March 13, 2024

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Program in Human Research Ethics and Oversight
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: January 11, 2024, EPA Human Studies Review Board Meeting Report

Dear Ms. Tadeo:

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a study protocol involving human subjects.

On January 11, 2024, the HSRB considered a study protocol for a laboratory efficacy test of an Oil of Lemon Eucalyptus and Picardin-based skin-applied repellent spray against ticks. Briefly, the goal of the proposed study is to determine the median complete protection time (CPT) for the product, and the data will be used to support product registration.

The HSRB's responses to the charge questions, along with detailed comments and recommendations for the EPA to consider are provided in the enclosed final meeting report.

Sincerely,

Lisa Corey

Julia A. Sharp

Lisa Corey, Ph.D.
Co-Chair, HSRB

Julia Sharp, Ph.D.
Co-Chair, HSRB

Report of the U.S. Environmental Protection Agency Human Subjects Review Board

HSRB Board Members

EPA Contact

Tom Tracy, Designated Federal Officer

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Contents

List of Acronyms –

CI	Confidence Intervals
CPT	Complete Protection Time
EPA	Environmental Protection Agency
FCC	First Confirmed Crossing
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
HSRB	Human Studies Review Board
IRB	Institutional Review Board
mCPT	Median Complete Protection Time
OLE	Oil of Lemon Eucalyptus, also known as Citriodiol
OPPTS	Office of Pollution Prevention and Toxics
WCG	Western Copernicus Group

HSRB Meeting Report

Laboratory efficacy test of an Oil of Lemon Eucalyptus (OLE)- and Picaridin-based skin-applied repellent spray against ticks (Ixodidae) using a human-subject test method," April 7, 2022, as amended, November 11, 2022. Unpublished document prepared by Carroll-Loye Biological Research, 5100 Chiles Road Suite 108, Davis, CA 95618. IRB approved 15 November 2022. 138 pp. MRID 51905311.

Introduction

On January 11, 2024, the Human Studies Review Board (HSRB) considered the scientific and ethical charge questions related to a study protocol titled "Laboratory efficacy test of an Oil of Lemon Eucalyptus (OLE)- and Picaridin-based skin-applied repellent spray against ticks (Ixodidae) using a human-subject test method." EPA sought the HSRB's review of the study protocol in accordance with 40 CFR 26.

Review Process

The Board conducted a public meeting on January 11, 2024. Advance notice of the meeting was published in the Federal Register as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-10408-01-ORD). This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale, and consensus in response to the charge questions on ethical and scientific aspects of the research.

For each agenda item, the Agency staff presented their review of the scientific and ethical aspects of the research. Each presentation was followed by clarifying questions from the Board. The HSRB solicited public comments and then proceeded to address the charge questions under consideration. The Board discussed the science and ethics charge questions and developed a consensus response to each question. For each of the charge questions, the Chair called for the Board to vote to confirm concurrence on a summary statement reflecting the Board's response.

For their evaluation and discussion, the Board considered materials presented at the meeting, research articles, and related materials, the Agency's science and ethics reviews of the research studies, the Agency's statistical analysis of the research data, comments from the Public, and oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/scientific-leadership/hsrb-january-11-2024.

Additional materials reviewed:

- December 14, 2022 EPA Human Studies Review Board Meeting Report (HSRB Final Report 3.1.23.pdf (epa.gov))

Charge Questions and Context

Charge to the Board – Science

Is the protocol "Laboratory efficacy test of an Oil of Lemon Eucalyptus (OLE)- and Picaridin-based skin-applied repellent spray against ticks (Ixodidae) using a human-subject test method" likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks?

HSRB Response

The research proposed in the protocol "Laboratory efficacy test of an Oil of Lemon Eucalyptus (OLE)- and Picaridin-based skin-applied repellent spray against ticks (Ixodidae) using a human-subject test method" is likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks, given the comments and recommendations provided by the EPA and HSRB are adequately addressed.

