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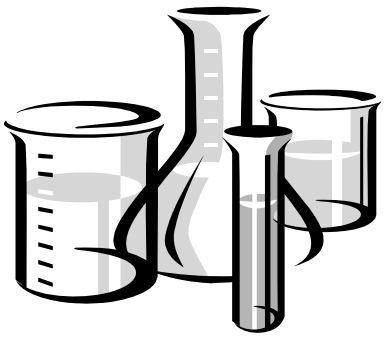
Product Performance Test Guidelines

OPPTS 810.3700: Insect Repellents to be Applied to Human Skin



NOTICE

This is one of a series of test guidelines established by the Office of Chemical Safety and Pollution Prevention (OCSPP) (formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS) prior to April 22, 2010),United States Environmental Protection Agency for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, *et seq.*), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*), and section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 346a), referred to hereinafter as the harmonized test guidelines.

The harmonized test guidelines serve as a compendium of accepted scientific methods for research intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting appropriate tests, and is also used by EPA, the public, and the companies that are required to submit data under FIFRA. These guidelines are not binding on either EPA or any outside parties, and EPA may depart from them where circumstances warrant and without prior notice. The methods described in these guidelines are strongly recommended for generating the data that are the subject of the guidelines, but EPA recognizes that departures may sometimes be appropriate. You may propose and alternatives to the methods described in these guidelines, with your supporting rationale. The Agency will assess such proposals on a case-by-case basis.

For additional information about the harmonized test guidelines and to access the guidelines electronically, please go to http://www.epa.gov/oppts and select "Test Methods & Guidelines" from the navigation menu at the top of the screen. You may also access the guidelines in http://www.regulations.gov grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, and EPA-HQ-OPPT-2009-0576.

OPPTS 810.3700: Insect Repellents to be Applied to Human Skin

(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) (7 U.S.C. 136, *et seq.*). This guidance applies to products in any formulation—such as lotion, liquid, or spray—intended to be applied directly to human skin. It does not apply to products applied to or impregnated into clothing or fabric, 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.

(2) General Considerations. Any protocol developed using this guidance must meet 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. Because these studies would support a FIFRA registration and involve intentional exposure of human subjects to the test repellents, review of each protocol by an Institutional Review Board (IRB), by EPA, and by EPA's Human Studies Review Board (HSRB) is required before the study is initiated. (See 40 CFR §26.1109, §26.1125, and §26.1601.)

This guideline does not supersede or overrule the regulations governing research conducted on human subjects contained in 40 CFR part 26, subparts K through Q, or any other Agency regulations. To the extent there are any unintended conflicts between this guideline and any EPA regulation, the regulation at issue governs.

(3) 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) EPA's Rule for the Protection of Human Subjects of Research. Any research conducted under this guideline is covered by the requirements of EPA regulations for the protection of human subjects of research set out at

40 CFR part 26, subparts K, L, and M. Persons conducting and submitting topical repellent efficacy studies should ensure compliance with all applicable requirements of that rule; the following paragraphs highlight a few of them:

(A) **Applicability.** Subparts K and L 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 under the pesticide laws—FIFRA and §408 of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 346a). (40 CFR §26.1101) Efficacy testing of topically applied repellents typically 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.) Subpart M applies to any person who submits data from research with human subjects for consideration in connection with any action that may be performed by EPA under FIFRA or section 408 of FFDCA.

(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 through 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, 40 CFR 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)).

(iv) State Requirements. Investigators and Sponsors should ensure research is conducted in compliance with any applicable state laws or regulations, which are independent of and additional to those cited here.

(4) 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 to all 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) Protection of subject privacy and confidentiality
(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 (l) provide guidance specific to different kinds of testing of repellents. Section (i) addresses empirical estimation of a typical consumer dose, and sections (j) through (m) address testing of repellency in the laboratory or field to particular species of arthropods.

(i) Specific guidance for dose-determination studies.
(j) Specific guidance for laboratory studies of mosquito or biting fly repellency.
(k) Specific guidance for field studies of mosquito or biting fly repellency.
(l) Specific guidance for laboratory studies of tick or chigger repellency.

The final section (m) 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 guideline and are not intended to be more generally applicable.

(1) The following events may indicate a failure of 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 either or both by the distance the tick or chigger moves onto treated skin or by how long the tick or chigger 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 either 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.

(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 efficacy failure as it is defined in each study—for example, the time from application until the first efficacy failure event confirmed within 30 minutes by a second similar event.

