA. 40 CFR §26.1125 provides:

"Any person or institution who intends to conduct or sponsor human research covered by §26.1101(a) shall, after receiving approval from all appropriate Institutional Review Boards (IRBs),

submit to EPA prior to initiating such research all information relevant to the proposed research specified by §26.1115(a), and, to the extent not already included:

(a) A discussion of:
(1) The potential risks to human subjects;
(2) The measures proposed to minimize risks to the human subjects;
(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue;
(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and
(5) The balance of risks and benefits of the proposed research.

(b) All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.

(c) Information about how subjects will be recruited, including any advertisements proposed to be used.

(d) A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.

(e) All correspondence between the IRB and the investigators or sponsors.

(f) Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB."

(E) Required post-testing submissions to EPA. 40 CFR §26.1303 provides:

"Any person who submits to EPA data derived from human research covered by this subpart shall provide at the time of submission information concerning the ethical conduct of such research. To the extent available to the submitter and not previously provided to EPA, such information should include:

(a) Copies of all of the records relevant to the research specified by §26.1115(a) to be prepared and maintained by an IRB.

(b) Copies of all of the records relevant to the information identified in §26.1125(a) through (f).

(c) Copies of sample records used to document informed consent as specified by §26.1117, but not identifying any subjects of the research."

(F) Prohibition of EPA reliance on unethical human research. 40 CFR §26.1705 provides:

" . . . EPA shall not rely on data from any research initiated after April 7, 2006, unless EPA has adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part, or if conducted in a foreign country, under procedures at least as protective as those in subparts A though L of this part."

(iii) Good Laboratory Practice Standards. Good Laboratory Practice Standards (GLP) as defined in 40 CFR part 160 apply to both laboratory and field studies of repellent efficacy. According to 40 CFR §160.17: "EPA may refuse to consider reliable for purposes of supporting an application for a research or marketing permit any data from a study which was not conducted in accordance with this part." 40 CFR §160.12(b) requires with any submitted research data "[a] statement describing in detail all differences between the practices used in the study and those required by this part." Additionally, newly-revised Part 158 specifies that "applicants must adhere to the good laboratory practice (GLP) standards described in 40 CFR part 160 when conducting studies." (40 CFR §158.70(b)).

(3) Organization of the Guideline. This guideline begins with definitions of special importance in understanding this guideline (Section b).

Sections (c) through (h) provide general guidance applicable to all topical repellent efficacy testing, whether conducted in the laboratory or in the field, and without regard to target species. Each of these six sections discusses one of the primary stages of repellent testing:

(c) Development of protocols for repellent studies
  (1) Scientific design of repellent studies
  (2) Ethical justification for repellent studies

(3) Subject selection and informed consent
(4) Respect for subjects
(5) Data collection and reporting
(d) Review of protocols for repellent studies
(e) Changes to IRB-approved research before execution
(f) Execution of repellent studies
(g) Reporting of completed repellent studies
(h) Retention of records

Sections (i) through (o) provide guidance specific to different kinds of testing of repellents—whether intended to estimate empirically a typical consumer dose (section (i)), or to test repellent efficacy in the laboratory or field to particular species of arthropods (sections (j) through (o).

(i) Specific guidance for dose-determination studies
(j) Specific guidance for laboratory studies of mosquito repellency
(k) Specific guidance for field studies of mosquito repellency
(l) Specific guidance for laboratory studies of stable fly repellency
(m) Specific guidance for field studies of biting fly repellency
(n) Specific guidance for laboratory studies of flea repellency
(o) Specific guidance for laboratory studies of tick and chigger repellency

A final section (p) lists references considered in the development of this guideline.

Three appendices are attached as well:

Appendix A: Checklist of Elements Required by 40 CFR §26.1125
Appendix B: Framework for Science and Ethics Reviews of Proposed Human Research
Appendix C: Checklist of Elements Required by 40 CFR §26.1303

(b) Definitions. The following definitions are of special importance in understanding this guideline. They apply only in the context of this guidance document and are not intended to be more generally applicable.

(1) The following events may be used in measuring repellent efficacy:

(i) A ***landing*** is the act of a flying or jumping insect or other arthropod alighting on human skin without probing or biting.

(ii) A ***probe*** is the act of penetrating human skin by the mouthparts of an insect or other arthropod without ingestion of blood.

(iii) A ***bite*** is the act of penetrating human skin by the mouthparts of an insect or other arthropod with ingestion of blood, typically associated with abdominal swelling and color change.

(iv) A ***crossing*** is the act of passage by a tick or chigger from an area of untreated skin to an area of treated skin. A crossing may be quantified by the distance the tick moves onto treated skin, or by how long the tick remains on treated skin.

(2) An ***unconfirmed event*** is a landing, probe, bite, or crossing not followed by another similar event within 30 minutes.

(3) A ***confirmed event*** is one landing, probe, bite, or crossing followed by another similar event within 30 minutes. The first event is confirmed by the second; the second event is the ***confirming event***.

(4) A ***human subject*** is a living individual about whom an investigator conducting research obtains data through intervention or interaction with the individual, or identifiable private information. By this definition, both untreated control subjects and treated subjects are considered human subjects of repellent efficacy testing research.

(5) ***Questing*** is the behavior of ticks or chiggers actively seeking a host.

(6) A ***repellent*** is a product intended to disrupt the host-seeking behavior of insects or other arthropods, driving or keeping them away from treated human skin.

(7) ***Complete Protection Time (CPT)*** is the time from application of a repellent until the first confirmed event showing efficacy failure.

(8) ***Dose determination*** is a testing procedure used to estimate a "typical consumer dose" of a topical repellent.

(c) Development of protocols for repellent studies. The first major stage of repellent testing is development of a protocol. Under EPA's amended rules for the protection of human subjects—the Human Research Rule—protocol development is the focus of far more attention than was the case before that rule took effect in April 2006. A detailed protocol must be approved by an Institutional Review Board and, accompanied by supporting documentation complying with 40 CFR §26.1125, must be reviewed by EPA and EPA's Human Studies Review Board before research is initiated.

Development of a protocol that meets the requirements of 40 CFR §160.120 and all other applicable scientific and ethical standards is critical to all later stages of repellent testing. General considerations in developing a protocol for repellent studies include its scientific

design, its ethical justification, subject selection and informed consent, respect for subjects, and data collection and reporting. Each of these topics is discussed in more detail in a specific sub-section below.

(1) Scientific Design of Research. To be scientifically justified, the proposed research should address an important research question that cannot be answered by existing data. In addition, the design should be such as to be likely to provide a definitive answer to the research question. The design should include a detailed description of the experimental design, including:

(i) Objectives. The objective of most repellent efficacy testing is to estimate how long after treatment a repellent will continue to protect users from the target pest. This period of effective repellency may be characterized as Complete Protection Time (CPT), or Relative Protection (RP) of one treatment as compared to another or to an untreated control. In dose-determination testing associated with repellent testing the objective is typically to estimate a "typical consumer dose" for use in later repellent testing. In all cases the scientific objective should be stated clearly.

(ii) Test materials. Repellent efficacy should be tested using the end-use formulation as registered or as proposed for registration. Test materials should be stored at ambient temperature and humidity before use.

(iii) Choice of endpoints and measures. Endpoints chosen for the study should be appropriate for the specific objectives of proposed research and likely to provide a robust answer to the research question while minimizing the risks to subjects. Considerations in endpoint selection for dose determination and repellency include:

(A) Dose determination. To estimate a "typical consumer dose" the endpoint of concern is a rate of application, typically expressed as mg/cm^2 or as ml/cm^2 of treated skin surface. Because each subject is likely to apply a different amount of repellent in uncontrolled trials, the endpoint should be calculated as the mean of multiple applications by many subjects.

(B) Repellency. The endpoint of repellency testing should be selected to show a failure of repellent efficacy for subjects treated with a "typical consumer dose." Failure in a test to determine Complete Protection Time (CPT) may be defined as the first event—i.e., the first landing, bite, or crossing—or as the first confirmed event—i.e., the first landing, bite, or crossing confirmed within 30 minutes by another similar event.

(*1*) In repellency testing with mosquitoes or biting flies the study design should choose either landings or bites as the event showing failure of repellent efficacy. The choice of landings for field testing reduces the risk of exposing subjects to vector-borne diseases. Even in laboratory testing using laboratory-reared, disease-free insects, the choice of landings reduces the risk of a reaction to bites. A proposal to use bites as an endpoint in either field or laboratory testing should be justified.

(*2*) In repellency testing with fleas, the preferred event to show failure of repellent efficacy is flea landings on treated skin.

(*3*) In repellency testing with ticks or chiggers the preferred event to show failure of repellent efficacy is a "crossing" from untreated skin onto treated skin.

(iv) Sample size. The sample should be large enough to be likely to yield a definitive answer to the research question being addressed. The proposed sample size should be justified statistically, taking into account the specific characteristics of the proposed research and the required accuracy and precision of the results. In general, a larger sample is needed if you expect protection over a longer duration, or greater variability between subjects. Because of the many uncontrollable factors tending to increase variability between subjects under field conditions, field tests may require a larger sample than laboratory tests. Other factors to consider are the number of treatments, type of experimental design, and heterogeneity of the sample population (e.g., ages, gender) and the environment (different habitats, different species and population densities). The greater the heterogeneity, the larger the sample required.

Two methods are commonly used to estimate sample size:

- Power calculations: Sample size is chosen to achieve a certain probability for correctly rejecting the null hypothesis of no treatment effects. Power is defined as one minus the probability of incorrectly accepting a null hypothesis.

- Confidence interval calculations: Sample size is chosen to achieve an acceptably small confidence interval 100 (1-α) % (for $\alpha = 0.05$) around a treatment mean estimate. The narrower the confidence interval, the more reliable the point estimate is and the larger the sample size must be.