Science Review

This protocol describes a study proposed to determine the duration and efficacy of a new skin-applied repellent product (Blox Tick Protection Spray), against three species of ticks (*Ixodes scapularis*, *Amblyomma americanum*, and *Dermacentor variabilis* or *Dermacentor andersoni* or *Rhipicephalus sanguineus*) in a laboratory setting. This product contains two active ingredients, i.e., oil of lemon eucalyptus (20%, by weight) and picaridin (20%, by weight). The efficacy will be evaluated using the median complete protection time (mCPT) from a sample of up to 32 human subjects (including 25 test subjects and seven alternates). This protocol was developed following the EPA guidance Office of Pollution Prevention and Toxics (OPPTS) 810.3700, and data generated from this protocol will be used to support product registration.

The three tick species will be tested on three different days. On each testing day, the product will be applied to the non-dominant forearm of each subject. The nominal application rate will be 1.1g/600cm^2, and the target skin area will be from wrist to elbow of the non-dominant forearm. The other, un-treated forearm of each subject will serve to determine whether ticks are actively questing prior to testing on the treated arm. After application and every 15 minutes, each subject will place an actively questing tick on the palm of the treated arm and monitor whether the tick moves more than 3 centimeters past the reference line toward the elbow (into the treated area) within 3 minutes. For each subject, the efficacy endpoint is defined as the complete protection time (CPT), i.e., the time between application of the test product and the beginning of the first confirmed crossing (FCC). FCC is defined as "*a (tick) crossing on a subject's treated forearm which is followed by another (tick) crossing within 30 minutes.*" For each tested tick species, the median CPT (mCPT) with 95% confidence intervals (CI) will be calculated from all the subjects and represents the product repellency efficacy.

Statistical Review

Kaplan-Meier Survival Analysis is proposed to estimate the mCPT for each set of tick species-specific CPT data, by using the statistical software JMP Pro 14.0 and/or the statistical programming language R and its associated application RStudio. The mCPT will be calculated for each tick species with 95% CI. The lower 95% CI and upper 95% CI will be calculated for each species using log-log transformed data. Kaplan-Meier Survival Analysis is appropriate for an estimated mCPT as a measure of central tendency for censored survival data.

The reviewed protocol proposes a sample size of 25 test subjects based on a Weibull distribution assumption of the tick repellent CPT and according to calculations using a precision expressed as the ratio: 95% LCL mCPT /estimated mCPT. The power analysis (described in detail on Pages 79-99) was based on a computer simulation procedure based on 4000 datasets randomly generated from a Weibull

distribution with a mCPT= 2 and ratio of the 5%-tile/median (P5MR) = 0.2. The sample size calculation is appropriate for the study goal.

Comments

In addition to the recommendations that have already been mentioned in the EPA science review, and the HSRB recommendations following the ethics review section, the HSRB has a few other comments on the science:

1) Two deviations from EPA OPPTS 810.3700 were noticed in the study protocol. First, positive controls are recommended in the test guideline but not included in the protocol. The HSRB understands positive controls are not mandatory. However, as mentioned on Page 9 of the OPPTS 810.3700 [Section (viii)], positive controls are desirable to support comparison of testing results from different days. It is the HSRB's understanding that different tick species will be tested on different days. Second, in this study the test material was applied using syringes, while the test consumer product will be used as aerosols. OPPTS 810.3700 suggests the test product be applied in the same method as directed on the future product label [Page 26, Section (1) of OTTPS 810.3700]. The HSRB understands that by using syringes the skin application rate may be estimated more accurately.
2) The source of dermal absorption rate for picaridin (10%) in Table 4 is not provided. This information cannot be found in the reference provided in Table 5 either.
3) There is no information provided regarding behavioral differences between study insects ("home" grown insects, presumably "well fed") compared to the same species in the wild (presumably hungry).
4) Kaplan Meier requires that random censoring be noninformative. The maximum likelihood and partial likelihood method selected does not handle informative censoring without an appreciable bias.
5) Descriptive analyses are not specified in the study protocol. Understanding the study participants and comparing the excluded participants, drop-outs, etc. with the participants completing the testing is essential for understanding the reproducibility, validity, and applicability of the study results, and for justification of the human participation benefit in the study.

Charge to the Board – Ethics

If amended to address EPA's and the HSRB's recommendations, is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

HSRB Response

The research proposed in the protocol "Laboratory efficacy test of an Oil of Lemon Eucalyptus (OLE)- and Picaridin-based skin-applied repellent spray against ticks (Ixodidae) using a human-subject test method" is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L, if the recommendations made by EPA and the HSRB are adequately addressed.

