July 10, 2018

EPA-HSRB-18-3

Dr. Jennifer Orme-Zavaleta
EPA Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: April 24-26, 2018 EPA Human Studies Review Board Meeting Report

Dear Dr. Orme-Zavaleta,

The United States Environmental Protection Agency requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of four studies in its April 2018 meeting. The four studies are the following: (1) a study of tick repellency: *Protocol for Laboratory-based Testing of a Tick Repellent Containing Oil of Lemon Eucalyptus*; two completed studies from the Antimicrobial Exposure Assessment Task Force (AEATF), (2) *A Study for Measurement of Potential Dermal and Inhalation Exposure During Application of a Latex Paint Containing an Antimicrobial Pesticide Product Using a Brush and Roller for Indoor Surface Painting*; and (3) *Determination of Removal Efficiency of 1,2-Benzisothiazol-3(2H)-one (BIT) from Hand Surfaces Using an Isopropyl Alcohol/Water Wipe and Wash Procedure*; and finally a publication addressing Wolbachia infected mosquitoes, (4) Popovici et al, *"Assessing key safety concerns of a* Wolbachia*-based strategy to control dengue transmission by* Aedes *mosquitoes."* The Board's responses to the charge questions and detailed rationale and recommendations for each of these studies are provided in the enclosed final meeting report.

Signed,

Liza Dawson, PhD
Chair
EPA Human Studies Review Board

INTRODUCTION

On April 24-26, 2018, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to the following four studies:

(1) A protocol entitled "A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks"

(2) A completed study entitled "Determination of Removal Efficiency of 1,2-Benzisothiazol-3(2H)-one (BIT) from Hand Surfaces Using an Isopropyl Alcohol/Water Wipe and Wash Procedure"

(3) A completed study entitled "A Study for Measurement of Potential Dermal and Inhalation Exposure During Application of a Latex Paint Containing an Antimicrobial Pesticide Product Using a Brush and Roller for Indoor Surface Painting"

(4) A published article titled "Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes"

REVIEW PROCESS

The Board conducted a public meeting on April 24th - 26th, 2018. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-9976-38-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 four studies.

For each of the studies, Agency staff presented their review of scientific and ethical aspects of the completed study, with each presentation followed by clarifying questions from the Board. The HSRB solicited public comments and then took up the charge questions under consideration. The Board discussed the science and ethics charge questions and developed a consensus response to each question in turn. 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 presentations given by EPA staff at the meeting, oral comments from Agency staff and from the investigators during the meeting discussions, and the Agency's written reviews, which were provided to the Board prior to the meeting.

Topic #1: A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks

Charge to the Board:

Charge to the Board - Science:
Is the protocol "A single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks" likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks?

Board Response:

The HSRB determined that with the modifications recommended by EPA and additional clarifications recommended by the Board, the study is likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks. An explanation of the HSRB's review and suggested changes is elaborated below.

HSRB Detailed Recommendations and Rationale:
This protocol follows a standard design seen in previous studies supporting product performance labeling for insect repellents. The stated objectives of the study are to 1) determine complete protection time (CPT) for a pump spray product containing 30% Citriodiol[1] against three tick species for a typical consumer application, 2) determine the 90% protective efficacy as defined by the sponsor and 3) determine the average "dose rate" (application rate of the product in mass of product per unit area of skin per application). EPA reviewers[2] supported the first objective, but recommended removing the second objective because it is not useful for product labeling. EPA reviewers clarified that CPT is defined as the protection period between product application and time point of repellency failure. Repellency is measured by crossing onto treated skin and remaining there for 1 minute minimum, and confirmed by the second crossing occurring within 30 minutes from the first crossing, by a second tick following the first. The EPA review states that this change was agreed on by the sponsor, but the protocol as reviewed by the HSRB still includes this second objective. The researchers also propose that before testing the efficacy of

[1] Citridiol is the brand name of a product containing 30% Oil of Lemon Eucalyptus (OLE), which is considered the active ingredient. OLE contains 65% p-menthane-3,8-diol (PMD), which is the compound with repellency activity.
[2] Fuentes, C., Bohnenblust, E., Arling, M. Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE. U.S. Environmental Protection Agency. March 30, 2018.

the product, a preliminary study will be conducted in which the consumer product will be tested to determine the quantity of product applied in a "consumer dose." The EPA recommends using the standard application rate for pump spray products, which has been the standard for similar studies, but in this case, the sponsor is advocating for the consumer dose-finding step instead.

According to the EPA scientific review, EPA has recommended a number of changes that the sponsor has agreed to make. However, the version of the protocol that was reviewed by the HSRB does not include all the recommended changes. For example, the determination of 90% protective efficacy is still in the protocol. The HSRB anticipates that all changes recommended by EPA and already agreed by the sponsor will be incorporated into the final protocol document.

The Board noted several inconsistencies in the protocol with respect to definitions of endpoints, number of subjects and procedures. The HSRB assumes that these issues will be resolved in subsequent drafts, however in the current version these issues remain unresolved. Specifically, the CPT needs to be clearly defined and consistent in the protocol according to the definition agreed by EPA and the study team, as noted above. In addition, the Board had questions about whether the crossing line is needed if CPT is based on time in treated area or is distance traveled across treated area still used in the definition. EPA reviewers clarified that the investigators will amend the protocol to remove the crossing line definition and assess repellency breakdown by time spent (1 minute) by a tick on treated skin. EPA concurred with this approach. EPA reviewers also clarified that the study will use the 1 tick every 15 minute approach and not the alternative, 5 ticks per 30 minutes.

The sample size needs to be consistent throughout protocol. The sample size was increased from 10 to 25 subjects for both measurement of product application rate and efficacy testing. Some of the subjects may participate in both phases of the study. The sample size needs to be consistently stated throughout the protocol.

The EPA's standard application rate for pump spray products is well supported; however, in this study the sponsor proposes to independently measure "typical consumer dose." The rationale for using this different dosing strategy is not clear. The Agency typically requires repellent studies to be conducted with standard dosing to facilitate comparisons among products, which is critical for effective and informative labeling regarding CPT. The sponsor argues that the typical consumer dose for their product is higher than that recommended by the Agency, however no clear evidence of this was presented to the Board. If the sponsor alleges there are unique features of the product formulation, consistency, and/or their delivery system that affect consumer dosing, then measuring application rate might be justified. In any case, the HSRB recommends that the results of the consumer dose testing should be reviewed by

EPA prior to conducting the CPT study. In addition, the 810.3700 testing guidelines provide specific guidance for Dosimetry testing. If the study does include measurement of application rate, the Board recommends that it include the following steps:

1. Conduct test on both arms to account for handedness
2. Quantify evaporation loss in mass that occurs between application and weighing (the alcohol vehicle will likely volatilize)
3. Confirm area of gauze bracelets when on the subject's wrist (i.e., when stretched around arm does the width change?)
4. Confirm that inside surface of gauze bracelets are not wet after application (i.e., breakthrough loss?)
5. Ascertain whether subjects purposely or inadvertently spray the product on the gauze "targets" or avoid the gauze and aim at the skin
6. Account for the "distribution" step during field application where people spread the material on the skin surface by hand (i.e., spray a dab and rub it around)
7. In Section 6.1 of the protocol, it states that a 'representative pump spray bottle with instructions for use on the product label' will be used for the consumer dose portion. The Board recommends that the actual consumer product with label be provided for the dosimetry so that it is a more accurate representation of what the consumer would purchase and then apply. If the bottle is different in some way, it could introduce some bias and/or variability. EPA reviewers also supported the use of the commercial container in the dose testing.
8. Section 8.1 states that volunteers should be instructed to apply after reading the label. The Board supports this requirement, as did EPA reviewers.