Calculation of sample size requires an estimate of the sample variance of the point estimate. Point estimates follow different distributions. Complete Protection Time might follow an exponential distribution, while standard deviations around point estimates of relative protection (based on percent reduction in number of bites) are likely to follow a Poisson model. This can only be approximated when the number of bites per subject is large; an approach not recommended for ethical reasons.

Withdrawal of test subjects from the study before failure of efficacy decreases the sample size and may compromise the validity of test results. The protocol should fully describe how sample size was determined, and how possible premature withdrawal of subjects from the test will be treated.

(v) **Allocation of subjects to treatments.** Subjects should be allocated to treatments randomly and, to the extent possible, blindly. A perfect random sample is difficult to achieve in practice. The best way to ensure random sampling is to use a table of random numbers. Such tables are available in most statistic books and related references. Multiple treatments per subject are permissible providing care is taken in the study design to prevent cross-contamination or confounding interference of different repellents tested in close proximity. It is particularly important for subjects receiving multiple treatments on different limbs not to rub or contaminate the repellent treatments, to keep results independent. The number of treatments per subject should be limited by the feeding behavior of the species targeted for testing, and whether the same subject is also serving as his/her own control. For example, for mosquito species feeding close to the ground on lower limbs, treatments should be applied only to legs. Multiple treatments of the same subject do not increase the sample size; a single subject can only be counted once in determining the sample size.

(vi) **Untreated controls.** To minimize risks to subjects in field tests both the number of untreated control subjects and the duration of their exposure should be minimized. Untreated controls should be exposed intermittently, and then only long enough to verify adequate pest pressure. It is statistically powerful and appropriate for treated subjects to serve as their own untreated controls.

(vii) **Preparation of subjects.** Before treatment or use as an untreated control, subject limbs should be washed with an unscented detergent and carefully rinsed and dried. Subjects should avoid alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) for at least twelve hours before and throughout the test. In field tests subjects should

avoid activities that might increase perspiration, and avoid abrading, rubbing, touching, or wetting the treated area.

(viii) **Treatment of subjects.** Subjects in trials designed to estimate a typical consumer dose should self-treat with the repellent, which should be provided in the type of container and delivery system (e.g., pump spray, aerosol spray, towelette) and with the directions for use intended for commercial distribution. Subjects in repellency trials should all receive a standard dose, delivered in a way that will ensure consistent application and uniform coverage.

(ix) **Statistical analysis plan.** Protocols should include a full description, explanation, and justification for the statistical methods to be employed to analyze test results, taking into account the specific study objectives.

For repellent tests to determine complete protection time—i.e., how long a product protects subjects from landings or bites—statistical testing should examine variability among subjects and between test sites and test sessions.

The statistical analysis plan should consider whether the experimental design and sample size are likely to result in a data set fitting the normal distribution. When results are normally distributed, it is generally appropriate to report the mean Complete Protection Time (CPT) across all treated subjects with its standard error. When the data do not fit a normal distribution, it may be possible to transform them to fit a distribution for which a parametric method of analysis can be employed. When the data do not fit and cannot be transformed to fit an underlying distribution, non-parametric analyses may be appropriate. The statistical analysis plan should address all these possibilities.

When the objective of the study is to test the hypothesis that the true CPT is equal to a certain value (CPT = x hours) against the one-sided alternative that the CPT exceeds that value (CPT > x hours), and the shape of the underlying data distribution is unknown, non-parametric tests such as Kaplan-Meier survival analysis may be adequate.

(x) **QA/QC plan.** Protocols should provide for periodic quality assurance inspections adequate to ensure the integrity of the study and consistent with the requirements of EPA's Good Laboratory Practices regulations (40 CFR part 160.)

(2) **Ethical Justification for Research.** Because repellent efficacy testing is never directly beneficial to the subjects of the research, the risks to subjects must be minimized for the research to be ethically justified. The benefits to society likely

to result from the knowledge to be gained in the research must also outweigh the risks to subjects. (40 CFR §26.1111)

(i) **Alternatives to research with human subjects.** If the research question can be answered without conducting new research with human subjects, human research is not justifiable. In general, the efficacy of skin-applied repellents can only be tested in research involving human subjects. Protocols should include a discussion of possible alternatives to human research, and explain why they are infeasible or would not answer the research question.

(ii) **Prerequisite research.** Before using human subjects in a test of repellent efficacy, at least basic acute toxicity testing with animals should be completed for the formulations to be tested and summarized in the protocol. Acute dermal toxicity, dermal irritation, eye irritation, and skin sensitization studies are especially important to support estimates of the margin of exposure (MOE) for subjects participating in a repellent test. All formulation components should be cleared by EPA for use in repellent formulations.

(iii) **Risks to subjects.** Protocols should include a complete discussion of risks to subjects, characterizing all risks and their likelihood, and describing all steps proposed to minimize them.

(A) **Risk characterization.** The nature of all risks to subjects should be described and the probabilities of occurrence of each type of risk should be estimated in the protocol. Potential risks in repellent testing typically include reactions to the test substance or to arthropod bites, acquisition of vector-borne illness, physical strain imposed by the requirements of the test, or possible psychological risk associated with a breach of confidentiality in handling the results of pregnancy testing.

(B) **Risk minimization.** As a condition of approval of proposed research, an Institutional Review Board (IRB) must determine that risks to subjects have been minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk. (See 40 CFR §26.1111(a)(1).) Different actions are effective in minimizing different risks; protocols should identify specific steps taken to minimize each identified risk to subjects.

Protocols should discuss specific steps proposed to ***prevent*** research-induced harm—for example, by training subjects, using appropriate eligibility criteria, ensuring adequate supervision of

subjects, and/or having a physician on call during testing. In addition, protocols should specify how any research-induced harm will be ***reversed***—for example, by the sponsor's assuming responsibility for the costs of medical treatment of research injuries, and for longer-term treatment of any insect-borne disease contracted during testing. Study protocols should include procedures for monitoring the safety of subjects, stopping rules specifying conditions under which a subject would be withdrawn from the research or the research would be terminated to protect subjects, and a medical management plan covering foreseeable contingencies.

Methods of risk minimization are ultimately study-specific, but ensuring that risks to subjects are minimized in repellent testing may require some or all of the following:

- Monitoring of potential field testing sites for the presence of disease vectors at least weekly during the month before testing. If vectors are found, the site should not be used for field testing.
- Serological or DNA-based assays of insects collected at the site of field testing to determine the presence or absence of disease organisms. Results of such testing should be reported to subjects after completion of the field test.
- Using the minimum number of untreated controls consistent with statistical soundness.
- Exposing untreated control subjects intermittently for only the minimum time required to confirm continued pest pressure.
- Training subjects to use an aspirator to capture landing insects before they have time to bite.
- Using only pathogen-free laboratory-reared insects in laboratory tests.
- Excluding subjects known to be sensitive to repellents or to insect bites.
- Ensuring that enough sub-investigators are present at all times so that the Principal Investigator can, if needed, attend to the safety of a subject without compromising the integrity of the research or endangering other subjects.
- Initiating post-study contact with subjects to inquire about any signs of study-related injury or illness.

(iv) Expected benefits of research. Participation as a subject in repellent testing has no direct benefit for individual subjects, yet is not risk-free. Thus the ethical justification for the research depends on the expected

benefits to society of the information likely to be gained from the research. Protocols should characterize all anticipated benefits of the information to be gained from the research, and to whom or to what segment in society each identified benefit is likely to accrue. In addition, protocols should estimate the likelihood of achieving each identified societal benefit. Payments to subjects or other incentives for participation should not be treated as benefits, either to the subjects or to society.

(v) **Relation of benefits to risks.** As a condition of approval of proposed research, an Institutional Review Board (IRB) must also determine that minimized "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. . . . The IRB should not consider possible long-range effects of applying knowledge gained in the research . . . [to be] within the purview of its responsibility." (See 40 CFR §26.1111(a)(2).)

(3) **Subject selection and informed consent.** Subject selection is important both to the scientific merit of research and to its ethical acceptability. Selection of representative subjects is critical to the generalizability of the results of research, and to be ethically acceptable, subject selection must be equitable. (40 CFR §26.1111(a)(3)) Fully informed and fully voluntary consent of subjects is fundamental to ethical human research. (40 CFR §26.1116)

(i) **Representative sampling.** The target population to which the results of repellent testing should ideally be generalizable is the population of repellent users. Within constraints dictated by practical and ethical concerns, subjects should be selected so as to be representative of this target population. Samples should include adults of various ages, of both sexes, and of a variety of races. Protocols should describe the demographic characteristics of the pool from which subjects are recruited.

(ii) **Inclusion/Exclusion factors.** A clear rationale should be presented for all exclusion/inclusion criteria. Because testing a topical repellent necessarily involves intentional exposure of subjects, children under 18 and pregnant or nursing women must be excluded. (See 40 CFR §26.1203.) Candidates over 55 should be excluded as subjects in field testing or their inclusion should be specifically justified, since older adults are more susceptible to arthropod-borne diseases. Vulnerable populations, including people of limited mental capacity, those not in good health or with compromised immune systems, those sensitive to chemical reactions, and students or employees of the investigators or sponsors should always be excluded. Recruitment should generally be among populations in the area in which the tests will be conducted; offerings of distant travel might unduly

influence choices to enroll, and transportation to distant localities might be coercive with respect to study withdrawal. If it is proposed, study protocols should clearly justify transporting study participants to distant locations.

(iii) **Methods of recruiting.** Protocols should describe in detail the proposed recruiting process, from the first contacts with potential candidates through all discussions of the research and the subjects' enrollment. Any advertisements or flyers proposed for use in recruiting should be appended to the protocol, and should reflect IRB review and approval. If recruiting is proposed to be done through telephone calls, the script for recruiting calls should be appended to the protocol, and should reflect IRB review and approval. If any candidates may prefer to speak or read a language other that English as their primary mode of communication, procedures for accommodating their needs in the recruiting process and in the conduct of the research itself should be described in detail.