(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—40 CFR part 26—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 (IRB) and, accompanied by supporting documentation meeting the requirements of 40 CFR §26.1125, must then be reviewed by EPA and the HSRB before research is initiated.

It is critical to all later stages of repellent testing that protocols contain the elements required by EPA's Good Laboratory Practices regulations at 40 CFR §160.120 and meet all other applicable scientific and ethical standards. The broad topics of scientific design, ethical justification, subject selection and informed consent, and protection of subject privacy and confidentiality are discussed in detail 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, addressing topics (i) through (x).

(i) **Objectives.** The objective of most repellent efficacy testing to support registration is to estimate how long after treatment a repellent will continue to protect users from the target pest; this period of effective repellency is measured as CPT. Sometimes the objective of repellent efficacy testing is to compare the efficacy of one treatment to another treatment or to an untreated control; this ratio is expressed as Relative Protection (RP). 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 either by weight as mg/cm^2 of treated skin surface or volumetrically as ml/cm^2 of treated skin surface. Because each subject is likely to apply a different amount of repellent in uncontrolled trials, the "typical dose" should be calculated as the mean of multiple applications by each of 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." Efficacy failure in a test to determine Complete Protection Time (CPT) may be defined either 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. The Agency encourages the use of the first confirmed failure event to estimate complete protection time for individual subjects, and the median CPT across all subjects in each treatment arm as the summary measure of CPT.

(*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 Agency encourages the choice of landings for field testing to reduce

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 an adverse 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 ticks or chiggers the appropriate event to show failure of repellent efficacy is a "crossing" from untreated skin onto treated skin.

(iv) Duration. Repellency testing should continue long enough to fairly assess the duration of protection provided by the repellent, and long enough that all or nearly all subjects experience efficacy failure.

If testing ends before some subjects experience a failure of efficacy the resulting data-set is said to be "right-censored." Right-censorship of data poses challenges for meaningful and accurate analysis. Right-censorship can be reduced through appropriate care in study design—for example, by planning a test of longer duration or, when appropriate, by pre-treating subjects well before their first exposure to target pests. Methods for analyzing right-censored datasets are discussed in paragraph (c)(1)(xi) below.

(v) Sample size. The sample should be large enough to be likely to yield a definitive answer to the research question being addressed, and its size should be justified statistically in each protocol, taking into account the specific characteristics of the proposed research and the desired accuracy and precision of the results. Researchers are encouraged to consult a statistician to help determine appropriate sample size.

Withdrawal of test subjects from the study before failure of efficacy decreases the sample size and may compromise the validity or utility of test results. The protocol should fully describe how the proposed sample size was determined, and how data for subjects who withdraw from the test prematurely will be treated.

Other factors which may affect sample size are the number of treatments, the experimental design, and the heterogeneity of the target population (e.g., by age, sex) and of the environment (different habitats, different conditions, different species and population densities).

(vi) Allocation of subjects to treatments. Subjects should be allocated to treatments randomly and, to the extent possible, blindly.

Multiple treatments per subject may be permissible if all treatments contain the same active ingredient at comparable concentrations, but care must be taken in the study design and execution to prevent cross-contamination or confounding interference. It is particularly important for subjects receiving different treatments on different limbs not to rub their limbs together or otherwise contaminate the repellent treatments, to keep results independent. The number of treatments per subject should be limited by the feeding behavior of the target species. For example, for mosquito species feeding close to the ground on lower limbs, treatments should be applied only to subjects' lower legs, and only two treatments per subject will be possible. Treating the same subject more than once with the same treatment does not increase the sample size; a single subject can only be counted once in determining the sample size.

When assessing relative protection provided by more than one product tested simultaneously, a Latin square design, in which each subject is tested with each treatment over the course of the study, is often appropriate.

(vii) **Untreated controls.** To minimize risks to untreated control subjects, both the number of untreated subjects and the duration of their exposure should be minimized. Untreated controls should be exposed intermittently, and then only long enough to confirm adequate pest pressure throughout the study. Recent research (Barnard, *et al.* 2002) has shown that treated subjects should not serve as their own untreated controls.

(viii) **Positive controls.** Positive controls are desirable to support comparison of results from different repellency studies or from testing on different days or at different locations. The recommended positive control material is 20% deet in ethanol, applied at a standard dose rate of 1 ml per 600 cm^2. Positive controls are particularly valuable when the objectives of the study include comparisons between formulations, and when testing spans different days. To minimize the likelihood of interference, subjects treated with the test material should not also serve as concurrent positive controls.

