EPA-HSRB-20-2

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

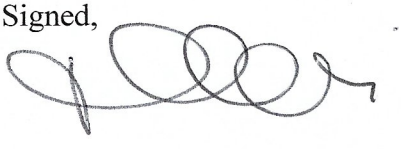
Subject: July 21-22, 2020 EPA Human Studies Review Board Meeting Report

Dear Dr. Orme-Zavaleta,

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a completed study and a study protocol involving human participants. On July 21, 2020, the HSRB considered a completed study of laboratory-based testing of a tick repellent containing Oil of Lemon Eucalyptus (OLE), submitted by the ARCTEC (Arthropod Control Product Test Centre) and sponsored by Citrefine International titled "Single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol ® (OLE) against three species of ticks." Briefly, the report summarized research to determine a typical consumer dose for a pump spray, skin-applied repellent and for laboratory testing to evaluate a repellent containing 30% OLE against three species of ticks. On July 22, 2020, the HSRB considered a study protocol submitted by the Antimicrobial Exposure Assessment Task Force II (AEATF): "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying" (AEA14). Briefly, the goal of the proposed study is to measure dermal and inhalation exposure to an antimicrobial pesticide when product is applied using hand-wand or electrostatic sprayers.

The HSRB's responses to the charge questions presented at the meetings on July 21-22, 2020 along with detailed rationale and recommendations for their conclusions are provided in the enclosed final meeting report.

Signed,



Jennifer Cavallari, ScD, CIH
Chair, EPA Human Studies Review Board

INTRODUCTION

On July 21-22, 2020, The United States Environmental Protection Agency (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to a completed study titled "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" and a study protocol from Antimicrobial Exposure Assessment Task Force, LLC (AEATF-II)-sponsored research –"A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying" (AEA14) and "Study Addendum: Addition of Electrostatic Sprayers". In accordance with 40 CFR 26.1601, EPA sought HSRB review of these completed studies.

REVIEW PROCESS

The Board conducted a public meeting on July 21-22, 2020. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-10001-03-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 completed and proposed research.

For each agenda item, the Agency staff presented their review of scientific and ethical aspects of the research, with each presentation followed by clarifying questions from the Board. The HSRB solicited public comments and next 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, study reports, related materials and documents provided by the study sponsors, the Agency's science and ethics reviews of the study, as well as oral comments from Agency staff and the investigators during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/osa/july-21-and-22-meeting-human-studies-review-board.

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- Science:

Did the research summarized in "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" generate scientifically reliable data, useful for deriving a typical consumer dose and estimating the amount of time the product tested repels ticks?

Response to the charge question:

The HSRB concludes that the research summarized in "Single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol ® (OLE) against three species of ticks" provides scientifically reliable data, useful for deriving a typical consumer dose and estimating the amount of time the product tested repels ticks.

The HSRB also has specific comments, recommendations and additional minor points that are described below.

HSRB detailed response and rationale:

The HSRB reviewed a completed study of laboratory-based testing of a tick repellent containing OLE, submitted by the ARCTEC and sponsored by Citrefine International. The study report provided the rationale, methodology, and results of a consumer dose rate study and tick repellency study.

The HSRB reviewed five documents:

- Study report "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."
- Science Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE
- April 24-26, 2018 EPA HSRB Meeting Report

- Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing OLE
- LSHTM Ethics Committee Terms of Reference

The primary objectives of the study were to determine the median complete protection times (mCPT) of EPA Reg. No. 84878-2 formulated insect repellent applied at the consumer dose rate against three adult species of ticks *Ixodes scapularis*, *Amblyomma americanum* and either *Dermacentor variabilis*, *Dermacentor andersoni*, or *Rhipicephalus sanguineus*. The secondary objective was to determine the average dose rate applied by consumers when using EPA Reg. No. 84878-2 formulated insect repellent to repel ticks.

The study protocol was reviewed by the HSRB in April 2018. There were several recommendations by EPA and the HSRB at this time including considerations for the consumer dosing study, choice of tick species, and increased clarity in CPT definition and study description. The HSRB-recommended changes were incorporated in the first protocol amendment. In total, there were five protocol amendments and 14 deviations (10 subject specific and 4 not subject specific).