(iv) **Compensation of subjects.** It is reasonable to compensate participants in repellent efficacy research for their time and trouble. The level of compensation should not be so high as to constitute an undue influence in the choice to participate, nor should it be so low as to make participation in the research attractive only to the economically disadvantaged. Compensation should not be used or administered so as to compromise the freedom guaranteed to subjects to withdraw from participation at any time for any reason, without sacrificing benefits to which they are entitled.

(v) **Informed consent.** It is a fundamental requirement for ethical research with human subjects that participation of subjects be both fully informed and fully voluntary.

(A) **Process.** Informed consent is much more than simply obtaining a subject's signature on a form. Informed consent is an extended process, beginning with recruiting, and continuing beyond the point of consent form signature throughout the conduct of the research. A single discussion of the recruiting and consent processes as a single, continuous process may be clearer than two separate discussions.

As a condition of approval of proposed research, an Institutional Review Board (IRB) must determine that informed consent will be sought from each prospective subject in accordance with, and to the extent required by §26.1116, and that informed consent will be appropriately documented, in accordance with and to the extent required by §26.1117. (See 40 CFR §26.1111(a)(4) and (5).)

The rule at §26.1116 requires that consent be sought "only under circumstances that provide the prospective subject . . . sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject shall be in language understandable to the subject." EPA recommends that all consent documents be written at or below an 8th grade reading level. If consent materials are translated into another language, it is important to ensure the translations are also at an appropriate reading level.

The rule at §26.1116 further defines the general requirements for informed consent, including basic elements always required (§26.1116(a)), other elements conditionally required (§26.1116(b), and a requirement to identify the pesticide and the nature of its pesticidal function (§26.1116(e)).

(B) Documentation. The rule at §26.1117 provides two options for documenting informed consent. The first option is a "written consent document that embodies the elements of informed consent required by § 26.1116." The second is "a short form written consent document stating that the elements of informed consent required by § 26.1116 have been presented orally to the subject." The short form requires that a witness be present in the consent interview. In repellent efficacy testing the short form is generally inappropriate; if use of a short form is proposed, it should be justified.

Ensure that any elements appearing in both the protocol and the consent form are in harmony. These elements may include, for example, statements of the purpose of the research, subject eligibility factors, risks and how they will be minimized, and the description of what will happen in the course of the research. The protocol and the consent form should be consistent in substance, although the presentation need not be the same because the readers and purposes differ. Consent forms should address candidates in the second person—as "you"—and should present all information from the subject's point of view.

In repellent testing, the consent form should tell subjects how many bites, if any, they are likely to get, and what symptoms of arthropod-borne disease they should be alert for after participating in a field study.

(4) Respect for Subjects.

(i) Protection of privacy and confidentiality. It is important to protect the privacy and confidentiality of subjects in repellent efficacy research, and to inform candidates in the recruiting and consent processes of the extent, if any, to which confidentiality of records identifying them will be maintained. This concern arises at several points in repellent research:

(A) Subject identification. Subjects should not be identified by name in data collection forms or study reports. Subject names unavoidably must appear on consent forms and on administrative documents, but subjects should be identified only by an arbitrary code on other study documents. The key linking identifying codes to subjects should be stored securely, away from other study records.

(B) Photographic images. If photographs or videos are made to document the research, care should be taken to minimize making recognizable images of subjects. If faces or other identifying marks cannot be excluded from a photograph or video image, the image should be altered to protect the identity of the subject(s).

(C) Pregnancy testing. Testing of female candidates for pregnancy should be handled with care and discretion. The investigator is responsible for ensuring that pregnant or nursing women are excluded as subjects (See 40 CFR §26.1203). It is not necessary to meet this responsibility by testing all female candidates for pregnancy. It is neither respectful nor informative to require women who are post-menopausal or surgically incapable of pregnancy to take a pregnancy test. Investigators should consider how best to meet their responsibilities while fully respecting female subjects.

When pregnancy testing is conducted, the circumstances of testing should protect the privacy of the candidate, both in the testing itself and in handling the results. Unexpected news of a pregnancy can cause significant psychological distress, which can be increased by any failure of discretion in handling the information. It is a good practice to over-recruit candidates, so that the design of the research would not be compromised by withdrawal of one or more subjects. This approach would permit candidates with positive pregnancy tests to withdraw without stating a reason. No records of the positive pregnancy test should be retained.

(ii) **Medical treatment of research-related injury or illness.** It is a good practice, consistent with respect for the subjects of research, for investigators or sponsors to commit to cover uninsured costs of medical treatment of subjects for injuries or illnesses resulting from their participation in the research.

(5) **Data Collection and Reporting.** Protocols should provide for collection and reporting of data covering all aspects of the research. In addition to the reporting elements discussed in section (g) of this guideline, protocols should provide for collecting and reporting:

(i) **Recruiting and enrollment statistics.** Data reported concerning subjects recruited and enrolled should include the following:

- Demographic characteristics of the pool from which subjects are recruited and of the subject sample selected,
- Numbers of candidates contacted, interviewed, and screened, and
- Numbers of subjects enrolled, withdrawn voluntarily or involuntarily, and completing the research.

(ii) **Elements required by GLP regulations.** Each protocol should provide for collecting and reporting all elements required by the Good Laboratory Practices regulation at 40 CFR §160.120.

(iii) **Additional elements required by Human Research Rule.** Each protocol must include or be accompanied by the supporting information required by 40 CFR §26.1125 and summarized in Appendix A to this guideline.

(d) **Review of protocols for repellent studies.** EPA's Human Research Rule requires extensive review of protocols before research is initiated. The rule requires that the complete protocol, consent form, and supporting materials be reviewed and approved by an Institutional Review Board (IRB) (See 40 CFR §26.1109), and having been approved by the IRB, that it be submitted, along with specified additional supporting documentation, to EPA for review by EPA and by the Human Studies Review Board (See 40 CFR §26.1125 and §26.1601.)

(1) **Review by IRB.** The membership, functions, general procedures, and decision criteria for IRBs are defined in the rule at 40 CFR §26.1107-§26.1111. Records to be maintained by IRBs are defined at 40 CFR §26.1115. Most IRBs review many other kinds of human research in addition to repellent efficacy studies. Extensive guidance to and about IRBs can be found on the website of the Office of Human Research Protections (OHRP), at www.hhs.gov/ohrp.

(i) **Required elements in submission to IRB.** Each IRB has its own application forms and procedures. The basic requirements are: a complete protocol, a consent form, and all materials intended for use in the recruiting process or to be provided to candidates or subjects. A protocol responsive to the guidance in section (c) of this document is likely to include all elements required by an IRB.

(ii) **Criteria for IRB approval.** The criteria for IRB approval of proposed research are defined in the rule at 40 CFR §26.1111. In summary, this passage of the rule requires that the IRB determine that proposed research meets all these criteria:

- Risks to subjects are minimized.
- Risks to subjects are reasonable in relation to the importance of the knowledge that may be reasonably expected to result.
- Selection of subjects is equitable.
- Informed consent will be sought from each prospective subject.
- Informed consent will be appropriately documented.
- When appropriate, adequate provision is made for monitoring collected data to ensure the safety of subjects.
- When appropriate, adequate provision is made to protect the privacy of subjects and to maintain the confidentiality of data.

In addition, the IRB ensures that when any subject is likely to be vulnerable to coercion or undue influence, additional safeguards are included in the study to protect their rights and welfare.

(iii) **Documentation of IRB approval.** IRBs have the authority to approve, require modifications in (to secure approval), or disapprove research proposals. (40 CFR §26.1109.) It is common for IRBs to grant conditional approval subject to the investigator's making specified modifications in the proposal; once any required modifications are made, the IRB will issue formal written approval of the research. This letter of approval, along with all other correspondence between the investigators or sponsors and the reviewing IRB, must be included with the subsequent submission to EPA. (See 40 CFR § 26.1125(e) and (f).)

(2) **Reviews by EPA and the Human Studies Review Board (HSRB).** After receiving approval from an IRB, the protocol and supporting material must be submitted to EPA for review by EPA staff and by the Human Studies Review Board. (See 40 CFR § 26.1125.)

(i) **Required elements in submission to EPA.** The elements to be included in a protocol submission to EPA are defined in the rule at 40 CFR

§26.1125 and summarized in Appendix A to this document. Records of two kinds are required:

(A) **Records describing the research proposal itself.** 40 CFR §§26.1125(a)–(d) call for a discussion of the risks and benefits of the proposed research, the consent forms as submitted to and as approved by the IRB, information about how subjects will be recruited, and a description of the circumstances and methods proposed for presenting information to subjects and seeking their consent. A protocol responsive to the guidance in section (c) of this document will already include this range of information.

In addition, 40 CFR §26.1125(e) and (f) call for submission of all correspondence between the IRB and the investigators or sponsors (§26.1125(e)) and official notification of IRB review and approval (§26.1125(f)). For purposes of §26.1125(e), "correspondence" does not include attachments transmitted by correspondence, such as the protocol or consent form, which are otherwise required to be submitted by other provisions of the rule.

(B) **Records of the IRB review.** 40 CFR §26.1125 requires submission of copies of records relevant to the proposed research which the IRB is required to maintain by 40 CFR §26.1108(a). There is some overlap between these records and those discussed above, but there are some important differences. 40 CFR §26.1108(a) calls for, among other records:

- Minutes of IRB meetings, in sufficient detail to show attendance; actions taken; votes for, against, and abstaining; the basis for requiring changes, and summarizing the discussion of controverted issues.

- A list of IRB members identified by name, earned degrees, representative capacity, indications of experience sufficient to describe each member's chief anticipated contributions to IRB deliberations, and any employment or other relationship between each member and the institution.