The HSRB also evaluated the study from the standpoint of risk characterization, which was summarized in Section 4.1 EPA's review of the study.[3] The product is currently EPA-registered as an insect repellant. The active ingredient is OLE, which contains 65% p-menthane-3,8-diol. (PMD). PMD has a relatively innocuous toxicology profile when applied topically, with no relevant toxicity endpoints of concern. Therefore, the Board was not concerned about risks of exposure of human subjects to this product.

During the scientific review, the Board also commented on whether there was a risk from exposure to tick bites. The consent form mentions risk of tick paralysis. Tick paralysis only occurs after a tick has fed for several days, it has an extremely low incidence, and is most common in children, who are excluded from

[3] Fuentes, C., Bohnenblust, E., Arling, M. Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE. U.S. Environmental Protection Agency. March 30, 2018.

this study. In this study, the risk tick paralysis is essentially eliminated by the very nature of the test method. Ticks are removed without biting and in no case would a tick be allowed to attach and feed for several days. Therefore, the Board does not consider this to be a risk in this study and recommends removing this statement from the consent form. The Board also notes that the risk of disease is mitigated by using laboratory-reared ticks.

The Board discussed the choice of tick species for testing. The sponsor has chosen *Rhipicephalus sanguineus* as a test species. EPA guidelines state that sponsors should use test species for which a label claim is sought, therefore the choice of R sanguieus suggests that the sponsor is seeking labeling for this species. The Board questions the choice of this species over *Dermacentor variabilis*. *D. variabilis* is the primary vector for Rocky Mountain Spotted Fever (RMSF) in the US and while *R. sanguineus* is an important tick vector in Europe, it is less important in the US and is mostly associated with dogs and kennels. It is not a frequent biter of humans as compared to *D. variabilis*. From a scientific perspective and an ultimate label claim, the Board considered *D. variabilis* to be far more relevant.

Statistical review

The randomization procedures for arm selection and for assigning subjects to tick species was not clear. The HSRB recommends clarifying how these randomization steps will take place. Also, it appears that a subject may be allowed to participate in testing for more than one tick species. If that is the case and the tests for each are sequential in time, the Board requests clarification about whether the subject is allowed to choose the tick species based on the timing of the test and their ability to participate.

Charge to the Board - Ethics:

Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response:

The Board responded that with the modifications recommended by the EPA and the HSRB, the study is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L.

HSRB Detailed Recommendations and Rationale:

The HSRB agreed with the ethics comments presented in the EPA review of the study.[4] In general, the protocol is well-designed, low risk, and reasonable in terms of risks and burdens on study participants. The Board had several comments related to clarifications of study procedures and explanations in the protocol document.

The risk level of the study is low based on two key considerations: the product used is nontoxic, and the ticks are laboratory-reared ticks and pathogen free. Regarding toxicity, the HSRB agrees with the EPA reviewers who stipulated that the toxicity information needs to be completely described in the protocol in Section 4.2. The study team needs to clarify if there is any significant risk of eye irritation. The EPA scientific review mentions that the two products registered with 30% OLE are categorized as Toxicity Category III for acute oral, acute dermal, and eye irritation, and Toxicity Category IV for dermal irritation. The risk assessments are based on p-Menthane-3,8-diol (PMD) which is the active ingredient in those products. Also in the EPA scientific review, reviewers state "The Agency concluded that there is reasonable certainty of no harm to populations or subpopulation (infants and children) from the use of PMD in insect repellent products applied to human skin." This low risk is important for the ethical analysis of exposure of greater or lesser quantities of product depending on the design of the study. During the HSRB scientific review, there was some discussion about whether a consumer dose model or a standardized dose model was preferable for the study. Since this product is extremely low risk, the fact that consumers might use more of it is not a major concern. The larger concern would be if either a) consumers using the product in daily life (not in the study) used less than the necessary dose and then had greater chance of tick bites or, alternatively, if b) the study itself used too low a dose and found inadequate protection when in fact a higher dose could have been protective—that is, provided a false negative test result based on inadequate dosing. Therefore, it seems reasonable that the scientific issues regarding consumer dose versus standardized dose should be adjudicated based on what is going to be more appropriate to consumer information and use in the field, and not based on the desire to minimize exposure to the product, because this is a very low risk product.

Additional ethics comments

The Board recommends the following changes in the protocol:

1. In section 5.2, Eligibility Criteria, inclusion criteria for females states "not pregnant or intending to become pregnant." Recommend adding "during the time period of the study."

[4] Fuentes, C., Bohnenblust, E., Arling, M. Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE. U.S. Environmental Protection Agency. March 30, 2018.

2. Inclusion criteria also list "no known allergy to Oil of Lemon Eucalyptus or any other repellent ingredients." Please clarify that this refers to ingredients in this product, not other repellents.
3. In the consumer dosing part of the study (dosimetry), Section 8.1, the study participants apply the product themselves and the gauze strips on the forearm are weighed to determine amount of product applied. The Board recommends clarifying if individuals will be instructed to use their dominant hand, alternate dominant and non-dominant hand, or just allowed to use whichever hand they choose. Use of dominant hand versus non-dominant hand might affect the application rate.
4. Section 8.2 describes the testing period after application of product, which lasts up to 10 hours. The Board suggestions clarifying provisions for meal breaks and determining whether washing hands after using the bathroom and/or before eating would disturb the product application on the forearm.
5. Section 10.3.2 addresses Serious Adverse Events and describes procedures for handling anaphylaxis; the Board requests clarifying whether Epi-Pens will be available.
6. In that same section, determination of relatedness of AEs are mentioned. The procedure for assessing relatedness of AEs, as well as the party responsible for this determination, need to be outlined.
7. Section 11.1, Research ethics approval, states that the protocol will be conducted in accordance with all applicable laws, regulations and IRB requirements. Regulations at 40 CFR 26 Subpart K and L, which are the relevant EPA regulations, should be cited here.
8. The same section also states that the LSHTM ethics Committee will review the study after the EPA, HSRB and WIRB approvals. The Board recommends clarifying a plan to handle any potential disagreement between the reviewing ethics committees.
9. Section 11.2 describes protocol amendments. Minor correction or clarification to the protocol will be documented in a file note in the Trial Master file. Any modifications to the protocol that constitute protocol amendments will need to be submitted to WIRB and/or the LSHTM ethics committee for approval. Depending on IRB procedures, submission of minor clarifications to the document may or may not be required.
10. 11.6 addresses payment for participation. The Board is in agreement with EPA's ethics review statements about payment, namely that a flat sum for consent and screening and an hourly rate for testing time seems appropriate and fair.
11. As noted during the scientific review, the Board recommends removing risk of tick paralysis from the description of risks for the study because the conditions for tick paralysis (biting and

attachment for several days) will not be present in this study. Risks that are not reasonably present should not be described as part of the risk profile of the study.