In the consumer dose rate study, the average dose rate of 3 applications per person was used to calculate an overall mean dose in 25 people. Subjects were instructed to read the instructions and apply product. The amount applied was estimated based on the measured amount deposited on three cloth arm bracelets and the portion of the arm length covered by the bracelets. The typical consumer dose rate of 0.793 $\mu L/cm^2$ was calculated using dosimetry test results.

To assess the duration of test substance efficacy, the test substance was applied at the specified amount to one of the subject's arms and spread over the entire surface of the lower arm by the experimenter using a single nitrile gloved finger. Subjects were asked to sit with both arms palm side up on a table. A tick of the appropriate species was first applied to the inside surface of the opposite, untreated, arm and observed for questing behavior. When a tick with questing behavior was identified, it was transferred to the wrist of the treated arm and observed for questing behavior. A tick that crossed onto the treated skin of the arm and spent more than 1 minute in

that area was considered not repelled; a tick that remained near the wrist or fell off the arm was considered repelled. This process was repeated at 15-minute intervals for up to 10 hours or until a crossing onto treated skin was recorded. This "first crossing" was then confirmed with a second crossing within 30 minutes. If the first crossing was confirmed, it was considered the time of effective repellency. If it was not confirmed, testing continued until a confirmed set of crossings was observed. The study concluded there was a median CPT of 4 hours 50 minutes against adult *Ixodes scapularis* ticks. The CPTs for the other species were longer (*Rhipicephalus sanguineus* was 8 hours 32 minutes and adult *Amblyomma americanum* was >10 hours). EPA concluded this study supports a label claim for the proposed pump spray product containing 30% w/w of the active ingredient, Citriodiol (OLE) that the product "repels ticks for 4 hours."

Overall, this is a well-conducted and straightforward study. The study report itself is well written and relatively easy to understand. Of note, compared to the Study Protocol reviewed by the HSRB in 2018, the addition of other tick species was particularly informative. The original protocol proposed using *Rhipicephalus sanguineus*, which would have resulted in a CPT of 8 hours and 32 minutes. Use of only this species would have overestimated CPT of other species, namely *Ixodes scapularis*.

The HSRB concurs with the EPA determination that the CPT supports a label claim of "repels ticks for 4 hours," using the current EPA labeling guidance based on the median CPT.

Recommendations

The HSRB recommends the following changes and clarifications to the study report "Single group trial to determine the CPT of an insect repellant formulation containing 30% Citriodiol ® (Oil of Lemon Eucalyptus) against three species of ticks" (or Study Report) and/or EPA Memorandum including the Science Review (or EPA Review) documents:

- In the EPA Review, the vehicle for the test substance should be stated (Section 4, EPA Summary). If there is a confidentiality issue, a genericized name of the primary diluent could be included. Further detail on whether evaporation is an issue due to low volatility of the vehicle (i.e., alcohol) should be addressed as appropriate.

- Both the Study Report and EPA Memo should provide discussion of the measured application rate versus the standard application rate (Page 10, EPA Review). The EPA Review states - The result is 0.00079278 mL/cm^2, converted to 0.7928 µL/ cm^2, rounded to 0.793 µL/cm^2. When adjusted for density, the result is 0.714 mg/cm^2. This translation should probably be made somewhere in the US EPA report. We would also note that the study report indicates the standard application rate is 0.83 mg/cm^2 (Page 75, Study Report). If the measured application rate is 0.714 mg/cm^2 with a standard error of 0.195 mg/cm^2, the results of the study are not really distinguishable from the standard application rate of 0.83 mg/cm^2. This finding is not mentioned in either document and should be addressed.
- In the EPA Review, (Page 3, EPA Summary) with respect to the description of the application study. "Average doses of application per subject (n = 3 applications/person) were used to calculate the grand mean dose of application across 25 subjects (n = 75)." This discussion should be expanded for clarity. There were three sets of three bracelets per subject. The mass of test substance deposited on the three bracelets placed on different thirds of the arm were summed for each of three trials. The results for each trial were then averaged to yield an individual mean. A grand mean was then calculated from the individual subject means.
- In the EPA Review (Page 4, EPA Summary) with respect to the formula used for calculation of the mass applied. The current formula as stated is confusing:

  formula: (sum of bracelet set circumferences) X 1/3 (length of the forearm)