- Written procedures for the IRB in the same detail described in §26.1108(a) and §26.1108(b).

These records can only be obtained from the IRB. An IRB may, however, submit them directly to EPA by prior arrangement.

(ii) **EPA Review.** Timely EPA review of submitted proposals for research is required by the rule at 40 CFR §26.1601. EPA will first review submitted proposals for completeness in terms of the requirements of 40 CFR §26.1125, and for substantiation of any claims of business confidentiality associated with the submission. Submissions which are incomplete, or which include unsubstantiated claims of business confidentiality, will not be reviewed further until these deficiencies are corrected. EPA plans to evaluate complete proposals in a single substantive review addressing both scientific and ethical aspects of the proposal, following the outline in Appendix B to this document.

EPA will provide its completed review to the submitter. EPA's review may call for revision of the proposal before it is reviewed by the HSRB. If EPA determines that the proposal is ready for HSRB review, it will be scheduled for HSRB review at the earliest opportunity. The submitter may respond to EPA's review in writing to the HSRB docket, and may also comment orally at the HSRB meeting.

(iii) **HSRB Review.** As provided in 40 CFR §26.1601(d), "following initial evaluation of the protocol by Agency staff, EPA shall submit the protocol and all supporting materials, together with the staff evaluation, to the Human Studies Review Board." The HSRB is an advisory committee, chartered under the Federal Advisory Committees Act, charged with advising EPA on scientific and ethical aspects of proposed and completed research with human subjects. The Board meets quarterly in public session, with opportunities for public participation; the materials they consider are kept in a public docket. The recommendations of the HSRB are documented in a public report of each meeting, prepared as soon as possible after the meeting—usually within 60-90 days. As soon as it is available, EPA will provide a copy of the HSRB's report to submitters of research proposals discussed by the HSRB. Additional information about the HSRB, including agendas and reports from past meetings and schedules of future meetings, can be found at the HSRB website: www.epa.gov/osa/hsrb.

(e) **Changes to IRB-approved research before execution.** After EPA and the HSRB have reviewed and commented on proposed research, revisions may be needed to the protocol, consent form or other materials before the research is executed. When IRB-approved materials are revised prior to initiation of the research to address concerns raised in EPA and/or HSRB reviews or for any other reason, those revised materials must be re-submitted to the IRB for review and approval before the research is initiated. This requirement applies to all changes to an IRB-approved protocol, to all changes to an IRB-approved consent form, and to all changes to any other materials approved by an IRB. (40 CFR §26.1108(a)(4)). Although an IRB has the discretion to conclude that some changes do not require full IRB consideration, investigators and sponsors do not have this discretion. EPA will treat as a breach of the regulations any planned changes to proposed research which are implemented without prior approval by the IRB.

(f) Execution of repellent studies

(1) Execution of protocol. When EPA and HSRB reviews are completed and the IRB has approved any revisions resulting from those reviews, the research can be initiated. Subjects can be recruited and data can be collected as specified in the protocol. Care should be taken to ensure and document that these events occur in the proper sequence.

(2) Quality Assurance (QA) oversight. Repellent efficacy testing, whether conducted in the field or in the laboratory, is subject to the Good Laboratory Practices regulations at 40 CFR part 160. These rules require that each testing facility include an independent QA unit, and that the QA unit monitor execution of each protocol and document its conduct in accordance with the GLP regulations. (40 CFR §160.35)

(3) Deviations from protocol. In executing even the best designed and most comprehensive protocols, unanticipated events may force deviations from the protocol. All such deviations from the protocol should be promptly and fully reported to the IRB, and both the deviations and their impact on the research should be discussed in the study report submitted to EPA. (40 CFR §160.185) EPA makes a distinction between planned changes to a protocol and deviations from a protocol. Planned changes must be treated as amendments to the protocol, and must be approved by the IRB before they are implemented. (40 CFR §26.1108(a)(4))

(4) Changes in IRB-approved research after initiation. Any amendment to the protocol, consent form, or other materials approved by the IRB must be submitted to the IRB for review, and must be approved by the IRB before it is implemented, unless immediate implementation is required to avert an imminent hazard to subjects. (40 CFR §26.1108(a)(4))

(5) Reports of adverse effects. Any adverse events affecting the subjects of a repellent efficacy test must be promptly reported to both the overseeing IRB and to EPA. (40 CFR §26.1108(b)(1))

(g) Reporting of completed repellent studies

(1) Study report. In addition to the standard elements described in the GLP regulation at 40 CFR §160.185 and to the appendices described below, the primary report of the study should include at least the following elements:

(i) Study identification: Title, identifying study number(s), sponsor, study director, investigators, name and location of the testing facility, and dates of the study.

(ii) Approved or intended label directions for use.

(iii) Demographic characteristics of all human subjects.

(iv) Test organisms: Genus, species, subspecies or strain (if information is available); development stage, age and sex of insects; rearing technique; preparation of insects for test (feeding/starving); method used to determine the disease-free status of laboratory organisms; insect density per cage; and landing pressure in each test cage or field site.

(v) Test cages/containers: Construction material, size, sleeve description.

(vi) Field site description: Location, type of habitat, predominant species.

(vii) Test conditions: Temperature, relative humidity, ambient light, air flow, wind speed, cloud cover.

(vii) A full description of the experimental design.

(ix) Test procedures, including but not limited to:

- Recruiting and enrolling of subjects: Number of potential recruits contacted, screened, interviewed, consenting to participate, withdrawing before completion of testing, and completing testing.
- Preparation of subjects: Training, demonstrating attractiveness, clothing and protective equipment worn, calculation of treated skin area and individual dose administered, washing, rinsing, and drying of limbs.
- Allocation of subjects to treatments; number of treatments per subject.
- Methods of application of repellent; individual dose applied.
- Time of treatment of each subject.
- Time of start and end of exposure of each subject.

(x) Complete accounting for all events during the testing period.

(xi) Results for each subject.

(xii) Conclusions

(xiii) Discussion

(2) Appendices. Appendices to the primary study report should include:

(i) Complete protocol as approved by the IRB.

(ii) Consent form and any additional recruiting materials as approved by the IRB.

(iii) All amendments to the protocol, consent form, or other materials as approved by the IRB.

(iv) Reports of all deviations from the protocol and assessment of their impact on the integrity of the research.

(v) Raw data and data collection sheets.

(vi) Reports of all statistical analyses conducted.

(vii) Documentation of ethical conduct of the research, as required by 40 CFR §26.1303, including all correspondence with IRB not previously submitted to EPA. The requirements of this section of the rule are summarized in Appendix C to this guideline.

(h) Retention of Records. The following record-keeping requirements apply to some or all records of research covered by this guideline:

(1) The record-keeping requirements in 40 CFR §26.1115 apply to Institutional Review Boards (IRBs) that review human research conducted under EPA's Human Research Rule.

(2) The record-keeping requirements of 40 CFR §169.2(j) apply to investigators who conduct pesticide research with humans subject to FIFRA §12(a)(2)(P).

(3) The record-keeping requirements of 40 CFR §169.2(k) apply to any person who submits the results of research to EPA in support of a petition for a tolerance or tolerance exemption or in support of registration or an application for registration.

(4) The record-keeping requirements of 40 CFR §160.190 and §160.195 apply to records of any study conducted under the Good Laboratory Practices rule.

(i) **Specific guidance for dose-determination studies.** All subjects participating in repellency testing should be treated with the same standard dose of the test material, expressed as weight of repellent per unit area (mg/cm^2 of treated skin,) or volumetrically as ml/cm^2 of treated skin. Recommended methods for choosing the standard dose depend in part on the active ingredient(s) and formulation(s) of the test material. For testing of lotion formulations containing DEET, a standard dose rate of 1 g/600cm^2 (equivalent to 1.67 mg/cm^2) has been used in many tests of repellency. Repellents in lotion form containing DEET can be tested for repellency at this standard dose rate, or at a "typical consumer dose" rate determined through a dose-determination study. For other ingredients and formulations no comparable standard dose has been identified, and it is recommended that a "typical consumer dose" be estimated empirically through dose determination testing as described below. The rationale for the dose rate actually used in repellent testing should be provided in all cases.

(1) **Test material.** The formulated product should be used as it is or will be marketed, in the same container and bearing the same directions for use.

(2) **Subjects.** Subjects used for dose determination should be recruited and informed as described above, and must provide written informed consent for their participation. The same or very similar eligibility criteria would apply as for repellency testing, although subjects participating only in dose determination should not be exposed to any insects or to any of the risks associated with exposure to insects. The number of subjects used for dose determination should be justified statistically. Their demographic characteristics should be as representative of the repellent use population as possible within ethical and feasibility constraints.

(3) **Measuring subject's skin area.** The surface area of subject's limb should be estimated in cm^2 by measuring the circumference in centimeters of the arm at the wrist and elbow, or the circumference of the leg at the ankle and knee, and at one or two equally spaced points between; then multiplying the average of these measures of circumference by the length in centimeters from the wrist to elbow or from the ankle to the knee.

(4) **Methods.** Each subject in dose determination should be instructed to apply the test repellent to his or her own limb—a forearm or lower leg—as they normally would apply a repellent to achieve complete coverage. Once the quantity applied has been measured and recorded, the applied repellent should be washed completely off the limb. This process should be repeated at least 3 times per limb.

The applied quantity of a liquid or lotion formulation should be determined by weighing the container before and after use.

Because less than all the spray released from the container will reach the target limb, a different method should be used to determine the applied quantity of a spray formulation. The target limb should be wrapped in gauze "bracelets" of known area so that the bracelets cover only part of the skin. Knowing the area of the bracelets, the rate of application to the skin per cm^2 can be approximated by weighing the bracelets before and after application. The exposed skin between bracelets can be used by subjects to determine when they achieve complete coverage. Dose determination with spray formulations should be conducted out-of-doors.