Comments on consent form

1. Under "Risks and discomforts" the issue of laboratory raised ticks is mentioned: "there is no risk of getting a disease from a tick bite during this study because the ticks we will use are raised in a laboratory…." Please add "and are disease-free" after "laboratory"
2. The section on compensation for injury has been suggested by EPA to be re-titled as "Payment of medical expenses in the event of a study related injury." While often in US based studies we have coverage of medical expenses and do not have compensation for injury, in this case, because clinical trial insurance is required in the UK and does cover compensation for injury, the original title is more accurate.
3. Contact information for questions about rights as a research subject lists WIRB, which is one of the reviewing IRBs. The Board suggests it would be more appropriate to list the LSHTM ethics committee given that it is the locally reviewing board and may seem more accessible to study participants from the London area.

Topic #2: Determination of Removal Efficiency of 1,2-Benzisothiazol-3(2H)-one (BIT) from Hand Surfaces Using an Isopropyl Alcohol/Water Wipe and Wash Procedure

Charge to the Board - Science:

Did the research in study AEA08 generate scientifically reliable data, useful for establishing the efficiency of the hand wash procedure used to remove BIT-treated paint from the hands?

Board Response:

The HSRB responded affirmatively that the research in study AEA08 generated scientifically reliable data, useful for establishing the efficiency of the hand wash procedure used to remove BIT-treated paint from the hands.

HSRB Detailed Recommendations and Rationale:

The goal of the completed research study AEA08 was to collect hand wash removal efficiency data to be used to correct/adjust the hand residue data collected in the AEATF II brush/roller paint study as well as the upcoming airless paint sprayer study. The study assesses the removal of BIT treated paint that has

been applied to participants' palms and allowed to dry. An aliquot of 50 uL of paint is applied at either 154 ppm or 547 ppm BIT on both hands of 20 subjects with 10 subjects for each concentration. Paint residues are removed and analyzed using the same hand wash and analysis methods used in the AEATF II brush/roller and airless paint sprayer studies. Statistical analysis of the results indicates statistically significant differences in the removal efficiency at 154 and 547 ppm indicating that separate removal efficiencies of 73.3% and 60.3%, respectively should be used. Since three concentrations of paint are used in the AEATF II brush/roller paint study, a linear association is assumed and a mean correction factor of 66.8% is recommended for the mid-level concentration between 368-382 ppm BIT.

The Board assessed the AEA08 study by reviewing the following documents:

- EPA Science review of study AEA08
- Appendix A AEA08 Statistical Review
- Final AEA08 Data
- AEA08 Analysis – SAS Code
- Ethics review of study AEA08
- IRB Minutes & Roster
- AEATF Study Report – AEA08 Handwash Removal Efficiency

The AETF II considered and implemented many of the EPA/HSRB review comments in the study, with the exception of the method of application of paint to the hand. It was suggested by the HSRB to paint the entire hand, rather than just the palm, due to the low dermal permeability of the palm. However, the AEATF II did not incorporate this suggestion, citing potential difficulty in carrying this out with possible problems with avoiding transfer of paint with a whole hand treated. There were minor protocol and SOP deviations that did not impact the validity of the scientific data.

The Board agrees with the EPA assessment that the AEATF II hand wash removal efficiency study was successfully executed and the removal efficiency data generated can be used to correct the hand exposure residues from the AEATF II painting studies. Since removal efficiency appears to be concentration dependent at the levels tested, caution should be used when extrapolating removal efficiency below or above the concentrations tested (154 and 547 ppm), as linearity may not be justified.

Based on the Board's evaluation, the Board supports the view that the study has generated scientifically reliable data, useful for establishing the efficiency of the hand wash procedure used to remove BIT-treated paint from the hands, *within the BIT concentrations tested (154 and 547 ppm).*

Clarification is needed on two points:

1) The Board questioned whether the spreader that is used to spread the paint is the same as the instrument used to deliver the BIT to the hand; if so, then any amount left on spreader is accounted for in the analysis of amount of paint remaining on rod. EPA and the study sponsor have clarified that this is correct. Please ensure that the report makes this clear.
2) These hand wash efficiencies are performed for 45 minutes. Application of a 45 -minute period wash efficiency may not fully represent experiments performed for 2 hours or more where level of absorption into the skin may vary.[5] Somewhere in the document the study sponsor should make note of this. Without additional studies at different times, absorption rate is difficult to access.

Statistical review

The AEATF II stated in their study protocol that their objective is to determine the removal efficiency of BIT in latex paint from human hands. The results of this study are being used to adjust for losses on the test subject's hands resulting from an identical hand wash removal sampling method used in both the paint brush/roller and airless sprayer exposure studies.

The statistical analysis indicates that the results of the correction factors for the two BIT concentrations (154 and 547 ppm) should not be combined since the means of the percentage removal efficiencies at the two concentrations are statistically significantly different at the 5% level.

The statistical analysis of data collected on 20 subjects, 10 per for each of the two BIT concentration, is adequate. Confidence intervals for the mean removal efficiency are constructed using SAS proc means. T tests are used to compare the means between low and high concentration groups using SAS proc t-test. The distributional assumptions are verified, normal distribution for an arithmetic mean and log-normal distribution for a geometric mean, by quantile-quantile plots and normality tests. These statistical procedures were deemed appropriate by the Board.

Charge to the Board - Ethics:

Does the available information support a determination that the research was conducted in substantial compliance with the requirements of 40 CFR part 26, subpart Q?

[5] EPA's protocol review (pp 7-8) discussed BIT rat dermal absorption study (MRID 46327901) results for absorption and amount remaining on rat skin at time intervals of 4, 8, 24, 48, and 72 hrs after treatment. Results indicate a substantial amount of BIT remained on the skin indicating a vigorous wash procedure would be needed. Absorption was 1.7% after 4 hours and 3.2% after 8 hours.

Board Response:

The HSRB concludes that study AEA08 submitted for review meets the applicable requirements of 40 CFR 26, subpart Q.

HSRB Detailed Recommendations and Rationale:

The purpose of this study was to determine the removal efficiency of BIT in latex paint from human hands, as described above. The Agency's rules at 40 CFR part 26 subpart Q that are applicable to this review include the following:

§26.1703: Except as provided in §26.1706, EPA must 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.

And

§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.

Intentional Exposure of Pregnant Women, Nursing Women, or Children

The Final Report and supporting materials submitted by AEATF II for EPA review indicate that no pregnant or nursing women enrolled in the study and all human subjects were between 18 and 67 years old. As a result, AEA08 satisfies Agency rules at 40 CFR §26.1703.

The Final Report notes that interested persons were asked if they were at least 18 years old during the initial phone interview. Potential subjects were also asked to bring a government-issued photo ID to the informed consent meeting so that the Study Director and/or Spanish-speaking Field Research Associate could verify the subject's identity and age of $\geq$ 18 (p. 16).

Female subjects were required to take an over-the-counter urine pregnancy test on the day of their monitoring event and prior to other study activities (p. 12). After the subject took the pregnancy test, she was asked if she wanted to continue study participation. The Final Report notes that all female subjects affirmed that they wished to continue participation, so a female member of the research team was present

and confirmed the negative results of their respective pregnancy tests (p. 20). Results of the pregnancy tests were not recorded.