(ix) **Preparation of subjects.** Before treatment with a test repellent or use as an untreated or positive control, subjects' 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. Subjects should avoid activities that increase perspiration, and avoid abrading, rubbing, touching, or wetting the treated area.

(x) **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, or lotion) and with the directions for use intended for commercial distribution. Subjects in repellency trials should all be treated at the same standard dose rate, and treatment should be delivered so as to ensure consistent application and uniform coverage.

(xi) Statistical analysis plan. Protocols should include a full description, explanation, and justification for the statistical methods proposed to analyze both dose determination and repellency test results, taking into account the specific study objectives and variables.

The statistical analysis plan should provide for testing results for normal distribution. When results are normally distributed, it may be appropriate to report the mean CPT across all treated subjects with its standard error. When the data do not fit a normal distribution—more typical of repellency datasets—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, such as Kaplan-Meier survival analysis[1], are suggested.

If the study objective is to determine CPT across all subjects, any right-censorship of repellency data will lead to underestimation of both the mean and the variance around it. Because right-censorship is common in repellency testing, EPA recommends use of the median CPT with its 95% confidence limits as the summary measure of CPT.

(xii) 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 expected benefits to society from the knowledge likely to be gained in the research must also outweigh the minimized 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. Investigators should consider possible alternatives to human research; protocols should discuss possible alternatives to human research and explain why they are infeasible or would not answer the research question.

[1] For guidance see, for example, WHO (2009) Guidelines for Efficacy Testing of Mosquito Repellents for Human Skin: WHO/HTM/NTD/WHOPES/2009.A. Annex 3: Estimation of Median and Confidence Interval of Complete Protection Time Using the Kaplan-Meier Survivor Function.

(ii) **Prerequisite research.** Before testing a repellent's efficacy with human subjects, acute toxicity to animals of the active ingredients should be tested and reported in the protocol. Acute dermal toxicity, dermal irritation, eye irritation, and skin sensitization studies are needed to estimate the margin of exposure (MOE) for subjects participating in a repellent test. All additional components in the materials tested should be cleared by EPA for use in repellent formulations.

(iii) **Risks to subjects.** Protocols should include a complete discussion of risks to subjects associated with their participation in the research, characterizing all risks and their likelihood, and describing steps proposed to minimize each of 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 (but may not be limited to) reactions to the test substance or to arthropod bites, acquisition of vector-borne illness, physical risks (e.g., stresses imposed by the requirements or conditions 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 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 having a physician on call during testing. In addition, protocols should specify how any research-induced harm will be ***reversed***—for example, by the investigator's or sponsor's committing to pay for uninsured costs of medical treatment of subjects for injuries or illnesses resulting from their participation in the research, and for longer-term treatment, if needed, of any arthropod-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 involve 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. Sites at which infected vectors are known to be present 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;
- Using bites as the endpoint in either field or laboratory studies only when fully justified;
- 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, even after risks have been minimized. Thus the ethical justification for the research ultimately 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.

Societal benefits include only those directly resulting from the knowledge likely to be gained from the research. Benefits potentially resulting from use of effective repellents should not be attributed to the proposed research. 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 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).) The protocol should discuss explicitly the balance of risks to subjects and anticipated societal benefits of the research.

(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. (FIFRA §12(a)(2)(P) and 40 CFR §26.1116)

(i) **Representative sampling.** The results of repellent testing should be as generalizable as possible to the target population of repellent users. Samples should include adults of various ages, of both sexes, and of a variety of races and ethnicities, and protocols should describe the demographic characteristics of the pool from which subjects will be recruited. Attractiveness of recruited candidates to the target species should be verified before they participate in repellency testing.

Testing with a sample known to be unrepresentative is discouraged; if it is proposed, it should be fully justified. Arguments based on the convenience of the investigators or on the difficulties associated with recruiting a more representative sample do not justify testing with an unrepresentative sample.

(ii) **Inclusion/Exclusion factors.** All exclusion/inclusion criteria proposed should be supported by a clear and explicit rationale. 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.) Because older adults are more susceptible to arthropod-borne diseases, candidates over 55 should be excluded as subjects in field testing, or their inclusion should be specifically justified.

Members of certain vulnerable populations, including people of limited mental capacity, those not in good health or with compromised immune systems, those sensitive to chemicals, and students or employees of the investigators or sponsors should always be excluded. Other potential subjects who may be in a vulnerable position, including the educationally or economically disadvantaged, or those who communicate with difficulty because of language differences or disabilities, should not be excluded arbitrarily if appropriate specific provision can be made to ensure their safety and welfare.