(5) Calculating standard dose for use in repellency trials. The mean dose applied by each subject to each limb and the grand mean across all subjects of mean doses applied to forearms and to lower legs should be calculated. The specific gravity of the test material should be used to convert the dose expressed as mg/cm^2 to a volumetric dose expressed as ml/cm^2. This volumetric dose should be used as the standard dose for repellency testing, scaling it to the treated surface area of each subject's limb or limbs.

(j) Specific guidance for laboratory studies of mosquito repellency. Laboratory studies may be valuable to establish preliminary threshold levels of repellency to mosquitoes and to standardize subjects' attractiveness before initiating field testing.

(1) Species. Mosquito tests should be conducted with *Aedes aegypti* and an *Anopheles* species such as *A. albomanus*, and a *Culex* species, *preferably Cx. quinquefasciatus.* Test insects should be identified by subspecies or strain if possible.

(2) Stage, age, and sex. Mosquitoes should be adult females five to ten days old. The age or age range of the test insects should be reported.

(3) Rearing techniques. Larvae should be reared under optimal conditions for the species. Most species should be reared at 27±3°C, relative humidity 80±10%, and photoperiod 16:8 hours (light:dark). Alternative rearing methods may also be acceptable (see, for example, Gerberg et al. 1998). Use of alternative rearing techniques should be fully explained and justified.

(4) Preparation of insects. Test insects should be fed 10% sucrose and no blood meal before being used in a test, and starved for 12 to 24 hours immediately before the test. Test insects should be established to be free of disease (e.g., malaria or West Nile virus); methods used to ensure they are disease-free should be reported.

(5) Disposition of insects. Test insects should be used for only one test and destroyed immediately after the trial.

(6) **Test cages.** Cages are typically 2' x 2' x 2' (8 cubic feet, equivalent to roughly 61 x 61 x 61 cm or 227,000 cm^3), square or rectangular, with one or more sleeved openings for the subject's arms. A mirror on the bottom of the cage helps observers see landings or bites on the underside of arms. Larger cages may be used for two test subjects at a time. Cage dimensions and design should be reported .

(7) **Insect density.** There should be at least one mosquito for each 1,133 cm^3 and at least 200 mosquitoes in each test cage.

(8) **Testing conditions.** Ambient temperature during the test should be maintained at 22° to 27°C, and relative humidity at 50 to 80%. Ambient light appropriate to the preferred feeding time for the test species should be provided—e.g., more light for day feeders like *Aedes aegypti*, and less for night feeders like *Anopheles spp.* and *Culex spp.* When testing in subdued light, care should be taken not to compromise the investigators' ability to observe mosquito activity. Investigators and subjects should avoid exhaling into the test cage; introduction of CO_2 could bias mosquitoes towards biting.

(9) **Negative controls.** EPA recommends that negative or untreated controls be used to verify continued landing pressure in all laboratory studies of mosquito repellency. Untreated controls should also be used to provide a standard of comparison when the objective of the study is to estimate percent repellency. Although some subjects may sometimes serve only as controls, to reduce variability between subjects the preferred negative control is the untreated forearm of a treated subject. Untreated control forearms should be washed, rinsed, and dried in exactly the same manner as treated forearms.

(10) **Positive controls.** Positive controls are not recommended when the objective of the study is to evaluate the performance of individual repellents. Positive controls may be appropriate when the objectives of the study include comparisons between formulations.

(11) **Treated area size and preparation.** The recommended treatment area is the subject's forearm (wrist to elbow..). This area should be washed with unscented soap, rinsed first with water and then with a solution of at least 50% ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area (cm^2) of each test subject's treated forearm and the specific dose administered to each subject (ml) should be reported. Adjacent areas above and below the treated area should be covered with a light-colored material that the test insects cannot bite through. Hands should be covered with latex gloves.

(12) **Establishing subject attractiveness to mosquitoes.** Before the test, subjects should expose their forearms to the mosquitoes in a test cage to establish their

attractiveness. Ten mosquito landings in 60 seconds or less should be sufficient to establish attractiveness of a subject.

(13) Establishing and confirming landing pressure. Before exposing any treated subjects, an untreated control forearm should be inserted into each cage and exposed to mosquitoes for up to 60 seconds to verify landing pressure. As mosquitoes land, they should be removed by shaking the arm before they have time to bite. EPA recommends that the control forearm be removed from the test cage as soon as it has received ten landings. Continued landing pressure should be confirmed at regular intervals throughout the testing period. If at any time fewer than ten mosquitoes land on the untreated control forearm within 60 seconds, fresh mosquitoes should be added to all cages in the study, and their aggressiveness should be confirmed before repellency testing continues.

(14) Exposure period. Approximately thirty minutes after being treated with the repellent, test subjects should insert their treated forearms into the cage of mosquitoes for the first time. Each exposure should last for a pre-determined duration and should be repeated at regular intervals—e.g., exposures may be for one minute each at 15-minute intervals or for five minutes each at 30-minute intervals, until breakdown of efficacy or the end of the study, whichever occurs first. Subjects should avoid rubbing their arms when inserting them into or removing them from the cage and between exposure periods.

(k) Specific guidance for field studies of mosquito repellency. Because of the increasing prevalence of West Nile virus and other mosquito-borne diseases, it is important that sponsors and investigators conducting field tests incorporate into field study design appropriate measures to minimize risks to subjects from exposure to mosquito-borne diseases. (40 CFR §26.1125(a)).

(1) Pre-test preparation. It is highly recommended that subjects participating in field research receive laboratory training in identification of landings, probes, and bites, and to learn to use mechanical aspirators to collect landing mosquitoes before they have time to bite. Subjects' attractiveness to mosquitoes should also be preliminarily established in the laboratory.

(2) Choice of field testing sites. Field tests should be conducted in at least two distinct habitats (e.g., forest, grassland, salt marsh, wetland, beach, barns, urban environments) where the predominant mosquito species are different. The test may be repeated in different locations within the same habitat, or in the same location more than once, on different days, using the same subjects to minimize variability. In this model, treatment replicates may be blocked by either location or date. Round Robin designs may be appropriate when testing more than one product.

Potential field testing sites should be monitored at least weekly for a month before testing is scheduled. To ensure that risks are minimized, as required by 40 CFR §26.1111, field testing should ***not*** be conducted in any area where West Nile virus or other arthropod-vectored diseases have been detected within a month before the date of testing.

(3) **Species identification**. Tests should be conducted with more than one mosquito species that occurs in the United States. If tests are conducted outside the U.S., the study report should justify the relevance of the test as a measure of repellency. Landing mosquitoes should be aspirated into a vial before and during the test and labeled with the time they were collected. After the field study, aspirated mosquitoes should be identified by genus and species—if possible, also by subspecies or strain. The number in each taxon trapped in each time period should be reported. After identification, mosquitoes should be subjected to serological or other analysis to determine the presence or absence of WNV or other disease organisms, and the results should be reported to subjects.

(4) **Negative controls.** EPA recommends that negative or untreated controls be used to verify continued landing pressure, and to provide a standard of comparison when the objective of the study is to estimate percent repellency. When the objective of the research is to estimate PT, no replicated controls are needed. A minimum of two untreated subjects to monitor landing pressure is adequate. An exposed, untreated forearm or lower leg can be considered a negative control. Untreated subjects used to monitor landing pressure should expose an untreated limb briefly at regular intervals during testing to confirm continued acceptable landing pressure. Although some subjects may serve only as controls, to reduce variability between subjects the preferred negative control is the untreated limb of a treated subject. Untreated limbs used as negative controls should be washed, rinsed, and dried exactly like treated limbs.

(5) **Positive controls.** Positive controls are not recommended when the objective of the study is to evaluate the performance of individual repellents. Positive controls may be appropriate when the objectives of the study include comparisons between formulations.

(6) **Treated area size and preparation**. The recommended treatment area is the subject's forearm (wrist to elbow) or lower leg (ankle to knee), depending on the feeding behavior of the predominant mosquito species at the selected test site. The treated area should be washed with unscented soap, rinsed first with water and then with a solution of at least 50% ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area (cm^2) of each test subject's treated limb and the specific dose administered (ml) to each subject should be recorded. With the exception of the treated area, subjects' heads, trunks, and limbs should all be covered with light-colored material through which insects cannot bite.

(7) **Establishing and confirming landing pressure.** Landing pressure should be measured before treatment and intermittently throughout the course of the test by untreated control subjects. Testing should not be conducted or continued unless at least five mosquitoes land on an untreated limb within five minutes.

(8) **Exposure period.** Continuous exposure throughout the testing period is preferred over intermittent exposure. Reliable results may be obtained for extended periods of exposure by treating all subjects up to several hours before the first field exposure, with exposures timed to coincide with periods of mosquito activity. This approach minimizes prolonged exposure of subjects to mosquitoes in the field, helps to reduce early withdrawal of subjects attributable to excessively long trials, and minimizes variability from non-targeted species randomly landing on subjects. Another study design for assessing long-term repellency involves treating different subjects at different times (e.g., 2, 4, 6, 8, or 10 hours before exposure), and then exposing all subjects at the same time when target mosquitoes are active. In this design the sample size should be increased to ensure sufficient replications per treatment.

(9) **Minimizing variation.** Many factors contributing to variability in repellent field studies cannot be controlled. But standardizing as many elements as possible, and designing studies with mosquito feeding patterns in mind can make a study design more efficient, potentially yielding conclusive results with minimum sample sizes. Standardization may be possible for such factors as mosquito species, time of testing, allocation to treatment, subject attractiveness to mosquitoes, etc. Standardization of environmental variables may include but is not limited to targeting a predominant mosquito species in its habitat, synchronizing exposure periods with mosquitoes' peak feeding activity, and allocating treatments consistent with the preference of the targeted mosquito species to feed on legs, arms, or both.