During the phone interview, interested subjects were informed that they must not be pregnant or nursing a child in order to participate in this study (p. 151). Subjects were also asked about their pregnant or nursing status when completing the Qualification Worksheet during their Informed Consent meeting (p.120).

Compliance with Subparts A through L

1. Independent Ethics Review

The study protocol and supporting materials were reviewed and approved by Schulman Associates Institutional Review Board (SAIRB), an IRB registered with the Office for Human Research Protections (OHRP, Registration #00000971). SAIRB is also fully accredited by the Accreditation of Human Research Protection Programs (AAHRPP). The Final Report submitted by AEATF II contains all correspondence between investigators and SAIRB, including documentation of SAIRB's initial approval of the study (dated February 9, 2015), approval of two subsequent amendments, and review of three protocol deviations. As noted in EPA's Ethics Review (March 15, 2018), these deviations did not have an impact on subjects' health, safety, or rights.

Additionally, the HSRB reviewed the study at its April 8-9, 2014 meeting. EPA and HSRB made specific recommendations to AEATF II, and their responses to those ethics-related recommendations are also included in Attachment 1 of EPA's Ethics Review of this completed study.

2. Selection of Subjects (Recruitment and Demographics)

IRB-approved advertisements were placed in two newspapers that served the study location: The *Fresno Bee* and *Vida en el Valle* (Spanish language). Investigators intended to place an advertisement in a third newspaper, the *California Advocate*, but newspaper staff did not provide a proof of the ad, so there was no ad placed in that paper. Interested individuals called the number listed in the advertisements to learn more about the study and to allow investigators to ascertain general eligibility. Those who were potentially eligible and still interested were then invited to attend an in-person Informed Consent meeting with members of the research team where they would undergo informed consent discussions and research team members would assess subject eligibility.

A total of 40 subjects enrolled in the study, with 20 subjects assigned to complete the handwashing activities. Other subjects were held in reserve or listed as alternates. As noted above, the 20 subjects who

completed the handwashing activities were between 18 and 67 years old, with 12 male subjects and 8 female subjects completing study activities. Two of the 20 subjects preferred to communicate in Spanish.

3. Minimization of Risks

Risks to subjects stemming from research participation included:

a. Risk of reaction to latex paint or BIT;
b. Risk of irritation from isopropyl alcohol;
c. Risk of discomfort with sitting with hands open for 45 minutes; and
d. Potential risk of unintentional release of confidential information.

Of note, there were no adverse events reported by any of the subjects. Risks to subjects were minimized by adherence to clear subject inclusion/exclusion criteria. The study enrolled only healthy subjects who had no known allergies or sensitivities to BIT, latex paint, or isopropyl alcohol. On-site medical personnel were available during each monitoring event in order to assist with any subject concerns (Final Report, p. 71). A television was set up in the testing facility and subjects rested their hands on a padded surface during the monitoring events. Although monitoring events were videotaped, all photos and videos were reviewed to ensure they did not show subjects' faces, tattoos, or other identifying features. Subjects' personal information is stored separately from other study data in Golden Pacific Laboratory's archive room.

4. Informed Consent

All subjects were required to review and sign an IRB-approved informed consent document. SAIRB provided certified Spanish translations of the English recruitment and consent materials. Potential subjects visited the study site for informed consent discussions and were allowed to bring friends or family members, if they chose to do so. Subjects were provided a copy of the informed consent document, product label, and safety data sheet (SDS) during this meeting. They were also encouraged by research staff to ask questions at this time and reminded that their participation was voluntary and could be stopped at any time.

Topic #3: A Study for Measurement of Potential Dermal and Inhalation Exposure During Application of a Latex Paint Containing an Antimicrobial Pesticide Product Using a Brush and Roller for Indoor Surface Painting

Charge to the Board - Science:

Did the research in study AEA09 generate scientifically reliable data, useful for assessing the exposure of painters who apply paint containing antimicrobial pesticides using brushes and rollers?

Board Response:

The HSRB has concluded that the study "Completed AEATF II study on Dermal and Inhalation Exposure to Antimicrobial Pesticides during Brush and Roller Application of Latex Paint (AEATF II Project ID AEA09; MRID 50521701" has generated scientifically reliable data, useful for assessing the exposure to antimicrobials for those (i.e., consumer and commercial painters) who use brush and rollers to apply latex paint containing antimicrobials.

Some suggestions are made in this report for the important inclusion of relevant data for understanding the study and use of the data in risk assessments.

HSRB Detailed Recommendations and Rationale:

The HSRB Board reviewed a number of study report documents that summarize the results for the complete AEA09 study to monitor dermal and inhalation exposure to antimicrobials during brush and roller application. Previously, the HSRB reviewed the protocol and related material in 2014. This review now covers the results of that approved study. A number of submitted documents were reviewed by the HSRB in order to respond to the charge.

Documents Reviewed or used as References

a) Science review of study AEA09
b) Appendix A AEA09 Clothing PF determination
c) Appendix B AEA09 Statistical Review
d) Final AEA09 Data
e) AEA Analysis-SAS Code
f) Ethics review of study AEA09

g) AEATF SOP Chapter 11
h) IRB Minutes & Roster
i) AEATF Study Report-AE09 BIT Brush & Roller

As noted above in this report, results of an associated study "AEATF II Paint Hand Wash Removal Efficiency Study" were also reviewed by the HRSB within the same timeframe on its own merit. Data from the AEATF II Hand Wash Removal Efficiency is used in this AEA09 Brush and Roller Study to adjust the hand wash results used in the determination of dermal exposure. The appropriate use of AEATF II data in the AEA09 study was also evaluated here.

AEA09 Study summary: This AEA09 study is intended to offer more detailed risk assessment data, over historically used risk assessment data from a PHED (Pesticide Exposure Handlers Database) dataset used, to assess paint brush exposures to antimicrobial products added to paint. In addition, this study is a part of a developing dataset for dermal and inhalation exposures for a number of antimicrobial handler scenarios. In year 2016-2017, eighteen subjects were monitored as they applied paint to surfaces (e.g., walls, ceilings, trims of doors and windows) for approximately 2 hours (range 48 to 172 minutes (average 113 minutes)) in warehouse rooms 10ft x 12ft, with 8ft ceilings in Fresno, California.

These subjects were told to apply about 2 gallons of paint to surface as they normally would, where the 18 subjects (considered monitoring events) were placed into three groups to apply BIT at concentrations within ranges of 144, 375 and 619 ppm (note: actual concentrations used by the 18 ME's were based on the analytical measures). The subject ages ranged from 20 to 64 years (five females), where these individuals had some previous experience applying paint. All subjects wore inner and outer dosimeters over entire body, a cap (with inner dosimeter), goggles and shoes with booties (if desired). Dosimeters, however covered the top areas of shoes.