Subjects should generally be recruited from populations in the area where testing will be conducted. An offering of distant travel may unduly influence a candidate's choice to enroll, and a subject who has accepted long-distance transportation may feel less than free to withdraw from a study. If it is proposed, transportation of subjects to distant locations should be justified, and specific mechanisms should be proposed to prevent any coercion or undue influence.

(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 will 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 than English, 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 an extended process, involving much more than simply obtaining a subject's signature on a form.

It begins with recruiting, and continues after signature of the consent form throughout the conduct of the research. A single discussion in the protocol of the recruiting and consent processes as a continuous whole is recommended.

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 consent discussions with individual candidates be conducted in private, and that all consent documents be written at or below an 8th grade reading level, as measured by the Flesch-Kincaid Grade Level score or another standard tool for assessing readability. (A tool to calculate the Flesch-Kincaid Grade Level score is a standard feature available to users of MS-Word.) If consent materials are translated into another language, it is important to ensure the translations are also at an appropriate reading level.

The informed consent process should describe how the investigator will confirm each candidate's understanding of the research and of what it will involve before he or she is enrolled.

The rule at §26.1116 further defines the general requirements for informed consent, including at §26.1116(a) the basic elements always required, at §26.1116(b) other elements conditionally required, and at §26.116(e) a requirement to identify the pesticide and the nature of its pesticidal function.

(B) **Documentation of informed consent.** 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, and prior discussion with EPA is strongly encouraged.

Ensure that any elements appearing in both the protocol and the consent form are in harmony. Elements common to both documents may include, for example, descriptions of the purpose of the research, subject inclusion/exclusion factors, risks to subjects and how they will be minimized, and what will happen in the course of the research. The protocol and the consent form should be consistent in substance, although the presentations should usually not be the same, because the readers and purposes differ. Consent forms should address candidates consistently in the second person—as "you"—and should present all information from the subject's point of view. By contrast protocols should be written in the third person, and should present all information from the investigators' 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) Protection of subject 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.

(i) 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.

(ii) 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 identifying marks cannot be excluded from a photograph or video image, the image should be altered to protect the identity of the subject(s).

(iii) 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. For example, 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 during the testing itself and in handling the results. Unexpected news of a pregnancy can cause significant psychological distress, which can be increased by any breach of discretion in handling the information. It is a good practice to recruit more candidates than are required by the study design, so that the design would not be compromised by withdrawal of one or more subjects. This approach permits a candidate with a positive pregnancy test to withdraw without stating a reason. No records of a positive pregnancy test should be retained.

(d) Review of protocols for repellent studies. EPA's Rule for the Protection of Human Subjects of Research 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 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 HSRB (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. Typical requirements are for 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 section (c) of this guideline is likely to satisfactorily address most requirements of a reviewing 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 from the research;

- 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; and
- 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 subjects are likely to be vulnerable to coercion or undue influence, additional safeguards are included in the study design 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 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 in the subsequent submission to EPA. (See 40 CFR § 26.1125(e) and (f).)

(2) Reviews by EPA and the HSRB. After receiving approval from an IRB, the protocol and supporting material must be submitted to EPA for review by EPA and by the HSRB. (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 guideline. Records of two kinds are required.

(A) Records describing the research proposal itself. 40 CFR sections 26.1125(a)–(d) call for a discussion of the risks and benefits of the proposed research, for the consent forms as submitted to and as approved by the IRB, for information about how subjects will be recruited, and for 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 guideline 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 also 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 research institution; and

- 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. By prior arrangement, however, an IRB may submit them directly to EPA.

(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 satisfaction of the requirements of 40 CFR §26.1125, and for substantiation of any claims of business confidentiality associated with the submission. If a submission is found to be incomplete, or to include unsubstantiated claims of business confidentiality, the submitter will be promptly notified; the submission will not be reviewed further until these deficiencies are corrected. EPA evaluates complete proposals in a single substantive review addressing both scientific and ethical aspects of the proposal, following the general outline in Appendix B to this guideline.

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 Committee Act, charged with advising EPA on scientific and ethical aspects of proposed and completed research with human subjects. The HSRB meets several times a year in public session, with opportunities for public participation. All materials they consider are kept in a public docket, except any which are subject to a substantiated claim of business confidentiality. HSRB recommendations are documented in a public report of each meeting, prepared as soon as possible after the meeting—typically 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 to the protocol, consent form or other materials may be needed 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 review by the full IRB, 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. Initiation of subject recruitment before obtaining IRB approval of the final protocol will be treated as a breach of the regulations.