(10) **Environmental conditions.** The time of day at which subjects are treated and the time at which exposure to mosquitoes begins and ends should be recorded and reported. Weather conditions during testing (including temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed) should be monitored intermittently throughout the study, and described in the study report. Testing should not be conducted if wind speed exceeds 10 mph.

(11) **Test subject placement and behavior.** Field test subjects should work in pairs to assist each other in identifying landings and collecting mosquitoes. Subjects may engage in normal outdoor activities during testing, such as walking, standing, sitting, and raising and lowering their arms. They should avoid heavy exercise and sweating, since these could affect test results. Pairs should keep at least three meters/ten feet apart from other pairs; clustering of subjects may affect the number of landings per subject.

(12) Establishing subject attractiveness to mosquitoes. Before the test, subjects should expose their forearms to the mosquitoes in a test cage to establish their attractiveness. Ten mosquito landings in 60 seconds or less should be sufficient to establish attractiveness of a subject.

(13) Data collection and reporting. Recording of events signally failure of efficacy during the exposure period should be supervised by investigators. The investigator, an associate, or another subject should record the number and timing of landings occurring during each exposure period for each subject at each site. All mosquito landings on subjects should be recorded; as much as possible, all landing mosquitoes should be collected for identification and analysis. Collected mosquitoes should be labeled with the time of landing.

(l) Specific guidance for laboratory studies of stable fly repellency

(1) Species. Stable fly tests should be conducted with *Stomoxys calcitrans*. If possible, the flies used for testing should be identified by subspecies or strain.

(2) Age of test flies. The protocol should provide for reporting the age or age range of the test flies used. Stable flies used for repellency testing should be three to ten days old.

(3) Rearing techniques. Larvae should be reared under optimal conditions for the species. Most species should be reared at 27° ±3°C, relative humidity 80% ±10%, and photoperiod 16:8 hours (light:dark). Alternative rearing methods are discussed by Gerberg et al. (1998.) Use of alternative rearing techniques should be fully explained and justified.

(4) Preparation of flies. Test flies should be fed 10% sucrose and no blood meal before being used in a test, and starved for 12 to 24 hours immediately before the test. Test flies should be established to be free of disease; methods used to ensure they are disease-free should be reported.

(5) Disposition of test flies. Flies should be used for only one test and destroyed immediately after the trial.

(6) Test cages. Cages are typically 2 x 2 x 2 feet (eight cubic feet, equivalent to roughly 61 x 61 x 61 cm, or 227,000 cm^3), square or rectangular, with one or more sleeved openings for the subjects' arms. Larger cages may be used for two test subjects at a time. Cage dimensions and design should be reported.

(7) Stable fly density. There should be at least one stable fly for each 5,034 cm^3 and at least 25 stable flies in each 2 x 2 x 2 foot test cage.

(8) **Testing conditions.** The temperature during the test should be maintained at 22° to 27°C, and relative humidity at 50 to 80%. Investigators and subjects should avoid exhaling into the test cage; introduction of CO_2 could bias flies towards biting.

(9) **Negative controls.** Negative or untreated controls should be used to verify continued landing pressure in all laboratory studies of stable fly repellency. Untreated controls should also be used to provide a standard of comparison when the objective of the study is to estimate percent repellency. Although some subjects may serve only as controls, to reduce variability between subjects, the preferred negative control is the untreated forearm of a treated subject. Untreated control forearms should be washed, rinsed, and dried exactly like treated forearms.

(10) **Positive controls.** Positive controls are not recommended when the objective of the study is to evaluate the performance of individual repellents. Positive controls may be appropriate when the objectives of the study include comparisons between formulations.

(11) **Treated area size and preparation.** The recommended treatment area is the subject's forearm (wrist to elbow). This area should be washed with unscented soap, rinsed first with water and then with a solution of at least 50% ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area (cm^2) of each test subject's treated forearm and the specific dose administered (ml) to each subject should be reported. Adjacent areas above and below the treated area should be covered with a light-colored material that the test insects cannot bite through. Hands should be covered with latex gloves.

(12) **Establishing subject attractiveness to stable flies.** Before the test, subjects should expose their forearms to stable flies in a test cage to establish their attractiveness. Five stable fly landings in 60 seconds or less should be sufficient to establish attractiveness of a subject.

(13) **Establishing and confirming landing pressure.** Before exposing any treated subjects, a subject's untreated control forearm should be inserted into each cage and exposed to stable flies for up to 60 seconds to verify landing pressure. As flies land, they should be removed before they have time to bite. The control subject's forearm should be removed from the test cage as soon as it has received five landings. Continued landing pressure should be confirmed at regular intervals throughout the testing period. If at any time fewer than five flies land on the untreated control forearm within 60 seconds, fresh flies should be added to all cages in the study, and their aggressiveness should be confirmed before repellency testing continues.

(14) **Exposure period.** Approximately 30 minutes after being treated with the repellent, test subjects should insert their treated forearms into the cage of stable flies for the first time. Each exposure should last for a pre-defined duration and should be repeated at regular intervals—e.g., exposures may be for one minute each at 15-minute intervals or for five minutes each at 30-minute intervals, until breakdown of efficacy or the end of the study, whichever occurs first. Subjects should avoid rubbing their arms when inserting them into or removing them from the cage and between exposure periods.

(m) **Specific guidance for field studies of biting fly repellency.** This section applies to field tests of repellency to black flies (gnats, southern buffalo gnats), ceratopogonids (no-see-ums, punkies, biting midges), sand flies, tabanids, or stable flies.

(1) **Pre-test preparation**. It is highly recommended that subjects participating in field research receive laboratory training in identification of landings, probes, and bites, and learn to use mechanical aspirators to collect landing flies before they have time to bite. Subjects' attractiveness to biting flies should also be preliminarily established in the laboratory.

(2) **Choice of field testing sites.** Repellency against black flies, stable flies, and other organisms that typically occur in only one habitat should be tested in two distinct areas, or in the same area on two separate days, if possible using the same subjects to minimize among-subjects variation among-subjects. Treatment replicates may be blocked by either location or date. Round Robin designs may be appropriate when testing more than one product.

(3) **Species.** Tests should be conducted with more than one biting fly species that occurs in the United States. If tests are conducted outside the U.S., the study report should justify the relevance of the test as a measure of repellency. If possible, landing flies should be aspirated into a vial before and during the test, and labeled with the time they were collected. After the field study, aspirated insects should be identified by genus and species, and if possible by subspecies or strain. The number of insects in each taxon trapped in each time period should be reported.

(4) **Negative controls.** Negative or untreated controls should be used to verify continued landing pressure, and to provide a standard of comparison when the objective of the study is to estimate percent repellency. An exposed, untreated forearm or lower leg can be considered a negative control. Untreated subjects used to monitor landing pressure should expose an untreated limb briefly at regular intervals during testing to confirm acceptable landing pressure. Although some subjects may serve only as controls, to reduce variability between subjects, the preferred negative control is the untreated limb of a treated subject. Untreated

limbs used as negative controls should be washed, rinsed, and dried exactly like treated limbs.

(5) **Positive controls.** Positive controls are not recommended when the objective of the study is to evaluate the performance of individual repellents. Positive controls may be appropriate when the objectives of the study include comparisons between formulations.

(6) **Treated area size and preparation.** The recommended treatment area is the subject's forearm (wrist to elbow) or lower leg (ankle to knee), depending on the feeding behavior of the predominant species at the selected test site. The treated area should be washed with unscented soap, rinsed first with water and then with a solution of at least 50% ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area (cm^2) of each test subject's treated limb and the specific dose administered (ml) to each subject should be recorded. With the exception of the treated area, subjects' heads, trunks, and limbs should all be covered with light-colored material through which insects cannot bite.

(7) **Establishing and confirming landing pressure**. Landing pressure should be measured before treatment and intermittently throughout the course of the test by untreated control subjects. Testing should not be conducted or continued unless at least five black flies land within five minutes, or at least one stable fly, ceratopogonid or tabanid lands within five minutes. Insects landing during this period should be aspirated into a vial for subsequent identification.

(8) **Exposure period.** Continuous exposure throughout the testing period is preferred over intermittent exposure. Reliable results may be obtained for extended periods of protection by treating all subjects up to several hours before the first field exposure, with exposures timed to coincide with periods of target insect activity. This approach minimizes prolonged exposure of subjects to insects in the field, helps to reduce early withdrawal of subjects attributable to excessively long trials, and minimizes variability from non-targeted species randomly landing on subjects. Another acceptable study design for assessing long-term repellency involves treating different subjects at different times (e.g., 2, 4, 6, 8, or 10 hours before exposure) and then exposing all subjects at the same time when target insects are active. In this design the sample size should be increased to ensure sufficient replications per treatment.

(9) **Minimizing variation.** Many factors contributing to variability in repellent field studies cannot be controlled. But standardizing as many elements as possible and designing studies with insect feeding patterns in mind can make a study design more efficient, potentially yielding conclusive results with minimum sample sizes. Standardization may be possible for such factors as insect species, time of testing, allocation to treatment, subject attractiveness to biting flies, etc. Standardization of environmental variables may include but is not limited to

targeting a predominant species in its habitat, synchronizing exposure periods with insects' peak feeding activity, and allocating treatments consistent with the preference of the targeted species to feed on legs, arms, or both.

(10) **Environmental conditions.** The time of day at which subjects are treated and at which exposure to target insects begins and ends should be recorded and reported. Weather conditions during testing (including temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed) should be monitored intermittently throughout the study and reported. Testing should be not be conducted if wind speed exceeds ten mph.

(11) **Test subject placement and behavior.** During field tests, subjects should work in pairs to assist each other in identifying landings and collecting insects. Subjects may engage in normal outdoor activities during testing, such as walking, standing, sitting, and raising and lowering their arms. They should avoid strenuous exercise and sweating, since these could affect test results. Pairs should keep at least 3 meters/10 feet apart from other pairs; clustering of subjects may affect the number of landings per subject.