  The HSRB suggests that the EPA consider presenting the formula as: the sum (each bracelet circumference x 1/3 of the forearm length represented by that bracelet) or something analogous. In the report, the authors refer to each arm region. Again, the formula as presented is valid based on the transitive property of algebra but the expression may confuse some readers with respect to why only 1/3 the length is used.
- In EPA Review and Study Report, the amount of test substance initially applied is given per subject but not the amount that was present after use of the glove to spread the test substance around (Tables 20-24, Study Report). Tables 25-29 show the amount of the applied test substance still on the glove. Ideally, these two tables would be combined to

show, based on the glove measurements, how much remained on the forearms for each subject. These would then be plotted against measured CPT to demonstrate that no relationship is seen between the amount of test substance on the subject's skin and CPT, or, alternatively, that no difference in mCPT was seen when subjects with very low amounts of test substance remaining on the forearm were excluded from the analysis. The analysis that EPA performed, examining the relationship between the amount of material on glove and measured CPT, should be added to the EPA Review and/or Study Report.

- In the EPA Review, it is stated that the difference in gloves before and after spreading was "negligible" (Page 4, EPA Review). This statement appears to be made based on the mean difference across all tests (Study Report table 8.5 to 14.1%). Whether a 14% loss should be termed "negligible" is debatable. However, for some individual tests the amount of material present on the glove after spreading was as much as 80% of the applied dose (see report Tables 25-28). We recommend against characterizing the loss to gloves as 'negligible". Applying only 20% of the test substance could presumably influence the results. Alternatively, the weighing of the gloves could have limited accuracy. Given that some gloves had ~20% lower weight after application, the accuracy of the measurement is a bit unclear.
- Page 4 (EPA Review). EPA's document should state that the amount was spread over the whole of the subject's forearm for clarity.
- Page 6 (EPA Review). The term "right censored data" is a bit vague and should be clarified. It would be helpful to include a parenthetical comment such as "(i.e., "no time point where the tick was repelled was recorded up to the end of the 10 hr study period")".

Statistical review

Overall, the statistical analysis of the data collected within the OLE tick study was adequate. The estimation of the consumer dose using the amount on the bracelet relative to the bracelet surface area seems appropriate. Appropriate descriptive statistics were computed for establishing the mCPT. Kaplan-Meier analysis is appropriate to establish the mCPT.

Recommendations

The HSRB suggests the following clarifications for the current study:

- Established EPA guidelines should be referenced with regard to rounding protocols. Specifically, the mCPT of 4 hours 50 minutes was rounded down to 4 hours. When appropriate, the EPA guidelines with brief rationale should be referenced.
- The CPT for *A. americanum* is listed in the results on page 15 of the EPA report but then it is stated that it 'could not be calculated but only estimated to be above 10 hours'. This may be unclear. mCPT is listed for all three species of ticks, even though more than half of the subjects tested with *A. americanum* were right censored. Confidence intervals were computed for the two other species. A suggested change for this description is: Testing using *A. americanum* resulted in 10 subjects experiencing CPT. Because more than half of the sample, 15/25=60%, of total subjects were censored (did not reach CPT), the median CPT for *A. americanum* could not be calculated. CPT for the 10 subjects (40% of subjects) that were not censored ranged from a minimum of 156 minutes, ~2:00 hours, to a maximum of 558 minutes, ~9:00 hours. The CPT for the 10 subjects that were not censored is estimated to be above 10 hours.

The HSRB has the following general recommendations to be considered for future studies.

- The Board discussed the use of median for determination of CPT. Some Board members recommend considering the use of alternate, more health protective, statistics outside of the median, for example the 10^{th} or 25^{th} percentile when calculating CPT. In addition to being less health protective, the median CPT does not capture the full range of variability in the data. The labeling should also be modified to reflect the percentile used in the calculation of the CPT: "…ticks are repelled for 4 hours for 50% of the population" if the mCPT is used, for example.
- One of the study protocol deviations was a mistake in calculating the average consumer dose. This resulted in overestimation of the average dose which was applied to the repellency study. The Study Report states:

  > An error in the consumer dose calculation was identified that resulted in subjects being provided with 0.008 $\mu L/cm^2$ too much TS on 40 occasions. The calculation error was reported to the EPA. In their judgement, and in that of the Study Director, the difference is negligible and unlikely to invalidate results obtained.