(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 to 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 deviations from the protocol may occur. 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 events. Any adverse events affecting the subjects of a repellent efficacy test must be promptly reported to both the overseeing IRB and to EPA, including adverse events not established to be related to product exposure or study participation. (40 CFR §26.1108(b)(1))

(g) Reporting of completed repellent studies

(1) Study report. In addition to the standard elements required by the GLP regulations at 40 CFR §160.185 and the appendices described below, the primary report of the study should include these elements, as applicable.

(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) Recruiting and enrollment statistics, including the following:

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

(iv) Test arthropods: Genus, species, subspecies or strain (if information is available) of target pests; and pest pressure in each cage or field site. For laboratory studies, the source of test arthropods, methods used to establish

and maintain their disease-free status, rearing techniques and conditions, developmental stage, age and sex of target arthropods and methods used to sex them; preparation of arthropods before testing, and density per cage or container.

(v) Test conditions: For field studies, the location and type of habitat, species distribution, climate and environmental conditions. For laboratory studies, a complete description of cages or containers, temperature, relative humidity, ambient light, and air flow.

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

- Preparation of subjects: Training, demonstrating attractiveness, clothing and protective equipment worn, calculation of skin area and individual dose, washing, rinsing, and drying of limbs;
- Allocation of subjects to treatments; number of treatments per subject; blinding key, if used;
- Method of dose determination;
- Method of application of repellent to each subject and individual dose applied;
- Time of application of repellent to each subject;
- Time of start and end of exposure of each subject.

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

(viii) Results for each subject.

(ix) Reports of all statistical analyses

(x) Conclusions

(xi) Discussion

(2) Appendices.

(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) 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 test material at a standard dose rate, expressed by 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 (N,N-diethyl-meta-toluamide) eet, a standard dose rate of 1 g per 600cm^2 (equivalent to 1.67 mg per cm^2) has been used in many tests of repellency. Repellents in lotion form containing DEET may be tested for repellency at this standard dose rate, or at a "typical consumer dose" rate determined empirically 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 through empirical dose-determination testing as described below. The rationale for the dose rate actually used in repellent testing should be documented in all study reports.

(1) **Test material.** The formulated product should be used as it is or will be marketed, in the same type of 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 testing would 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. The sample should include adults of both sexes and varied race, and should be as representative as possible of the repellent user population, consistent with ethical and feasibility constraints.

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

(4) **Methods.** Each subject should be instructed to apply the test repellent to his or her own limbs—to forearms or lower legs, or both—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 for each limb treated by each subject.

The applied quantity of a lotion or towelette formulation can be determined by weighing the container or towelette before and after use. Because less than all the spray released from a pump-spray or aerosol container will reach the target limb, a different method is needed to estimate 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. So long as the area of the bracelets is known, the quantity of a spray formulation applied to the skin per cm^2 can be estimated by weighing the bracelets before and after application. Skin should be left exposed between bracelets to help subjects 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 by weight as mg per cm^2 to a volumetric dose expressed as ml per cm^2; this calculation should be included in the study report. The 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. The protocol should include the specific gravity of all test materials and should specify this calculation, and should specify doses both by weight and by volume.

(j) Specific guidance for laboratory studies of mosquito or biting fly repellency.

(1) Species. Mosquito tests should be conducted with *Aedes aegypti*, an *Anopheles* species such as *An. albimanus*, or a *Culex* species, preferably *Cx. quinquefasciatus.* Stable fly tests should be conducted with *Stomoxys calcitrans*. The source of test insects should be reported, and if possible, they should be identified by subspecies or strain.

(2) Stage, age, and sex. Mosquito testing should be conducted with adult females 5-10 days old; methods of sexing should be reported. Stable flies should be male or female adults 3-10 days old. The age range of test insects should be reported.

(3) Rearing techniques. Mosquito and biting fly larvae should be reared under optimal conditions for the species—typically at 27° ± 3°C, relative humidity 80% ± 10%, and photoperiod 16:8 hours (light:dark). Alternative rearing methods for mosquitoes are discussed by Gerberg et al. (1998). Use of alternative rearing techniques for biting flies and/or mosquitoes should be fully documented and justified.

(4) Preparation of insects. Test mosquitoes should be fed 10% sucrose and no blood meal before use in a test, and starved for 12 to 24 hours immediately before the test. Test stable flies may be fed dry sugar cubes with a separate water source; they should also receive no blood meal before use in a test, and should be starved for 24 hours immediately before the test. Test insects should be established to be free of disease; the source of insects and the methods used to ensure they are disease-free should be reported.