From the study results, three dermal exposure scenarios are determined across the BIT concentrations, for 18 subjects by manipulating the single or combined results from the analysis of inner and outer dosimeters and include: 1) long pants/long sleeves, no gloves, 2) long pants/short sleeves, no gloves, and 3) short pants/short sleeves, no gloves. These scenarios are intended to represent the following possible individuals who apply paints by the brush and roller methods; 1) commercial painter, 2) commercial/residential painters, and 3) residential painters, respectively. Total dermal exposure measures include the combination inner and outer dosimeters as explained above, hat dosimeters, painter's hat, face/neck wipes, sweat wipes, and handwashes. Exposures are reported in mg/lb ai, and estimated using lognormal simple random sampling. Inhalation exposure (in mg/lb ai) for the breathing zone was determined using two types of personal monitors (e.g., inhalable OVS) over the time-period of exposure.

For inhalation exposure, an 8 hr TWA mg/m^3/lb ai was also determined. The antimicrobial active ingredient used is BIT (1,2-benzisothiazoline-3-one). BIT has a vapor pressure of 4.4E-7 mmHg at 20 degrees C and its volatility is considered low. Exposure during pouring of the paint and application using airless spray cans is not included.

If the data are rich enough there are opportunities to look at exposure not only active ingredient applied, but based on time of painting, surface area covered (range 267 ft^2 to 888 ft^2), and by age and experience of participant. Not all these factors may be relevant for a simpler evaluation of risk. The influence of environmental conditions (e.g., temperature) can also be addressed. The product used however is of low volatility and not expected to be unduly influenced by temperature. EPA stated that its main intention is to use the appropriate unit exposures (mean or 95% percentile) in the following equation to determine potential dermal or inhalation exposure:

Potential exposure = UE (mg/lb ai or mg.m^3/lb ai) x absorption (%) if applicable x maximum label rate (% ai by weight) x weight of treated product/article (pounds).[6]

EPA scientific review. EPA's review determined that the study meets EPA standards for pesticide exposure monitoring and is considered acceptable and appropriate for use in occupational/consumer exposure/risk assessments for individuals applying paint with a brush and roller method. In addition, the primary quantitative objective was to report results for dermal exposure within a 3-fold accuracy for the geometric mean, arithmetic mean and 95th percentile with 95% confidence. This objective was met except occasionally for the empirical 95th percentile. The second objective was to evaluate whether there was an 80% power to detect log-log linearity with a slope of 1. This objective was met if the widths of the confidence intervals for the slope based on the lognormal model are at most 1.4

HSRB scientific review. HSRB's review identified two main areas of importance: (1) AHETF's response to the HRSB 2014 review of the protocol; and (2) deviations from the original approved protocol and study design. The Board developed recommendations for clarifications in the meeting report as well as additional issues to consider regarding future data analysis and uses of the data.

The EPA and the HRSB had several comments on the original AEA09 study in 2014. These included offering edgers and paint cups to subjects, using a separate color paint for walls and trim, providing details on ventilation and airflow characteristics, adjusting the upper concentration of BIT, using 6 concentrations of BIT instead, use of various types of painting equipment and wall textures, defining volatility of BIT in protocol, use of a sock or hood instead of cap, use of an exhaust fan to provide fresh

[6] Page 4 of 36 in EPA's science review

air, removing the necessity for background wipes, specifying that the paint will not be diluted and the sheen of the paint, and other suggestions and corrections on typo.

Most of the comments were adjusted to meet the EPA and HSRB recommendations. The following comments were either not changed or were confusing from Table 2 of the Science Review: 1) testing was done at 3 concentrations; 2) the same equipment was offered for all participants; 3) there was no mention of using different wall textures; 4) a dosimeter added underneath a hat instead of using a hood; 5) the report was not clear with regard to the issue of the fan exhaust; and 6) mention of CDPR and EPA conflict on whether the exhaust fan should remain on during activities (page 7: Table 2 of EPA's Science Review of this Brush and Roller Study . Typically EPA wants to model exposure with low level of air exchange rates; but when conducting a human exposure study CDPR would propose a high air exchange rate for worker/subject safety.

Clarifications requested by the HSRB to be included in the study report:

1) On the issue of effective ventilation in the test room during monitoring, the Board needs clarification on the fraction of time that the door was open and the air recirculation rate through the air conditioning unit during the monitoring events.
2) The issue of whether study subjects should receive their individual research results was never resolved by study group, HRSB or EPA. HSRB and EPA have agreed that this is a broader discussion that may apply to all studies and will be addressed at a later time.
3) This brush and roller study lasted approximately two hours and subjects applied 1.75 to 2.25 gallons of paint BIT concentrations within three ranges. EPA or the study report should clarify again how the decision was made to use these amounts of paints at these concentrations and how this may be relevant to how individuals use these products. Mentioned in the April 2018 meeting was the reliance on available data in the literature on usage rates for consumers. Please reference this survey or data in the study report again, or in the EPA review. As mentioned in the meeting, some data indicates that the average consumer paints for 4 events per year and may apply up to 8 gallons a year over a surface range of 172 to 511 ft^2 /gallon.[7]

[7] EPA Science review of protocol page 7 describes the selection of the amount of paint. In summary, EPA's assessments use 2 gallons of paint applied by brush/roller when estimating exposures for residential applicators (5 gallons for commercial painters) (http://www.epa.gov/pesticides/science/residential-exposure-sop.html). The EPA's 2011 Exposure Factors Handbook (http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252) provides estimates for consumer painting citing a mean of 4 painting events per year, a mean annual volume of 2.9 gallons painted with a 90^{th} percentile annual volume of 6.7 gallons used.

4) Although the inhalation measurements were below the level of quantifications (LoQ and LoD are the same in this study), the HSRB requests better explanation of the equation used for 8-hr time weighted average. In this case it appears that a two- hour period of data collection is divided by 8-hrs, reducing the TWA. Either the exposure over the 2 hours can be used as the TWA average (this assumes that the other 6 hours, exposure would be about the same), or alternatively, some worst-case or conservative estimate could be used. This calculated TWA cannot be applied to individuals painting more than 2 hours. HSRB believes this TWA calculation (although represented here in order to normalize the data) is best left to the risk assessment phase to avoid confusion and will depend on the exposure period over which it is applied. The EPA Guidelines for Statistical Analysis of Occupational Exposure Data[8] provides an explanation of TWA. At minimum, the HSRB recommends adding a statement that these TWA must be adjusted if consumers paint for longer than two hours.
5) HSRB recommends removing or altering the "exposure equation" on page 4 of 36 (of EPA Science Review), this equation is confusing and represents exposure and dose. The report is referring to exposure here, and should not introduce a dose concept here without additional explanation.
6) HSRB recognizes the challenges of accounting for loss of the compound in particular by the dermal route and how well the hand wash-efficiency study represents that phenomenon. Dermal absorption alone will depend on a number of factors including; 1) surface area of application, 2) time of residence on the skin 3) properties of the compound, 4) properties of the vehicle, 5) concentration of the compound, 6) condition and thickness of skin, and 7) environmental conditions. HRSB agrees that absent endless experiments under variable conditions, use of these hand-wash efficiency measurements is a reasonable approach to account for loss mechanisms for BIT during the study period. EPA or the study report should better clarify the use of the hand wash efficiency measures (i.e., to account for all loss mechanisms such as absorption and sticking to skin, loss to air, etc.). These hand wash efficiencies were performed for 45 minutes and the BIT in a paint formulation was applied to the palm of the hands at a different concentration than used in the study. HRSB noted that BIT is a low volatility compound, low molecular weight 151 g/mole, with a low K_{ow}-0.76 (low octanol-water partitioning). Given its properties and the fact that the stratum corneum is lipophilic, BIT would be expected to have a low absorption rate into the skin and should largely remain on the skin surface. Hand wash efficiencies were not as high as might be expected, indicating other loss mechanisms.