As the difference was within the standard error of the mean application rate, the HSRB agrees that this discrepancy would have little impact on the conclusions of the repellency study. Nonetheless, the fact that one participant's data was excluded and went unnoticed is concerning. The HSRB recommends the implementation of study protocols to double check the computation of values.

CHARGE TO THE BOARD - ETHICS

Does the available information support a determination that the research was conducted in substantial compliance with procedures at least as protective as those in the applicable requirements of 40 CFR part 26, subparts K-L?

Response to the charge question:

The available information supports that study, "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" was conducted in substantial compliance with procedures at least as protective as those in the applicable requirements of 40 CFR part 26, subparts K-L

HSRB detailed response and rationale:

The Board reviewed the scientific and ethics reviews and recommendations for this trial as completed by EPA staff, as well as the final study report, which included extensive records from the IRB and ethics committee. The trial was conducted in the United Kingdom under the dual oversight of Western Institutional Review Board (WIRB) and by the London School of Hygiene and Tropical Medicine (LSHTM) Intervention Research Ethics Committee via a reliance agreement. WIRB and LSHTM Intervention Research Ethics Committee both approved the protocol and all amendments, as well as the consent forms and recruitment materials. The trial excluded subjects under 18 years of age, pregnant females and lactating females.

The trial conduct was consistent with the protocol and its safety provisions. There were a number of protocol deviations, however, none of which substantially affected the health or welfare of the research subjects. There were 8 adverse events, 6 involving bites of ticks or colony mosquitoes,

all of which resolved. Two adverse events involved a general ill feeling following study participation. Neither of these was assessed as study related and both resolved.

In its conduct, the OLE tick study met applicable ethical standards for the protection of human subjects of research, and requirements for documentation of ethical conduct of the research were satisfied.

- The study excluded subjects under 18, pregnant females and lactating females as required by 40 CFR 26 Subpart Q section 26.1703 and 26.1705.
- The study was conducted in substantial compliance with all applicable provisions of subparts K and L of 40 CFR 26 (Subpart K addresses "Basic Ethical Requirements for Third-Party Human Research for Pesticides Involving Intentional Exposure of Non-Pregnant, Non-Nursing Adults" and Subpart L details "Prohibition of Third-Party Research involving Intentional Exposure to a Pesticide of Human Subjects who are Children or Pregnant or Nursing Women").

A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products" and the "Study Addendum: Addition of Electrostatic Sprayers"

Charge to the Board- Science:

Is the protocol "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products" and the "Study Addendum: Addition of Electrostatic Sprayers" likely to generate scientifically reliable data, useful for assessing the exposure of those who apply products containing antimicrobial pesticides using hand wand or electrostatic sprayers?

Response to the charge question:

The research presented in the protocol "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products" and the "Study Addendum: Addition of Electrostatic Sprayers" is likely to generate scientifically reliable data, useful for the assessment of exposures of those who apply products containing antimicrobial pesticides using hand wand or electrostatic sprayers given the comments and recommendations provided by the EPA and HSRB are adequately addressed.

The HSRB also has specific comments, recommendations and additional minor points that are described in the discussion below.

HSRB detailed response and rationale:

The HSRB reviewed the protocol "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying" (AEA14) and an addendum titled "Study Addendum: Addition of Electrostatic Sprayers" (AEA 14 Study Addendum), both submitted by the Antimicrobial Exposure Assessment Task Force II (AEATF II). The main protocol was submitted to EPA in March 2020 by AEATF. The Electrostatic Sprayer (ESS) Addendum was submitted to EPA in early June 2020, to specifically address the use of these sprayers for SARS-CoV-2.

Summary of Study Protocol

The main protocol was a four-volume study titled "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products" (AEA14). This study was designed to gather a baseline set of data to evaluate potential dermal and inhalation exposures for individuals who apply antimicrobial products (e.g., sanitizers, disinfectants, and fungicides/mildewcides) using pressurized hand-held wand-type spray equipment (noting that this study focused on manual spraying vs. automated spraying) to areas ranging from outdoor residential areas to industrial and institutional uses for both food contact and non-food contact areas of food/beverage handling and food processing facilities (e.g., meat/poultry processing, dairies, creameries, and cheese-making), livestock/animal housing, mushroom houses, and food storage and transportation. This study was designed to evaluate exposures from five (5) different scenarios (2 outdoor spraying, 1 indoor spraying, and 2 indoor spraying), each containing 18 monitoring events (MEs).