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

(6) Test cages. Cage size may vary, but is typically 2’ x 2’ x 2’ (8 cubic feet in volume, roughly equivalent to 232,000 cm^3), square or rectangular in plan, with one or more sleeved openings for the subject’s arms. A mirror on the bottom of the cage helps observers see insects landing on the underside of subjects’ arms. Larger cages may be used for two subjects at a time, so long as they are treated with the same repellent. Cage dimensions and design should be reported in detail.

(7) Insect density. There should be at least 200 mosquitoes in each 2’x2’x2’ cage (equivalent to one for each 1,160 cm^3) or at least 50 stable flies per cage (one for each 4,640 cm^3.) After each exposure period all stable flies should be removed by vacuuming from each cage, and 50 fresh stable flies should be added to each cage.

(8) **Testing conditions.** Ambient temperature during the test should be maintained at 21° ± 8°C, and relative humidity at 55-85%. 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 insect activity. Investigators and subjects should avoid exhaling into the test cage; introduction of CO_2 could bias insects towards biting.

(9) **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 with a solution of ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area of each test subject's treated forearm (in cm^2) and the volumetric dose administered to each subject (in ml) should be reported. Skin adjacent to the treated area should be covered with a light-colored material that test insects cannot bite through. Hands should be covered with impenetrable (e.g., latex) gloves.

(10) **Untreated controls.** One or two untreated control subjects should verify continued landing pressure in tests of CPT. Results for these untreated subjects should not be compared to those for treated subjects; multiple untreated controls should be used as a standard of comparison only in studies of relative protection/percent repellency. To minimize potential interference, untreated subjects should not be treated on their other forearm. Forearms of untreated subjects should be washed, rinsed, and dried exactly like treated forearms.

(11) **Positive controls.** A positive control group treated at a rate of 1 ml per 600 cm^2 with 20% DEET in ethanol is recommended to calibrate the test system.

(12) **Establishing subject attractiveness to test insects.** Before the test, subjects should expose their untreated forearms to the target insects in a test cage to establish their attractiveness. Five mosquito landings in one minute or less or 2 stable fly landings in two minutes or less are sufficient to establish attractiveness.

(13) **Establishing and confirming landing pressure.** Before exposing any treated subjects during each exposure period, an untreated subject's forearm should be inserted into each cage to confirm landing pressure. Landing insects should be removed from the subject's arm, by shaking it or by other means, before they have time to bite. The untreated subject should remove his or her forearm from the cage as soon as it has received the requisite number of landings. If at any time fewer than 5 mosquitoes land on the untreated control forearm within one minute, all mosquitoes should be removed from all cages in the study and fresh insects should be added to each cage. If at any time fewer than 2 stable flies land on the untreated control forearm within two minutes, all stable flies should be removed

from that cage and 50 fresh flies should be added. The aggressiveness of the fresh insects should be confirmed before repellency testing continues.

(11) Exposure period. Approximately 30 minutes after treatment with the repellent, test subjects should insert their treated forearms into the cage for the first time. Exposures of pre-defined duration should be repeated at regular intervals—e.g., 5-minute exposures at 30-minute intervals—until efficacy failure for each subject 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 or biting fly repellency.

(1) Pre-test subject preparation. Subjects in field studies of repellency should be trained in the laboratory to identify landings, probes, and bites of the target species and to use aspirators to collect landing insects before they have time to bite.

(2) Choice of field testing sites. Field tests for mosquito repellency should be conducted in at least two distinct habitats (e.g., forest, grassland, salt marsh, wetland, beach, barns, or urban environments) where the predominant mosquito species differ. So long as the environments and species distribution differ, it is not important for test sites to be widely separated geographically. Repellency against black flies, stable flies, or other biting flies typically occurring in only one habitat may be tested in only one habitat.

Potential sites for testing mosquito repellency should be monitored at least weekly for a month before testing is scheduled. To minimize risks to subjects, field testing should ***not*** be conducted where West Nile virus (WNV) or other mosquito-vectored diseases have been detected within the previous two weeks. Because biting flies are not known to vector human diseases in the USA, no comparable site monitoring is required for studies of repellency to biting flies.