[8] https://www.epa.gov/sites/production/files/2015-09/documents/stat_guide_occ.pdf, page 35

7) The report should be clear that the hand wash efficiencies are also used to "conservatively" adjust the face/neck wipes (conservative because these do not contain a rinse step).
8) Page 20 of the Full Brush and Roller study indicates that 5 of the 20 wall wipes that were collected were showed BIT residues ranging from 0.148-0.346 ug/sample (considered background). This would indicate that transfer from the painted walls from previous subjects was low and would not influence results. This is an important point to mention in the science review, especially given this was another point of consideration in the paint-sprayer study evaluated by EPA in 2018. It was decided that it was unnecessary to perform the wiping of walls. This brush roller study demonstrates that the paint dries and is not likely to transfer to the next subject.
9) In the reading of the full study report and the EPA scientific review, it was not very clear that the BIT measured concentrations on the hats and dosimeters were added together to determine the exposures for these painting scenarios. For the three scenarios listed, the Board requests that it be mentioned again that the three scenarios also assume no head protection.
10) The second objective was to evaluate whether there was an 80% power to detect log-log linearity with a slope of 1. This objective was met if the widths of the confidence intervals for the slope based on the lognormal model are at most 1.4. Please have the study sponsors more clearly report what the new power would be if calculated.

There were two protocol amendments: 1) edits to the reference methods and 2) clarifications to the guideline for transporting samples from the field to laboratory. In addition, there were eight protocol, 1 method, and 7 SOP deviations that were recorded. These were not listed in EPA science review and should be, although deemed minor and not affecting quality and use of data. HRSB does however agree with EPA that these deviations and amendments either do not affect the validity of the study and use or data or were appropriately addressed to ensure that the data can be used in a scientific manner.

HSRB recommended some clarifications be added to the Study report or some items to keep in mind in using the data for risks assessment.

1) The largest deviation occurred due to contamination of inner dosimeters by the same antimicrobial used in the study as the active ingredient. This occurred for ten of the inner dosimeters and was realized during the analysis phase. EPA made suggestions to imputation (i.e., replacement of values) for BIT transfer from painting for the contaminated samples in order to make use of complete dermal exposure results. Suggestions were to 1) repeat the study, 2) use a mean derived penetration factor from the other samples, 3) default penetration factor of 50%, or 4) use an upper bound penetration factor of 100%. A separate clothing penetration factor analysis

was presented looking at the impact on the dermal exposure measurement using the various imputation choices. Use of a mean (i.e., simple arithmetic mean, 12.3% (note Lognormal approach)) derived penetration factor was chosen across 48 samples (different body parts, and ai applied). Penetration factor is defined as the ratio of inner dosimeter of BIT residue divided by outer BIT residue for the same ME, across the remaining eight ME that did not have contaminated inner dosimeters. Because the majority of exposures occurred through the hands, this imputation was not seen to affect the validity of the study and use of the data for risk assessment. HSRB recommends a clearer acknowledgement in the study report that this reduces the variability in dermal exposures and likely has the greatest impact on the MEs with the lowest hand contribution to dermal exposure values. This ME scenario is the consumers painting in short pants and short sleeves, and in particular the ME's in this category at the lowest hand contribution.

2) EPA in the April 2018 HSRB meeting better clarified which 10 inner dosimeters are missing (i.e., what BIT concentrations). Although this information is found in tables of the study report, please include this information more clearly in the text of the study report. EPA in the April 2018 meeting mentioned that this contamination of inner dosimeters occurred for four MEs in the low BIT concentration range, two in the mid BIT concentration range and four in the upper concentration range.
3) In general, there were numerous issues with the dosimeters. This included inner dosimeters that were contaminated, and contamination with some outer dosimeters (page 19 of full report) where 7 of the -twenty-seven outer dosimeters field fortification at the low levels had high recoveries for BIT ranging from 127% to 203%. There is a mention of some pre-washing of the outer dosimeters to lower that background concentration as discovered in the pre-study. HSRB agrees with EPA that in the future new protocols need to be established to avoid this error. One strategy is to pre-test samples of dosimeters again in the actual studies to ensure they are free of the test compound.
4) One ME (ME 15) (one of the mid-level concentrations) (Figure 1. Regression plot of Long Dermal Exposure of the Statistical Review, other figures 28-28) appeared to be an outlier. This ME 15 was asked to adjust his behavior (5 minutes into activity) but still ended up with the highest dermal exposures in the mid concentration range. HSRB has agreed that this apparent outlier should remain in the dataset for two reasons. One is that what may appear as an outlier may in fact represent the variability in how individuals use these products and this variability should be captured in the regression. In addition, because this ME's exposure appears higher than

his counterparts in this BIT range, keeping his data in the regression would in fact be conservative; this ME's data has a higher dermal exposure estimate.

5) The scenarios for risks are based on how individuals would dress, but might also depend on weather conditions. Meaning, if consumer painters open windows to paint as instructed on cans during the winter, they would also choose to wear long pants/long sleeves. In addition, for a commercial small painter, they might wear short sleeves/short pants.
6) Face and neck wipes are adjusted by multiplying by a factor of 1.11 to correct for the area of the face covered by safety glasses. The Board requests a reference of how this 11% is derived for this parameter estimate (i.e., was the area under the face covered by safety glasses actually measured or is this an estimate based on the typical surface area found under goggles).

HSRB observations on the test chamber:

The ventilation rate was measured prior to using the test chambers for painting. The ventilation was reported to be 11-12 air changes per hour (ACH) with door open and 7 ACH with door closed. The high ventilation rate was required by the regulatory state agency where the study was conducted to reduce exposure to subjects. We assume that the testing was conducted with the door closed and ventilation fan turned on but there may have been periods of time with door open—the Board does not have definitive information on this. Either way, the ACH during testing is much higher than would be expected under normal conditions even with windows open in a home. Decreasing ACH to a typical range (0.2 – 2) would proportionally increase the exposure concentration given the same emission rate into the room.

In addition to the high ventilation rate, an air conditioner (AC) was used in the test rooms that recirculated room air at some unknown rate. Give high water emissions in the room during painting (elevated humidity), the AC will likely condense liquid water on the coils. Considering the physiochemical properties of BIT (high solubility ~ 22 g/L and low Henry Law Constant ~ 2E-10), the condensed water on the AC coils will likely scrub any BIT from the air that passes over the coils. So, in summary, if the AC recirculation is considered a removal pathway for BIT along with the high room ventilation, then the study could significantly underestimate inhalation exposure.