The second document was an addendum to the AEA14 study (above), titled "Study Addendum: Addition of Electrostatic Sprayers" (AEA14 Study Addendum). This document addressed the use of electrostatic sprayers (ESS) for SARS-CoV-2, the virus that causes COVID-19. This exposure assessment added a new (sixth) monitoring scenario to the existing AEA14 study protocol to measure exposure to workers (n=18) spraying antimicrobial product indoors using ESS.

The proposed sample size is a total of 108 monitoring events (MEs) distributed among the scenarios and subdivided by consumers vs. occupational, electrostatic sprayers, and baseline PPE (long-sleeved shirt, long pants, shoes, socks, protective eyewear, hard hat and a respirator) vs PPE (long-sleeved shirt, long pants, rain pants, rain jacket, rubber boots, hard hat, chemical resistant gloves, protective eyewear and a respirator). Dermal and inhalation exposure will be measured using whole-body dosimeters (WBD) (inner and outer), head patches, face/neck wipes, hand wipe/washes, and personal air monitors.

An AEATF II survey found that a majority of pressurized hand-wand products are intended for professional use only, with a few for professionals/consumers, with none labeled solely for consumer use. A majority (~60%) of use sites labeled for treatment by pressurized hand-wand

spraying are for indoor use sites (e.g., food handling and processing facilities, livestock production, and mushroom houses) with ~20% being for outdoor use (e.g., outdoor wood surfaces, cooling towers, and exterior hard surfaces). Approximately 20% of the use sites could be for either indoor or outdoor use (e.g., livestock housing, wood preservation, and mold control). The AEATF study protocol did note that there were some potential use sites for consumer treatment, such as indoor DIY treatments for mold and outdoor treatments of siding and shingles for fungal and mildew control.

The protocol for AEA14, signed by the Sponsor's Representative on March 19, 2020, specified five (5) different exposure scenarios for a total of 90 monitoring events (MEs) (n=18 different test subjects for each scenario) for both consumer and occupational exposure scenarios. As noted earlier, the June 2, 2020, AEA14 Study Addendum added a sixth (6) monitoring scenario to monitoring exposure to workers spraying products indoors using ESS (2b below), which increased the total MEs to 108. The following exposure scenarios are proposed in the study protocol and addendum (see Table 3 and text of Vol. 1 of Study Protocol; Table 1 and text of Addendum; EPA 2020 Review Table 5):

1. Outdoor spraying (baseline PPE):

    1a. Consumer: 18 MEs (manual hand-held tank sprayers and hose-end sprayers).

    1b. Occupational: 18 MEs (mechanically pressurized sprayers).

2. Indoor spraying: surface spraying in dry environments (baseline PPE):

    2a. Occupational: 18 MEs (manual hand-held tank & backpack sprayers and battery-powered backpack sprayers).

    2b. Occupational: 18 MEs [hand-held, backpack, and cart-mounted electrostatic sprayers (ESS)].

3. Indoor spraying: environmental sanitizing/disinfecting in wet environments

    3a. Occupational: 18 MEs (mechanically pressurized sprayers and central distribution spray systems/Venturi-injection) (baseline PPE).

3b. Occupational: 18 MEs (mechanically pressurized sprayers and central distribution spray systems/Venturi-injection) (full PPE).

Each ME will consist of measuring potential dermal exposure (using inner and outer dosimeters, face/neck wipes, hats, and hand wipes/washes) and breathing zone air levels for a single subject working within a specified set of conditions. The measurements will be conducted in either a simulated or actual work site or combination of both. Study participants in this exposure monitoring study will only be handling diluted products.

The two test chemicals that will be used in the study are EPA-registered quaternary ammonium antimicrobial compounds, or "quats," which are commonly used in consumer and professional sanitizing products. The products that are to be used in this study are:

- Maquat® 5.5-M (EPA Registration Number 10324-80) which will be used for the outdoor and indoor non-food contact spraying; and
- Maquat® 7.5-M (EPA Registration Number 10324-81) that will be used for the indoor food-contact spraying.