Ethics Review

This is a laboratory evaluation of Blox Tick Protection spray against ticks (Ixodidae) using a human-subject test method. This protocol is very similar to a previous human-subject test protocol testing a different repellent against ticks from Carroll-Loye Biological Research that the HSRB reviewed the results of in late 2022. The HSRB appreciates that Carroll-Loye and EPA have attempted to address in this protocol a number of topics raised during the HSRB review of that earlier study. The current study is well described in the draft EPA scientific and ethics reviews, so this ethics review will focus on addressing the ethics charge to the HSRB. The HSRB agrees with the recommendations made in the draft EPA ethics review, several of which reflect concerns raised by the HSRB with the earlier tick study by Carroll-Loye that may be found in the December 14, 2022 EPA Human Studies Review Board Meeting Report (HSRB Final Report 3.1.23.pdf (epa.gov)).

The HSRB notes that this study is to be conducted under good laboratory practices (GLPs) as noted in the protocol and the draft EPA science review. At the review of the results of the earlier tick protocol (see the December 14, 2022 HSRB meeting report), there was a public comment by the study sponsor noting that a problem with GLPs is that they do not explicitly address protection of human research subjects and the relationships and responsibilities between sponsors, investigators, and institutional review boards (IRBs). The HSRB noted this, and also noted that there are another set of guidelines, ICH E6(r2) on Good Clinical Practice (GCP) that explicitly addresses the protection of human research subjects and the relationships and responsibilities of sponsors, investigators, and IRBs. The HSRB also noted that tick studies, such as the one reviewed then (and similar to this study) strongly resemble phase 1 clinical trials that are routinely conducted under GCPs. The HSRB recommended in its December 14, 2022 report: "EPA should explore, in collaboration with stakeholders the possibility of utilizing both GLPs and GCPs, as appropriate and perhaps with modifications, to evaluate/guide the development, conduct, reporting and regulation of human pesticide studies." The HSRB remains concerned about this given the public comments from over a year ago and the fact that this protocol is being conducted under GLPs and "the most current ethics," without any ethics standard being cited. Yes, it must be "likely to meet the applicable requirements of 40 CFR part 26, subparts K and L," but that regulation does not spell out basic ethical principles of human subjects research – including regarding adverse events – and the responsibilities of the sponsor, investigator and IRB. Adding that the study will be conducted **consistent** with standards of good clinical practice, such as ICH E6(r2) would help close that gap. While this might not be something that EPA can require, the HSRB encourages the sponsor and investigator in this study to consider adding this on their own. The HSRB also reaffirms its general recommendation from the December 14, 2022 report as copied above.

Applicable Ethical Standards with comments:

The following provisions of 40 CFR 26 Subpart Q define the applicable ethical standards which read in pertinent part:

> §26.1703: Except as provided in §26.1706, EPA shall not rely on data from any research subject to this subpart involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

§26.1705: Except as provided in §26.1706, EPA must not rely on data from any research subject to this section unless EPA determines that the research was conducted in substantial compliance with all applicable provisions of subparts A through L of this part.

In addition, §12(a)(2)(P) of the FIFRA applies. This passage reads:

In general, [i]t shall be unlawful 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.

Comments regarding §26.1703:

Section 3.2.1 of the protocol lists the inclusion criteria, one of which is that subjects are 18 to 60 years of age. As a consequence, children will not be participating in this study.

Section 3.2.2.8 of the protocol indicates that women who affirm they are pregnant or lactating at screening will be excluded from the study. Women who are enrolled as subjects in the study will perform pregnancy self-checks using an OTC test kit provided by a researcher on the day of any study visit in which repellent will be applied or in which the subject will be exposed to ticks. Only nonpregnant women will be allowed to participate in the study.

Comments regarding §26.1705:

For practical purposes, this section of the regulations references the Federal government's Common Rule for the protection of human research subjects (also referred to as the U.S. Department of Health and Human Services regulations 45 CFR 46), with some modifications primarily due to the fact that these studies are regulated by EPA, not funded, sponsored, or conducted by EPA. Essentially the Common Rule requires that for a study such as this one that involves human subjects address the following (this list incorporates only the requirements that are relevant to this study):

The research must be scientifically sound (covered separately in the HSRB science review).