(3) Species identification. Mosquito tests should be conducted where more than one species is present. If tests are conducted outside the U.S., the relevance of the study for U.S. regulatory purposes should be justified in the study report. Landing insects should be aspirated or trapped before and during the test, and labeled with the time of collection. After the field study, collected insects should be identified by genus and species, and if possible, by subspecies or strain. The number in each taxon collected 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. The results of these analyses should be reported to subjects and included in the study report.

(4) **Untreated controls.** Untreated control subjects are necessary in all study designs. To minimize the potential for interference, untreated controls should be subjects who are not treated with a repellent on any limb.

When the objective is to estimate CPT, two untreated control subjects are sufficient. They should expose an untreated arm or leg briefly at regular intervals during the test to confirm continued acceptable landing pressure. Results for the untreated controls should not be compared to results for treated subjects. When the objective is to evaluate relative protection, more untreated control subjects are needed to provide a standard of statistical comparison.

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

(5) **Positive controls.** A positive control group treated at a rate of 1 ml per 600 cm^2 with 20% deet in ethanol is recommended to calibrate the test system.

(6) **Minimum landing pressure to initiate or continue test.** 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 landing pressure of the target species is at least one mosquito landing within one minute, or at least one stable fly, black fly, ceratopogonid or tabanid landing within five minutes. Insects landing on untreated subjects should be collected for later identification, and labeled with the time of collection.

(7) **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. A smaller treated area on the appropriate limb of at least 250 cm^2 is also acceptable. The treated area should be washed with unscented soap, rinsed with a solution of ethanol or isopropyl alcohol in water, and dried with a clean towel. The surface area treated for each test subject 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) **Exposure period.** Exposure of treated subjects may be intermittent—for example, for 1 or 2 minutes at 15-minute intervals, or for 5 minutes at 30-minute intervals—to reduce risks to subjects and allow them to rest between exposures.

When testing a repellent with a long period of effectiveness, it is critical to the reliability of the data to minimize "right-censoring" of the data—i.e., the number of subjects who do not experience a failure of efficacy before the end of the test. Reliable results may be obtained for extended periods of protection by treating subjects up to several hours before the first field exposure, with subsequent

exposures timed to coincide with periods of target insect activity. This approach reduces prolonged exposure of subjects to insects in the field, helps to reduce early withdrawal of subjects attributable to excessively long trials, and increases the likelihood that most or all subjects will experience a failure of efficacy.

Another acceptable 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.

(8) **Minimizing variation.** Many factors contributing to variability in repellent field studies cannot be controlled, but minimizing variation when possible and designing studies with insect feeding patterns in mind can make a study design more efficient. Standardization is possible for such factors as time of testing, allocation to treatment, subject attractiveness to target insects, etc. Other factors which may be managed to minimize variation include targeting a predominant species in its habitat, synchronizing exposure periods with peak feeding activity of target insects, and treating limbs consistent with the preference of the target species to feed on legs, arms, or both.

(9) **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 periodically throughout the study and reported. Testing should be not be conducted or continued if wind speed exceeds 16 kph/10 mph.

(10) **Test subject placement and behavior.** Subjects may work in pairs to assist each other to identify landings and collect insects. Because clustering of subjects may confound results, at least 3 m/10 ft should be maintained between pairs. Subjects should avoid strenuous exercise and sweating, since these could affect test results.

(11) **Data collection and reporting.** Under the supervision of the investigator, an associate or another subject should record the number and timing of each landing or bite during each exposure period for each subject. If possible, all landing insects should be collected for identification and labeled with the time of collection.

(l) **Specific guidance for laboratory studies of tick and chigger repellency.** Because reliable field tests have not been developed, EPA recommends testing for repellency against ticks and chiggers in the laboratory. If field tests are conducted, proposed label directions for re-application frequency should reflect field study results.

(1) **Summary of recommended method.** Each subject should place the fingers of one hand on a flat surface, with the elbow above the wrist and the forearm held at an angle of 30° or more to the surface. With a suitable instrument (such as an

artist's paintbrush, forceps, or a cotton swab) the investigator should place a tick or chigger, one at a time, on the subject's wrist, at a release point marked 3 cm below the boundary of the treated area of the forearm. The tick or chigger should be oriented gently (e.g., with paint brush, forceps, or cotton swab) toward the treated area. After its first movement up the arm toward the margin of the treated area, each tick or chigger should be allowed 3 minutes to move across the boundary onto the treated area. A tick or chigger that crosses at least 3 cm into the treated area (toward the elbow) is reported as 'not repelled'. One that does not cross into the treated area, or that crawls into the treated area but immediately turns back or falls off, is reported as 'repelled.' Fresh ticks or chiggers are exposed to the treated area one at a time, at regular intervals for the duration of the test.