Both of these products contain the same mixture of four quaternary ammonia compounds. Maquat 5.5-M contains 5.5% total quats, and Maquat 7.5-M contains 7.5% total quats. The main quat compound in these products is ADBAC (n-Alkyl dimethyl benzyl ammonium chloride, CAS# 68424-85-1), which is present at a concentration of 2.2% of Maquat 5.5-M and 3.0% of Maquat 7.5-M. Both of these products are reported in the study protocol to be concentrated cleaners/disinfectants/sanitizers registered for use on a variety of use sites including hospitals, food processing, homes, breweries, dairies, health clubs, day care centers, and livestock housing. Both are labeled for surface sanitizing as well as mold and mildew control on surfaces including exterior household surfaces such as brick, vinyl, plastic, sealed concrete, painted or sealed woodwork, and sealed stucco.

The original study protocol states the following regarding the concentrations to be used in the study (Vol. 1 of 4, p. 35, March 19, 2020):

> Both products (the concentrates) have a Danger signal word with PPE requirements of protective eyewear and chemical-resistant gloves. A ready-to-use dilution, Maquat 86-M

(EPA Registration Number 10324-85), containing 0.086% total quats (860 ppm, the same four quats as in Maquat 7.5M and Maquat 5.5-M) has a Caution signal word. It is also registered for institutional, commercial, and residential uses as well as spraying on exterior hard surfaces and has no PPE requirements. As such, when diluted to 860 ppm or less, Maquat 5.5-M and Maquat 7.5-M can be used without the need for PPE. The highest proposed use rate in the study is 0.06% (600 ppm total quats). As comparison, the consumer household cleaner, Formula 409®, contains 0.3% (3,000 ppm) ADBAC.

However, a June 17, 2020, update of AEA14 ("Test Substances and Concentrations used in AEA14) proposed changes to the concentrations to be used in the study, as well as identifying labels for the sub-registrants (Nisus DSV and 4Quat). According to the updated Study Addendum (Table 2: Target Use Concentrations), the updated concentrations of total quats to be used range from 215 to 1,160 ppm, depending on the monitoring scenario and volume sprayed (gallons).

In addition, based on an inhalation risk assessment conducted for the six exposure scenarios, the Study Addendum presented data on Margins of Exposure (MOE) which all exceeded the target level of concern (LOC) of 10, and which reportedly indicated acceptable risks.[1]

The EPA, in their June 22, 2020, review of the AEATF hand wand sprayer and ESS study protocols, evaluated the potential risks and concluded the MOEs were acceptable:

- For dermal exposure, EPA stated: "In AEATF's revised submission, the highest concentration of total Quats that will be sprayed is 1160 ppm (1160 ppm total Quats of which ADBAC accounts for 40%), equivalent to 0.0464% ADBAC. This is still over an order of magnitude less concentrated than the 0.8% ADBAC solution the guinea pigs were exposed to. Additionally, the AEATF II conducted a dermal assessment using a film thickness approach where the dermal MOE at the highest ADBAC concentration was 277 with a Target MOE of 10. (V1:68)."

[1] The MOE is the ratio of the toxicity effect level (e.g., a no observed effect level or a lowest observed effect level) to the estimated exposure dose. A lower MOE suggests that a chemical is more likely to pose an unreasonable risk. For this assessment, EPA determined an acceptable LOC is greater than 10 (i.e., all exposures were at least 10 times lower than the toxicity value) and all exposures exceeded this LOC.

- For inhalation exposure, EPA stated: "The estimated inhalation MOEs for the subjects spraying in the three scenarios (including a 10x protection factor for respirators where appropriate) range from 27 to 23,600 with a Target MOE of 10 (AEATF 2020)."
- EPA also stated: "The potential dermal and inhalation risks have been evaluated by EPA through a comparison of available toxicity data on ADBAC and DDAC and the anticipated dermal and minimal inhalation exposure. The comparison indicates minimal dermal and inhalation risks. ... Individuals who may be at an increased risk for adverse effects are not eligible to become subjects in this study, including individuals known to be allergic or sensitivities to chemical-based cleaning or disinfecting products, isopropyl alcohol, soaps, or latex gloves, or as well as those with known skin conditions that could be exacerbated by study participation or with cuts/abrasions on areas that will be exposed during testing. (V2:29)."

We agree with EPA (EPA June 22, 2020 Science and Ethic Review, p. 18), and would also like to encourage continued collaboration with EPA to ensure the study sufficient power (80% vs. 75%) for example, by increasing the spread of concentration applied, and evaluating independence/correlation if subjects may be eligible to take part in multiple parts of the study.