(12) **Establishing subject attractiveness to biting flies.** Before the test, subjects should expose their forearms to stable flies in a test cage to establish their attractiveness. Five stable fly landings in 60 seconds or less should be sufficient to establish attractiveness of a subject.

(13) **Data collection and reporting.** Recording of landings or bites during the exposure period should be supervised by investigators. The investigator, an associate, or another subject should record the number and timing of all landings or bites occurring during each exposure period for each subject at each site. All landings on subjects by target insects should be recorded; as much as possible, all landing insects should be collected for identification.

(n) **Specific guidance for laboratory studies of flea repellency.** Field tests are not routinely required for flea repellents, but may be conducted and submitted voluntarily. If field tests are conducted, label directions for re-application frequency should reflect field results.

(1) **Species.** Tests should be conducted using the cat flea, *Ctenocephalides felis*.

(2) **Stage, age, and sex**. Adult male or female fleas five to ten days old should be used. The age or age range of the test insects should be reported.

(3) **Rearing techniques**. Fleas should be reared at 27° ±3°C, relative humidity 50-80%, and photoperiod 16:8 h (light:dark). Use of alternative rearing techniques should be fully explained and justified.

(4) **Preparation of insects.** Adults fleas should not be blood-fed.

(5) **Disposition of insects**. Fleas should be used for only one test and should be destroyed after the trial.

(6) **Test containers**. Containers should be square, circular, or rectangular; plastic or glass; with an opening on the top to insert the subject's arm. Five-gallon buckets or containers made from truncated garbage bins are preferred to cages, because fleas can easily escape from most cages. The bottom of the container should be covered with a rough material such as clean sand. A container should be used to test only one subject and one treatment at a time. Report container dimensions and design.

(7) **Flea density.** There should be at least one flea per ~757 cm^3 and at least 25 fleas in each test container. Larger densities of fleas may be difficult to control.

(8) **Testing conditions.** The temperature during the test should be kept at 22°-27°C, relative humidity at 50-80%, and the lights should remain on.

(9) **Negative controls**. Negative or untreated controls should be used to verify landing pressure. When the repellent is applied to the forearm of a subject, the preferred negative control is the untreated forearm of the same subject. Untreated control limbs should be washed, rinsed, and dried exactly like treated limbs.

(10) **Positive controls.** A positive control is useful to relate the performance of a new formulation to that of another known formulation.

(11) **Treated area size and preparation**. The test subject's forearm (wrist to elbow) should be used as the treated area. Areas above and below the treated skin should be covered with a material the flea's mouthparts cannot penetrate.

(12) **Establishing subject attractiveness to fleas.** Before treatment the subject should expose his or her forearm to the fleas in the test container to establish attractiveness to the fleas. The Agency recommends at least one probe within 30 seconds for the subject to qualify as a test participant.

(13) **Establishing and confirming landing pressure**. Before exposing a treated forearm, an untreated forearm should be inserted into the container and exposed to fleas for up to 60 seconds to verify landing pressure. The forearm should be removed from the test container as soon as one probe has occurred. If no landings or probes occur within 60 seconds, additional fleas should be added to the cage until one probe occurs within 60 seconds.

(14) Exposure period. Within thirty minutes after treatment, and at regular intervals of no more than thirty minutes thereafter, the subject's forearm should be inserted into the container of fleas for five minutes. For each exposure period, the number of fleas landing should be recorded. The cycle of exposing an untreated control arm followed by a treated arm to the fleas should be repeated until the control arm no longer receives one flea landing within 60 seconds. Subjects may then continue the test using a second container, until the repellent fails or the end of the study, whichever occurs first. Test subjects should avoid rubbing the repellent-treated area when putting their arms into the container and between exposure periods.

(15) Data collection and reporting. Recording of landings or probes during the exposure period should be supervised by investigators. The investigator, an associate, or another subject should record the number and timing of all landings or probes occurring during each exposure period for each subject.

(o) Specific guidance for laboratory studies of tick and chigger repellency The Agency recommends laboratory testing for ticks and chiggers, because reliable field tests have not been developed. The Agency is investigating potentially appropriate field tests that may be recommended in the future. Although field tests are not routinely conducted for testing performance of skin repellents against ticks and chiggers, if such tests are conducted, label directions for re-application frequency should reflect field results.

(1) Summary of recommended method. Subjects should place their fingertips on a flat surface with palms raised above the surface and the forearm held perpendicular to the surface to create a vertical test area. The investigator should place ticks or chiggers, one at a time, on the subject's forearm with a suitable instrument (e.g., an artist's paintbrush, forceps, or a cotton swab) approximately one cm from the edge of the treated area of the forearm, near the wrist. All tools employed to handle ticks should be cleansed to avoid contamination. The tick or chigger should be guided gently (e.g., with paint brush, forceps, or cotton swab) toward the treated area. After the first movement toward the margin of the treated area, ticks or chiggers should be allowed one minute to cross the boundary onto the treated area. Ticks or chiggers that cross onto the treated area (toward the elbow) and remain there for at least 60 seconds should be reported as 'not repelled'. Some repelled ticks may crawl onto the treated area but immediately turn back or fall off; these should be reported as 'repelled.' A new group of five ticks or chiggers should be exposed to the treated area every 30 minutes.

(2) Negative control. The negative control should be the untreated forearm of a treated subject, used to screen ticks or chiggers for questing behavior. Only actively questing ticks or chiggers should be selected for testing. The control forearm should be washed, rinsed, and dried exactly like the treated forearm

before exposure to the test organism. The release line and boundary line should be marked on the untreated forearm exactly as they are marked on the treated arm. The test organism should be picked up carefully to prevent damaging its body or forelegs (e.g., with a soft artist's paint brush, forceps, or a cotton swab), and placed on the release line on the wrist of the subject's untreated arm. A tick or chigger that moves steadily from the release line across the boundary line and upward along the subject's untreated forearm is actively questing, and appropriate for use in repellency testing.

(3) **Species.** Tick tests should be conducted using laboratory colonies of the tick species the label claims to repel. Common tick species in the United States include the blacklegged tick (deer tick, *Ixodes scapularis*), western blacklegged tick (deer tick, *Ixodes pacificus*), lone star tick *(Amblyomma americanum*), American dog tick *(Dermacentor variabilis*), and relapsing fever tick (softbacked tick, *Ornithodoros turicata*). Chigger tests should be conducted using laboratory colonies of chiggers in the *Trombiculidae* family; *Eutrombicula splendens;* or *E. cinnabarrs*. Test arthropods should be identified by genus and species, and if possible by subspecies or strain.

(4) **Stage, age, and sex.** When testing with blacklegged (deer tick), lone star, or soft-backed ticks, either adult or nymphal life stages are appropriate for testing. Only the adult American dog tick is recommended, since nymphs of this species do not feed on humans. Tests with chiggers should use immature chiggers. The age or age range of all test animals should be reported.

(5) **Rearing techniques**. Test arthropods should be reared at 22° ±3°C, relative humidity 50-80%, and photoperiod 16:8 (light:dark). Any alternative rearing techniques should be justified.

(6) **Preparation of test arthropods**. Ticks and chiggers should be disease-free. The source of test animals and the methods used to ensure they are disease-free should be reported.

(7) **Disposition of test arthropods**. Ticks or chiggers should be used for only one test and should be destroyed immediately after the trial.

(8) **Tick or chigger density.** At least five previously untested ticks or chiggers should be exposed to the treated forearm in each exposure period.

(9) **Testing conditions**. Temperature should be kept during the test at 22° to 27°C, and relative humidity at 50 to 80%. The lights should be kept on.

(10) **Treated area size and preparation**. Two lines should be drawn on the inside of subjects' wrists. The 'boundary line' should be located at the edge of the treated area near the wrist of the repellent-treated forearm. Another line—the 'release

line'—should be drawn 1 cm away from the boundary line, outside the treated area. With the forearm held vertically, a fresh tick or chigger is released, one at a time, at the release line on the treated arm. A "crossing" is recorded if the test organism crosses the boundary line, and remains in the treated area for at least one minute.

(11) **Exposure period**. At 30 minute intervals a new group of 5 ticks or chiggers should be tested for active questing and then exposed to the treated area, continuing until efficacy failure or the end of the study, whichever occurs first.

(12) **Data collection and reporting.** Recording of crossings and repelled test arthropods during the exposure period should be supervised by investigators. The investigator, an associate, or another subject should record the number and timing of all events occurring during each exposure period for each subject.

(p) **References.** The following publications were consulted for supporting guideline recommendations.

(1) American Society for Testing and Materials (2006) E 939-94 Standard Test Method of Field Testing Topical Applications of Compounds as Repellents for Medically Important and Pest Arthropods (Including Insects, Ticks and Mites): I Mosquitoes.

(2) American Society for Testing and Materials (2003) E 1488-02 Standard Guide for Statistical procedures to use in Developing and Applying test methods.

(3) Barnard, D.R.; Ulrich, R.B.; Xue, R.; and Debboun, M. (2007) Chapter 5: Standard methods for testing mosquito repellents. In *History of Insect Repellents: Principles, Methods and Uses*. Debboun, Frances, and Strickman, eds. CRC Press. (495 p.)

(4) Barnard, D.R. (1998) Mediation of DEET repellence in mosquitoes (Diptera: Culicidae) by species, age, and parity. J. Med. Entomol. 35(3): 340-343.

(5) Barnard, D.R.; Posey, K.H.; Smith, D.; and Shreck, C.E. (1998) Mosquito density, biting rate and cage size effects on repellent tests. Med. Vet. Entomol. 12:39-45.