The study protocol, informed consent document, and all recruitment materials must be reviewed and approved by an appropriately constituted IRB.

The regulatory requirements for IRB membership, functions and operations, review of research, record keeping and communications with the investigator are all specified in the Common Rule.

For a study such as the one being reviewed, the IRB may approve research covered by the Common Rule if it determines that all of the following requirements are satisfied:

1) Risks to subjects are minimized:
    a. By using procedures that are consistent with sound research design and that do not unnecessarily expose subjects to risk, and
    b. Whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.

2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits of therapies subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (e.g., the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.
3) Selection of subjects is equitable. In making this assessment the IRB should consider the purposes of the research and the setting in which the research will be conducted. The IRB should be particularly cognizant of the special problems of research that involves populations vulnerable to coercion or undue influence, such as children, prisoners, individuals with impaired decision-making capacity, or economically or educationally disadvantaged persons.
4) Informed consent will be sought from each prospective subject or the candidate's legally authorized representative, in accordance with, and to the extent required by, § 26.116.
5) Informed consent will be appropriately documented or appropriately waived in accordance with § 26.117.
6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.
7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.

As noted in the draft EPA ethics review, the protocol for this study, informed consents, and study recruitment materials as well as all similar materials for the amendment were reviewed and approved by a Western Copernicus Group (WCG) IRB. The original protocol and related materials were conditionally approved on April 21, 2022 with final approval on May 3, 2024. Amendment 1 and related materials were approved on October 25, 2022. Amendment 2 and related materials were approved on November 15, 2022**.** WCG is a commercial IRB that is registered with the Department of Health and Human Services Office for Human Research Protections and meets the Common Rule regulatory requirements for membership, functions and operations, review of research, record keeping, and communications with the investigator.

There are no benefits to individuals who participate in this study. Risks to participants will be minimized in the study protocol in several ways, including via the inclusion criteria (for example, limiting the age to 18-60), via the exclusion criteria in the protocol if participation in the research might pose a risk to their health (for example, via an allergic reaction to tick bites), the use of colony-sourced ticks, via testing ticks for viral vectors prior to testing sessions (though see below), COVID precautions, confidential handling of pregnancy testing results, etc. Participants will be monitored during the study for adverse events associated with tick bites, as well as other risks due to the environment, such as heat exhaustion. The protocol has some limited follow-up provisions, but EPA has recommended a follow-up contact approximately 7 days after the last test period and this is consistent with a general recommendation in the December 14, 2022 HSRB report. The risks associated with participation in this study, per the protocol, are reasonable given the knowledge that will be gained (about the efficacy of the tested product).

In the earlier tick study (see 14 December 2022 HSRB report), a serious issue arose concerning the sourcing of ticks and testing for tick borne illnesses that put some study participants at additional risk. EPA has made recommendation #7 in the EPA science review and recommendation #3 in the EPA ethics review to address that concern. The HSRB supports these recommendations as they will help to minimize risks to study participants.

The protocol does a good job of describing how subjects will be recruited and selected. This section is similar to that in the previous tick protocol, and there were a number of issues concerning that (e.g., requiring English, recruiting focused on students, recording of demographics) included in the December 14, 2022 HSRB report and that are also relevant to this protocol. EPA has noted these and made recommendation #4 and #8 in the EPA's ethics review that the HSRB supports. These should improve the equitable selection of subjects for this study as well as the ability to track demographics more transparently. The recommendations also address potential concerns regarding coercion when students are recruited.

Section 3.3 of the protocol discusses the methods for obtaining informed consent and these are also well reviewed in the draft EPA ethics review. The IRB approved ICF is included in the IRB record provided. Recommendation #4 in the EPA ethics review addresses concerns regarding English only consents and the HSRB agrees with the EPA ethics review on this point. If the EPA recommendations are addressed the HSRB believes this study will seek informed consent in accordance with, and to the extent required by § 26.116. Informed consent will also be appropriately documented in accordance with § 26.117.