While the general definition of a "crossing" is constant, the details of the method of scoring a crossing must be operationalized in each protocol in a way appropriate to the species and life stage of ticks to be tested. A release point 3 cm distant from the treated area and scoring of a crossing when a tick moves at least 3 cm into the treated area have worked effectively in tests with nymphal *Dermicentor variabilis* and *Ixodes scapularis,* and are provided here as examples.

(2) **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. It is permissible to test two species concurrently, alternating so that each subject qualifies and tests a tick or chigger from each species within each exposure period.

(3) **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 arthropods should be reported.

(4) **Rearing techniques**. Ticks and chiggers should be reared at 25° ± 3°C, at high relative humidity (> 90%), and photoperiod of approximately 16:8 hours (light:dark). Any alternative rearing techniques should be fully described and justified.

(5) **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.

(6) **Disposition of test arthropods**. Each tick or chigger should be used only once and should be destroyed immediately after use.

(7) **Number of ticks or chiggers.** Ticks or chiggers are tested for questing behavior and for repellency one at a time, according to the study design. Some studies using a single species of tick test 5 ticks in each 30-minute exposure period; other studies test one tick of each of two species in each 15-minute exposure period. Either pattern is acceptable.

(8) **Testing conditions**. Temperature should be maintained during the test at 20° to 25°C, with relative humidity at ≥ 35% and indirect ambient light 50 to 80%. The lights should be kept on.

(9) **Subject preparation**. Before treatment, both forearms of each subject are washed with unscented detergent and carefully rinsed and dried. One forearm from wrist to elbow is treated with the test material, and two lines are drawn on the subject's wrist: a 'boundary line' at the edge of the treated area, and another line—the 'release line'—3 cm distant from the boundary line, outside the treated area toward the fingers. Similar lines are drawn in the same positions on the wrist of the subject's other, untreated arm—one line at the wrist, and another 3 cm away toward the fingers.

(10) **Untreated control.** The untreated forearm of each treated subject is used to screen ticks or chiggers for questing behavior; only actively questing ticks or chiggers should be selected for repellency testing. With an appropriate instrument, such as an artist's paint brush, forceps, or a cotton swab, each tick or chigger should be picked up carefully to prevent damaging its body or forelegs 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. A tick or chigger failing this test should be immediately destroyed.

(11) **Positive controls.** A positive control group treated at a rate of 1 ml per 600 cm^2 with 20% deet in ethanol is recommended to calibrate the test system.

(12) **Test procedure.** With the treated forearm held upright above the table, one fresh tick or chigger at a time, immediately after demonstrating active questing on the subject's untreated arm, is placed at the release line on the treated arm. A "crossing" is recorded if the test organism crosses the boundary line at least 3 cm into the treated area within 3 minutes, and remains in the treated area for at least one minute.

(13) **Exposure period**. At intervals of 15 to 30 minutes according to the study design, fresh ticks or chiggers are placed one at a time at the release point on the

untreated arm and tested for active questing. Qualified ticks or chiggers are then placed at the release point on the treated arm and exposed to the treated area. The cycle continues until efficacy failure or the end of the study, whichever occurs first.

(14) Data collection and reporting. Recording of crossings and repelled test ticks or chiggers 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 in each exposure period for each subject.

(m) References. The following publications were consulted in developing this guideline.

(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 *Insect Repellents: Principles, Methods and Uses*. Debboun, Frances, and Strickman, eds. CRC Press. (495 p.)

(4) Bernard, D. R., Bernier, U. R., Posey, K. H. and Xue, R-D (2002). Repellency of IR 3535, KBR 3023, para-menthane-3,8,-diol, and deet to Black Salt Marsh Mosquitoes (Diptera:Culicidea) in Everglades National Park. J. Med. Entomology39(6): 895-899

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

(6) 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.

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

(8) 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.

(9) 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.

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

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

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

(13) 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.

(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 (2009) Guidelines for Efficacy Testing of Mosquito repellents for Human Skin: CDS/NTD/WHOPES/2009.4.

(18) WHO (1996) Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides: CTD/WHOPES/1C/96.1.

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		
	§1125(a) a discussion of: (2) The measures proposed to minimize risks to the human subjects;		
	§1125(a) a discussion of: (3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue		
	§1125(a) a discussion of: (4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and		
	§1125(a) a discussion of: (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.		

Appendix B: Framework for Science and Ethics Reviews of Proposed Human Research

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.				