The study found that inhalation of BIT was insignificant and this result is consistent with the high solubility and low H of BIT. However, with the high effective ventilation rate (i.e., ACH plus AC recirculation), it is difficult to determine whether inhalation exposure is negligible or the room air was being scrubbed. This issue should be considered in future experimental design. It is unfortunate that unrealistically high ventilation rate in the test room was required during monitoring. We understand that

BIT has a very low volatility and may in fact be insignificant. However, all we can know for certain is that it inhalation exposure was below the reporting limit. Therefore, to account for the high effective ventilation rate the agency should start with the measured result (i.e., ½ LOQ) and then adjust that value to account for the effective ventilation rate (exhaust and AC recirculation).

Statistical review:

Overall the statistical analyses by ICF were wide ranging and very thorough. Commonly used statistical techniques were utilized to describe the results. Several issues needed to be addressed before the analyses could be carried out; e.g., whole body dosimeter contamination, and a number of assay results with values below the limit of quantitation (LOQ), also referred to as "non-detects." Reasonable choices were made for dealing with those issues.

The analysis generated a large number of numerical tables of results for the arithmetic and geometric means standard deviations and a set of percentiles of the distributions. A good summary of the patterns and anomalies in the results were given in the report.

The analysis compared the results based on the normal distribution and the log-normal distribution. Because of the skewness in the data the log-normal distribution was clearly the better choice overall.

An additional analysis of the data based on a gamma distribution may be useful. The gamma distribution is another continuous skewed distribution that has a much more flexible set of distributional shapes than does the log-normal. Recently the gamma distribution has become more commonly used in some areas of statistical applications.

Charge to the Board - Ethics:

Does the available information support a determination that the research was conducted in substantial compliance with the requirements of 40 CFR part 26, subpart Q?

Board Response:

The HSRB concludes that study AEA089 submitted for review meets the applicable requirements of 40 CFR 26, subpart Q.

HSRB Detailed Recommendations and Rationale:

The purpose of this study was to determine potential dermal and inhalation exposure levels for consumers (i.e., non-professional painters) using a brush and/or roller to apply latex paint containing one of three concentrations of an antimicrobial pesticide product (1,2-Benzisothiazolin-3-one, or BIT). Subjects (18 in total) were asked to paint rooms (including walls, trim, baseboards, and ceilings) according to their typical practices. Each monitoring event involved approximately 2 gallons of paint, lasting 48 to 172 minutes, and involving concentrations of BIT at 144, 375, or 619 ppm.

The Agency's rules at 40 CFR part 26 subpart Q that are applicable to this review include the following:

§26.1703: Except as provided in §26.1706, EPA must 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.

Intentional Exposure of Pregnant Women, Nursing Women, or Children

The Final Report and supporting materials submitted by AEATF II for EPA review indicate that no pregnant or nursing women enrolled in the study and all human subjects were between 20 and 64 years old. As a result, AEA09 satisfies Agency rules at 40 CFR §26.1703.

The Final Report notes that interested persons were asked if they were at least 18 years old during the initial phone interview. Potential subjects were also asked to bring a government-issued photo ID to the informed consent meeting so that the English-speaking Field Research Associate or Spanish-speaking Field Research Associate could verify the subject's identity and age of $\geq$ 18 (p. 24).

Female subjects were required to take an over-the-counter urine pregnancy test on the day of their monitoring event and prior to other study activities (p. 31). After the subject took the pregnancy test, she was asked if she wanted to continue study participation. The Final Report notes that all female subjects affirmed that they wished to continue participation, so a female member of the research team was present and confirmed the negative results of their respective pregnancy tests (p. 31). Results of the pregnancy tests were not recorded.

During the phone interview, interested subjects were informed that they must not be pregnant or nursing a child in order to participate in this study (p. 316). Subjects were also asked about their pregnant or nursing status when completing the Qualification Worksheet during their Informed Consent meeting (p.85).

Compliance with Subparts A through L

5. Independent Ethics Review

The study protocol and supporting materials were reviewed and approved by Schulman Associates Institutional Review Board (SAIRB), an IRB registered with the Office for Human Research Protections (OHRP, Registration #00000971). SAIRB is also fully accredited by the Accreditation of Human Research Protection Programs (AAHRPP). The Final Report submitted by AEATF II contains all correspondence between investigators and SAIRB, including documentation of SAIRB's initial approval of the study (dated March 4, 2016), as well as approval of two amendments and acknowledgment of a series of protocol, method, or Standard Operating Procedure (SOP) deviations. The EPA Ethics Review noted EPA concerns about the process for reviewing and approving protocol amendments, though it should be noted that the amendments and the issues surrounding their approval did not have any impact on subjects' health, safety, or rights (March 30, 2018, p. 6-7). The EPA Ethics Review provides details about these deviations and notes that EPA does not believe they had any impact on subject safety or welfare, and the HSRB concurs with EPA's assessment.

Additionally, the HSRB reviewed the study at its April 8-9, 2014 meeting. EPA and HSRB made specific recommendations to AEATF II, and their responses to those ethics-related recommendations are also included in Attachment 1 of EPA's Ethics Review of this completed study.

6. Selection of Subjects (Recruitment and Demographics)

IRB-approved advertisements were placed in two newspapers that served the study location: The *Fresno Bee* and *Vida en el Valle* (Spanish language). Investigators intended to place an advertisement on May 20, 2016 in a third periodical, the *California Advocate*. However, investigators could not confirm placement of this ad and reported this potential omission as a deviation. Interested individuals called the number listed in the advertisements to learn more about the study and to allow investigators to ascertain general eligibility. Those who were potentially eligible and still interested were then invited to attend an in-person Informed Consent meeting with members of the research team where they would undergo informed consent discussions and research team members would assess subject eligibility.

A total of 37 subjects enrolled in the study, with 18 subjects completing monitoring events. As noted above, the 20 subjects who completed the handwashing activities were between 20 and 64 years old, with 13 male subjects and 5 female subjects completing study activities. All subjects preferred to communicate in English.

7. Minimization of Risks

Risks to subjects stemming from research participation included:

e. Risk of reaction to latex paint or BIT;
f. Risk of irritation from isopropyl alcohol;
g. Risk of heat-related discomfort related to wearing dosimeters while painting;
h. Risk of using a ladder while painting; and
i. Potential risk of unintentional release of confidential information.

Of note, there were no adverse events reported by any of the subjects.

Risks to subjects were minimized by adherence to clear subject inclusion/exclusion criteria. The study enrolled only healthy subjects who had no known allergies or sensitivities to BIT, latex paint, or isopropyl alcohol. All subjects had at least one year of professional painting experience, so that they were familiar with the activities they were asked to perform. On-site medical personnel were available during each monitoring event in order to assist with any subject concerns and to observe for signs of heat-related illness. The testing environment's heat index was also monitored if the temperature at the study site exceeded 82 degrees Fahrenheit; testing was to be stopped if the heat index exceeded 95 degrees Fahrenheit, though this did not happen (Final Report, p. 184). Although monitoring events were videotaped, all photos and videos were reviewed to ensure they did not show subjects' faces, tattoos, or other identifying features. Subjects' personal information is stored separately from other study data in Golden Pacific Laboratory's archive room.