The protocol discusses the collection of adverse events under section 3.7 Medical Monitoring, Assistance, and Management. While the protocol states, "insect repellent efficacy research is non-medical by nature, subjects do not present a medical condition as part of participation, and health effects of treatment are neither intended nor anticipated as likely," this seems more than a little disingenuous as the HSRB notes that insect repellents carry warnings for consumers about adverse events they may experience and the protocol includes exclusion criteria, such as "hypersensitivity to tick bites" and "known to be allergic to topical repellents, essential oils of plants, or common cosmetics" that make it clear that adverse effects may occur during these studies, and further there are the provisions in the protocol regarding having a physician "on call" and first aid training for study staff. So there clearly is a need and a requirement here to collect adverse events in a systematic way, and to ensure research participant safety if an adverse event occurs. Despite this, it is not clear from the discussion in section 3.7 that there is a systematic process for collecting adverse events. It appears that adverse events are solely based on complaints volunteered by study participants, there is no general inquiry regarding study participant's status ("how are you doing") that would give a study participant the chance to volunteer information on adverse events. In addition, the lack of a follow-up contact with study participants means that any adverse event that occurs after study participants leave the test site (such as a tick-borne illness or delayed allergic reaction) will be missed unless the study participant takes the time to contact the investigator. EPA has appropriately commented on this and recommended the investigator include a 7-day post-testing follow up call. This is consistent with a general recommendation made by the HSRB in its December 2022 report that, "protocols should both provide

for unsolicited follow-up calls by participants in the event of them experiencing a delayed adverse event after test days (up to 30 days), as well as a scheduled follow-up visit or call at 15 days after the last test day the participant is involved to systematically document post-test day adverse events should they occur." The HSRB agrees with the use of 7 days rather than 15 days based on the EPA analysis and reaffirms this recommendation with the following modification, "protocols should both provide for unsolicited follow-up calls by participants in the event of them experiencing a delayed adverse event after test days (up to 30 days), as well as a scheduled follow-up visit or call up to 15 days after the last test day the participant is involved in to systematically document post-test day adverse events should they occur."

The HSRB also recommends that EPA, when consulting with future investigators on protocols, encourage investigators to have a clear, succinct, section of the protocol that lays out the "collection, handling, and reporting of adverse events" (which would include medical monitoring and care, if required) without the unhelpful and confounding discussion of why the investigator believes adverse events are not a concern. As pointed out above, collection, handling, and reporting of adverse events is not an option in these studies, it is a requirement and should be treated as such. It also is required that a protocol address this topic in order for an IRB to determine that, "When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects," as required for IRB approval. The HSRB strongly supports EPA recommendations for these reasons.

Subjects will be paid $25/hour for their participation in this study. This seems to be an appropriate amount of compensation and not excessive. The HSRB agrees with EPAs recommendations regarding compensation.

The study will use codes to refer to patients and there will be no link back from the code to the individual participant. Appropriate provisions are made to keep the results of pregnancy tests confidential. Records of the study will be kept in a secure location. The HSRB agrees with EPAs recommendations regarding research participants' privacy and protection of confidential information.

Based on the review of the materials provided, as well as the 14 December 2022 HSRB report, the HRSB believes that, if amended to address EPA's and the HSRB's recommendations, the research is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L.

Recommendations

- The HSRB recommends rationale be added in the study report to explain the two deviations and how they were determined not to affect the scientific reliability of the study outcome.
- The HSRB recommends the reference for the picaridin dermal absorption rate (10%) be added in the study protocol.
- At the bottom of Page 10 in the EPA review, it is stated that "a calculated MOE>100 indicates no risk." There is no such thing as no risk. The HSRB recommends that EPA change this to de minimis or extremely low or some other descriptor.
- In Section 3.2 (a) "poor physical condition" is listed as exclusionary for subject selection. The HSRB recommends that EPA define and specify who determines this. In the next item of the

bulleted list, “unwillingness to submit to brief query about personal condition” is listed as an exclusion. The HSRB recommends that EPA specify what “personal condition” refers to.