(6) Carroll, S. P. (2007) Chapter 12: Evaluation of topical insect repellents and factors that affect their performance. In *History of Insect Repellents: Principles, Methods and Uses*. Debboun, Frances, and Strickman, eds. CRC Press. (495 p.)

(7) Frances, S.P. (1994) Response of a chigger, *Eutrombicula hirsti* (Acari: Trombiculidae) to repellent and toxicant compounds in the laboratory. J. Med. Entomol. 31(4): 628-630.

(8) Frances, S.P.; Eikarat, N.; Sripongsai, B.; and Eamsila, C. (1993) Response of *Anopheles dirus* and *Aedes albopictus* to repellents in the laboratory. J. Am. Mos. Con. Assoc. 9(4): 474-476.

(9) Gerberg, E. J. ; Barnard, D.R.; and Ward, R.A. (1998) Manual for Mosquito Rearing and Experimental Techniques. AMCA Bull. 5.

(10) Govere, J.M.; and Durrheim, D. N. (2007) Chapter 8: Techniques for evaluating repellents. In *History of Insect Repellents: Principles, Methods and Uses*. Debboun, Frances, and Strickman, eds. CRC Press. (495 p.)

(11) Huntsberger, D. and Billingsley, P. (1981) Elements of Statistical Inference. 5th edition. Allyn and Bacon. Inc., Boston.

(12) Klun, J.A. and Debboun, M. (2000) A new module for quantitative evaluation of repellent efficacy using human subjects. J. Med. Entomol. 37(1): 177-181.

(13) Rutledge, L.C., and Gupta, R.K. (1999) Variation in the protection periods of repellents on individual human subjects: an analytical review. J. Am. Mosq. Cont. Ass. 15(3) 348-355.)

(14) Schofield, S.; Tepper, M.; and Gadawski, R. (2007) Field evaluation against mosquitoes of regular and polymer-based DEET formulations in Manitoba, Canada, with comment on methodological issues. J. Med. Entomol. 44: 457-62.

(15) Smith, C.N. (1955) Insect repellents. Quarterly Report, Entomological Research. Entomology Research Branch, U.S. Department of Agriculture. 8 p.

(16) Verwey, R.E. (1996) Laboratory method for testing insect repellents on human test subjects against chiggers in the laboratory. Unpublished document prepared by S.C. Johnson & Sons, Inc., Racine, WI. 3 p.

(17) WHO: CTD/WHOPES/1C/96.1: "Report of the WHO informal Consultation on the Evaluation and Testing of Insecticides."

Appendix A: Checklist of Elements Required by 40 CFR §26.1125

40 CFR 26.1125 Prior submission of proposed human research for EPA review

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
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.		
		(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.		
		(3) Records of continuing review activities.		
		(4) Copies of all correspondence between the IRB and the investigators.		
		(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.		
		(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).		
		(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).		
The following Information, to the extent not already included:	§1125(a) a discussion of:	(1) The potential risks to human subjects (2) The measures proposed to minimize risks to the human subjects; (3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue (4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and (5) The balance of risks and benefits of the proposed research.		
		§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		
		§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		
		§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.		
		§1125(e): All correspondence between the IRB and the investigators or sponsors.		
		§1125(f): Official notification to the sponsor or investigator . . . that research involving human subjects has been reviewed and approved by an IRB.		

Questions to be Addressed in EPA Protocol Review

Protocol Identification

(a) Title
(b) Date
(c) Principal Investigator and any sub-investigators
(d) Participating Laboratories
(e) Sponsor
(f) Reviewing IRB

1. Societal Value of Proposed Research

(a) What is the stated purpose of the proposed research?
(b) What research question does it address? Why is this question important? Would the research fill an important gap in understanding?
(c) How would the study be used by EPA?
(d) Could the research question be answered with existing data? If so, how?
(e) Could the question be answered without newly exposing human subjects? If so, how?

2. Study Design

(a) What is the scientific objective of the study? If there is an explicit hypothesis, what is it?
(b) Can the study as proposed achieve that objective or test this hypothesis?

2.1 Statistical Design

(a) What is the rationale for the choice of sample size?
(b) What negative and positive controls are proposed? Are proposed controls appropriate for the study design and statistical analysis plan?
(c) How is the study blinded?
(d) What is the plan for allocating individuals to treatment or control groups?
(e) Can the data be statistically analyzed?
(f) What is the plan for statistical analysis of the data?
(g) Are proposed statistical methods appropriate to answer the research question?
(h) Does the proposed design have adequate statistical power to definitively answer the research question?

2.2 How and to what will human subjects be exposed?

(a) To what will subjects be exposed?
(b) What is the rationale for the choice of test material and formulation?
(c) What is the rationale for the choice of dose/exposure levels and the staging of dose administration?
(d) What duration of exposure is proposed?

2.3 Endpoints and Measures

(a) What endpoints will be measured? Are they appropriate to the question(s) being asked?
(b) What steps are proposed to ensure measurements are accurate and reliable?
(c) What QA methods are proposed?
(d) How will uncertainty be addressed? Will reported point values be accompanied by measures of uncertainty?

3. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern? How was it identified?
(b) From what populations will subjects be recruited?
(c) Are expected participants representative of the population of concern? If not, why not?
(d) Can the findings from the proposed study be generalized beyond the study sample?

3.2 Equitable Selection of Subjects

(a) What are the inclusion/exclusion criteria? Are they complete and appropriate?
(b) What, if any, is the relationship between the investigator and the subjects?
(c) If any potential subjects are from a vulnerable population, what is the justification for including them?
(d) What process is proposed for recruiting and informing potential subjects?
(e) If any subjects are potentially subject to coercion or undue influence, what specific safeguards are proposed to protect their rights and welfare?

3.3 Remuneration of Subjects

(a) What remuneration, if any, is proposed for the subjects?
(b) Is proposed remuneration so high as to be an undue inducement?
(c) Is proposed remuneration so low that it will only be attractive to economically disadvantaged subjects?
(d) How and when would subjects be paid?

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 materials?
(b) What is the nature of the risks to subjects of the proposed research?
(c) What is the probability of each risk associated with the research? How was this probability estimated?

4.2 Risk minimization

(a) What specific steps are proposed to minimize risks to subjects?
(b) How do proposed dose/exposure levels compare to established NOELs/NOAELs for the test materials?
(c) What stopping rules are proposed in the protocol?
(d) How does the protocol provide for medical management of potential illness or injury to subjects?
(e) How does the protocol provide for safety monitoring?
(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?
(g) How and by whom will medical care for research-related injuries to subjects be paid for?

5. Benefits

(a) What benefits of the proposed research, if any, would accrue to individual subjects?
(b) What benefits to society are anticipated from the information likely to be gained through the research?
(c) How would societal benefits be distributed? Who would benefit from the proposed research?
(d) What is the likelihood that each identified societal benefits would be realized?

6. Risk/Benefit Balance

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

7. Independent Ethics Review

(a) What Institutional Review Board (IRB) reviewed the proposed research?
(b) Is this IRB independent of the investigators and sponsors of the research?
(c) Is this IRB registered with OHRP?
(d) Is this IRB accredited? If so, by whom?
(e) Does this IRB hold a Federal-Wide Assurance from OHRP?
(f) Are complete records of the IRB review as required by 40 CFR §26.1125 provided?
(g) What standard(s) of ethical conduct would govern the work?

8. Informed Consent

(a) Will informed consent be obtained from each prospective subject?
(b) Will informed consent be appropriately documented, consistent with the requirements of 40 CFR §26.1117?
(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?
(d) What is the literacy rate in English or other languages among the intended research subjects?
(e) What measures are proposed to overcome language differences, if any, between investigators and subjects?
(f) What measures are proposed to ensure subject comprehension of risks and discomforts?
(g) What specific procedure will be followed to inform prospective subjects and to seek and obtain their consent?
(h) What measures are proposed to ensure fully voluntary participation and to avoid coercion or undue influence?

9. Respect for Subjects

(a) How will information about prospective and enrolled subjects be managed to ensure their privacy?
(b) How will subjects be informed of their freedom to withdraw from the research at any time without penalty?
(c) How will subjects who decline to participate or who withdraw from the research be dealt with?

Appendix C: Checklist of Elements Required by 40 CFR §26.1303

§ 26.1303 Submission of Completed Human Research for EPA Review

Any person who submits to EPA data derived from human research covered by this subpart shall provide at the time of submission information concerning the ethical conduct of such research. To the extent available to the submitter and not previously provided to EPA, such information should include:

Requirement			Y/N	Comments/Page References
(a) Copies of all of the records relevant to the research specified by §26.1115(a) to be prepared and maintained by an IRB	§1115(a)(1): Copies of • all research proposals reviewed, • scientific evaluations, if any, that accompany the proposals, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.			
	§1115(a)(2): Minutes of IRB meetings which shall be 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.			
	§1115(a)(3): Records of continuing review activities.			
	§1115(a)(4): Copies of all correspondence between the IRB and the investigators.			
	§1115(a)(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.			
	§1115(a)(6): Written procedures for the IRB in the same detail as described in § 26.1108(a) and § 26.1108(b).			
	§1115(a)(7): Statements of significant new findings provided to subjects, as required by § 26.1116(b)(5).			
(b) Copies of all of the records relevant to the information identified in §26.1125(a)-(f)	§1125(a) A discussion of:	(1) The potential risks to human subjects;		
		(2) The measures proposed to minimize risks to the human subjects;		
		(3): The nature and magnitude of all expected benefits of such research, and to whom they would accrue;		
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and		
		(5) The balance of risks and benefits of the proposed research.		
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.			
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.			
	§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.			
	§1125(e): All correspondence between the IRB and the investigators or sponsors.			
	§1125(f): Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB.			
(c) Copies of sample records used to document informed consent as specified by §26.1117, but not identifying any subjects of the research				
(d) If any of the information listed in paragraphs (a) through (c) of this section is not provided, the person shall describe the efforts made to obtain the information.				