8. Informed Consent

All subjects were required to review and sign an IRB-approved informed consent document. SAIRB provided certified Spanish translations of the English recruitment and consent materials, though the Spanish-language consent documents were not needed. Potential subjects visited the study site for informed consent discussions and were provided copies of the consent document, Qualification Worksheet, product label, and Safety Data Sheet. They were encouraged to take those materials home to consider participation and to discuss with friends or family members, if they chose to do so. They were

also encouraged by research staff to ask questions at this time and reminded that their participation was voluntary and could be stopped at any time.

Topic #4: A published article titled "Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes"

Charge to the Board - Science:

Is the research described in the published article "Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes" scientifically sound, providing reliable data for the purpose of contributing to a weight of evidence determination in EPA's assessment of the risks to human health associated with releasing *Wolbachia*-infected mosquitoes?

Board Response:

The Board concluded that the research described in the article by Popovici et al was ***not*** scientifically sound and does ***not*** provide reliable data to contribute to a weight of evidence determination for assessment of human health risks due to release of *Wolbachia*-infected mosquitoes.

HSRB Detailed Recommendations and Rationale:

The key findings in the Popovici paper that are proposed to be used in the weight of evidence approach rest on two assays that attempted to measure the immune response of humans to a potential exposure to *Wolbachia*, via mosquito bites from *Aedes aegypti,* in the HSRB's view, there is nothing approaching a rigorous description of the research methods in the article, which therefore precludes the ability to make a rational judgement regarding the utility of the data, either as quantitative data or as qualitative data. There are gross omissions of methodology, including the detection chemistry in the immunoblot and the ELISA. The fundamental element upon which one would base a judgement of utility in these data is the sensitivity of the assays. In the absence of a measure of sensitivity, negative data tells us nothing about the presence or absence of a target in a sample, and that is the case in this published article. Not much is illuminated by the fact that experimentally prepared rabbit antibody to Wsp can detect *Wolbachia* Wsp, because no information is given about how much or how little human anti-Wsp antibody it would take to register as a band. Concerns in this regard are further amplified by the variable intensity of the same molecular weight ladder in Panel 1a, lane 3 and lane 10, and by the fact that the same ladder generated different banding patterns in panel B, compared to panel A, and by the fact that the band in lane 3 (Wsp) looks like it is lighting up pixels that are outside (to the left) of what looks like the immunoblot

membrane. It appears that each sample was cut out of the immunoblot membrane individually. It is not clear if the bands were from different gels, from different blots, from different incubations, from different months or some combination of these variations. All of these factors can compromise immunoblot precision and accuracy. The Board reiterated that the fact that a signal can be generated to Mosquito thorax extracts using rabbit antibody reveals nothing about the sensitivity of the assays. That data could easily be interpreted as less human antibody against Mosquito thorax, but of a higher affinity compared to more human antibody against Wolbachia, albeit at a lower affinity. The fundamental point is that a reasonable level of assay validation has not been done.

Similarly, in Figure 2, the fact that antibodies to salivary gland proteins can register a signal but antibodies to Wolbachia antigens cannot tells us nothing about how much or how little human antibody to Wolbachia was in a sample. Fundamentally, in both the immunoblot experiment and in the ELISA experiment no use of an informative positive control was made, giving us no indication of the ability of the assay to respond to a positive target if one was present in the sample.

The HSRB therefore disagrees with the EPA conclusion that "... the results provide evidence that humans who regularly blood-feed *Ae. aegypti* mosquitoes do not develop an immune response to antigens present in *Wolbachia* extracts." That may or may not be true. The Board's conclusion is that the results of this study failed to detect an immune response to Wolbachia using assays of unknown sensitivity and methods that were entirely inadequately described. For these reasons, the Board concludes that the research as described falls below the threshold of being scientifically sound, providing reliable data for the purpose of contributing to a weight of evidence determination in EPA's assessment of the risks to human health associated with releasing Wolbachia-infected mosquitoes.

Statistical review

An experimental design in this study involved 17 human volunteers in a blood-feeder (BF) group and 5 human volunteers in a non-blood-feeder (NBF) control group. To compare the BF and NBF groups, a Mann Whitney test was used and a significant p-value of 0.0129 was reported for a higher IgG level specific to *A. aegypti* salivary gland extracts for BFs than NBFs. The other tests were not statistically significant.

Some details seem to be lacking such as the volunteer selection procedure, randomization protocols (if any), and a sample size calculation/power analysis. Given that the sample size is quite small and the test is nonparametric, the conclusion that there is no evidence for a difference between BF and NBF groups may be attributed to a low power for detecting a difference.

In addition, the article indicates that the study occurred over a 4-year time frame and involved multiple episodes of blood feeding of mosquitoes. It is likely that some or all of the 17 volunteers had been exposed in several blood feedings over a period of years. It is not clear how the temporal aspects of the data were taken into account in the study design and the data analysis.

Charge to the Board - Ethics:

Does the available information support a determination that the research was conducted in substantial compliance with the requirements of 40 CFR part 26, subpart Q?

Board Response:

The Board concludes the available information supports the determination that the research was conducted in compliance with requirements of 40 CFR part 26, subpart Q.

HSRB Detailed Recommendations and Rationale:

This study on its face is relatively straightforward with regard to human subject issues. Volunteers were recruited to provide blood meals to mosquitoes raised in the laboratory setting, for the purpose of developing a strain of *Wolbachia*-infected *Aedes aegypti* mosquitoes. As an additional secondary objective, the laboratory also conducted a study of immune responses to mosquito and *Wolbachia* antigens in these volunteers. Procedurally, there is no problem with the study conduct: informed consent was sought and documented; IRB review was conducted and relevant documentation is available.

Overall this study poses low risk to human volunteers. However, the Board notes that the scientific issues regarding the validity and reliability of the antibody assays affect both the scientific assessment and also the risk assessment for volunteers. It is believed and asserted that Wolbachia does not stimulate immune response in the volunteers because no *Wolbachia* antigens are transferred during mosquito biting. The immune assays seek to provide objective evidence of this assertion. The question of whether or not the evidence is convincing was addressed in the scientific review. The designation of low risk to human volunteers in part depends on the view that the *Wolbachia* infection does not increase risks above and beyond that which would be experienced by volunteers being bitten by other, non-experimental laboratory reared mosquitoes. Because the study is attempting to demonstrate the lack of effect of *Wolbachia*, the reasoning is a little circular—the data reported in the study are purported to support the claims of low risk. However, in spite of the weakness of this particular study, it does appear that scientific evidence from other studies supports the view that presence of *Wolbachia* infection in the biting mosquito does not increase the risks to humans being bitten; other research also supports the view that mosquito biting in

itself is not risky, if the mosquitoes are free of pathogens, which they are in this case. Therefore, there are no major ethics concerns regarding risk, in spite of the questionable antibody assessments in the study.

Regarding recruitment of members of the lab to serve as human subjects: in general, this is discouraged since it is difficult to ensure no subtle or unspoken pressure regarding participation. As a general practice, it is not recommended. However, the fact that the study overall is low risk, the IRB approved the study and the recruitment plans, and investigators made an effort to ensure that individuals were not penalized for non-participation, all mitigate this potential harm. If the study were risky, the ethical assessment might shift significantly.

Overall the Board found no ethics barrier to the use of these data from human volunteers for decision-making. However, the overall acceptability of the study depends on the scientific review regarding soundness of the scientific methods used in the study.