- In the EPA review (Section D., EPA Science Comments and Recommendations, Page 12) it is stated that “Justification is based on lack of evidence in the open scientific literature for the influence of human hosts’ sex on tick preference.” A lack of evidence does not establish there is no impact of sex. The HSRB recommends that EPA provide further support for this decision.
- The study protocol (Page 16 and Page 49) maintains that bite behavior of mosquitoes can be used to predict that of ticks and that sex and ethnicity are not factors. One of the papers they cite (Martinez et al., 2020) specifically cites the role of genetics (hence ethnicity) in mosquito bite behavior. The HSRB recommends that the applicant should eliminate any use of mosquito data to support tick protocols. In their revised protocol they are recording ethnicity, and this will be an opportunity (although with limited subject size) to address this issue.
- At the bottom of Page 23 in the study protocol, it is stated that there will be no biological samples of any kind, but it is presumed the pregnancy test requires urine collection. The HSRB recommends that this discrepancy be addressed.
- The HSRB recommends that EPA discuss any differences in behavior between study insects (“home” grown insects, presumably “well fed”) and the same species in the wild (presumably hungry).
- The HSRB recommends that the cause of censoring be recorded and described in the study report.
- The HSRB recommends that the protocol include information on additional variables collected and descriptive statistics (e.g., mean time of first crossing) that will be included in the study report.
- The HSRB recommends that throughout the protocol document, attention to the correct use of significant figures should be addressed i.e., reporting the dose as 0.001974/cm^2 is not scientifically supportable.
- There are some inconsistencies between the protocol and amendment approval dates between the science review and the ethics review. The dates from the ethics review from above have been used because they appear to be correct based on the WCG certificates of action in the IRB review volume. The HSRB recommends that this be double checked.
- When consulting with future investigators on protocols, the HSRB encourages investigators to have a clear, succinct, section of the protocol that lays out the “collection, handling, and reporting of adverse events” (which would include medical monitoring and care, if required) without the unhelpful and confounding discussion of why the investigator believes adverse events are not a concern. As pointed out above, collection, handling, and reporting of adverse events is not an option in these studies, it is a requirement and should be treated as such.
- The HSRB recommends that measurements made with the test arm and non-dominant arm are described in clear terms. To elaborate, “control” may not be the appropriate term. The measurements with the “control” forearm are observational (qualitative), namely “actively

questing") while the measurements with the treated arm are quantitative (distance moved, figure 1) and are not identical but for treatment as is often the objective with a control. On a broader and related matter, the HSRB also recommends that the science behind the methodology (experimental details) used be explained or citations provided in the protocol.

Recommendations for Future Studies

In addition to the specific recommendations for this study, the Board has several recommendations for future studies:

- The HSRB suggests that EPA consider changing "Product Performance Test Guidelines OPPTS 810.3700: Insect Repellents to be Applied to Human Skin" guidance to include a question to female candidates as to whether or not they are postmenopausal or surgically unable to become pregnant and exclude those women from mandatory pregnancy tests.
- The efficacy endpoint is defined as the time of the FCC for each species for each subject. FCC is defined as a tick crossing on a subject forearm which is followed by *another tick crossing within 30 minutes*. The requirement of a second tick crossing within 30 minutes is prone to introducing multiple errors.

The HSRB recommends that a scientific justification of second crossing should be added to the guidance for study protocol composition to balance the following concerns:

a. Different ticks might simply choose crossing on a subject forearm for other unknown or uncontrollable reasons other than related to the product application. The time and the event of the first tick crossing occurrence are important aspects of the overall understanding of the product repellency.
b. The second crossing requirement might result in underestimating the overall risk/event of crossing, which could bias the results towards overestimating the effect of the product (false-positive results on the product repellent efficacy, with longer estimated mCPT than correct).

- When there is no evidence-based research of repellency products that combine ingredients to determine if the product reduces the number of tick crossings during a certain period of time, the benefits of combining the ingredients is not clear. The HSRB recommends that EPA revisit "Product Performance Test Guidelines OPPTS 810.3700: Insect Repellents to be Applied to Human Skin" to clearly state and justify the objectives of these types of studies. Specifically, HSRB recommends that EPA consider defining 'performance' clearly.
- Additionally, HSRB recommends that EPA justify and clarify the definition of 'complete protection time' and the use of the median (as opposed to the 90th percentile, for example) in "Product Performance Test Guidelines OPPTS 810.3700: Insect Repellents to be Applied to Human Skin."
- The HSRB recommends that EPA include additional scientific reference to support the methodologies employed in study protocols. For example, this may include providing a reference to a guidance document or literature which supports the decisions and assumptions underlying the protocol methods.