Overall, the HSRB agrees with AHETF and EPA that the updated antimicrobial-specific hand-wand sprayer data are needed to be more reflective of the uses monitored in this study compared to the existing data based on agricultural chemicals. The inclusion of measurements from electrostatic sprayers is also necessary to assess exposure of people using this type of equipment for disinfecting and sanitizing, which has increased significantly recently.

Planned Diversity

The idea of planned diversity in the conditions are to bias towards higher exposure elements. EPA states it "believes that the design of the AEATF II hand wand and electrostatic sprayer scenarios will represent the middle and upper portions of the daily exposure distribution expected for consumer and occupational workers applying antimicrobial products (e.g., sanitizers/disinfectants/algaecides/mold remediation/etc.) to hard surfaces, food processing equipment, etc." (EPA June 22, 2020 Science and Ethic Review, p. 13)

Mixing, loading, and cleaning will be conducted by study personnel and not the participant. The diversity will include different ambient conditions, product used, type of sprayer used, size/dimensions of the areas, indoor vs. outdoor, and personal experience, to name a few. The study protocol encourages participants to work "as they normally would do." Several aspects may contribute to the diversity, but also increase uncertainty. For example, certain scenarios are not evaluated and may represent higher exposures (e.g., mushroom houses). Importantly, the study provides a benchmark for each scenario in terms of surface area and volume of product used. Cleaning is not evaluated in the study with the rational that it could decrease exposure with "rinsing residues from hands." However, cleaning might also increase exposure to other body parts.

Monitoring

The primary measures of exposure are dermal and inhalation. The study protocol describes a procedure for rest room breaks or rest breaks with a drink.

Surrogate Test Chemical

Finally, quaternary ammonium compounds and in particular, ADBAC, are common chemicals that are found in a variety of products. It is possible that use of consumer products (e.g., shampoos, wipes, deodorants, lotions) could present an additional source of target analyte that would overestimate exposure.

Recommendation:

With respect to monitoring, the subject should be instructed to avoid eating during the activity. Should eating during the activity be allowed, the protocol should be specific as to how to account for loss due to this activity (e.g., similar to the description of rest room use by the participant).

Statistical Review

The AEATF II stated that their study protocol proposes to examine potential dermal and inhalation exposure to both consumers and occupational workers during the spraying of surfaces

using an antimicrobial product in the following scenarios: (1) outdoor spraying, (2) indoor "dry" environments, and (3) indoor "wet" environments. The results of this study are being used to examine potential dermal and inhalation exposure during pressurized hand-wand or electrostatic spraying of antimicrobial product.

The goal of 80% power is estimated to be almost met for every scenario. Exceptions are 75% power that is estimated to be met for dermal exposure in Scenario 1a and Scenario 2a.
The statistical analysis of data is adequate. Analysis of variance (ANOVA) is most appropriate for measuring whether there are any statistically significant differences between the means of two or more independent (unrelated) groups. The group geometric means will be compared using ANOVA. All 18 MEs will be used in a scenario. For scenario 1a, the geometric means between the hose-end and manually pressurized back-pack sprayer will be compared using ANOVA. For scenario 2b, the geometric means between the hand-held, backpack will be compared using ANOVA.

The EPA proposed a simple linear regression model for the logarithm of the exposure with an intercept term and with a slope coefficient multiplied by the logarithm of the amount (pounds) of active ingredient handled (AaiH). A simple linear regression model is most appropriate for predicting the value of a variable based on the value of another variable. The primary statistical model will assume a slope of one, which means that the normalized exposure has the same log-normal distribution for all 18 MEs. The fitted model will be used to estimate the arithmetic means, geometric means, and 95th percentiles of the normalized exposure overall, and for each group, together with bootstrap confidence intervals. If the linear models do not fit the data well, other models (e.g., quadratic models, log-log-logistic models, logistic models, and quantile regression models) will be considered. Confidence intervals for the slope will be utilized to examine if the slope is different from 1 or from 0.

The primary statistical modeling will substitute values below the limit of quantitation (LOQ) by half the LOQ, but the results will be compared with alternative approaches for censored data (e.g., the maximum likelihood method). Summary tables and graphs (e.g., exposure plotted against the AaiH showing the fitted regression models, Q-Q plots of the normalized exposures)

will be developed. The potential for bias and uncertainty will be examined in scenarios 3a and 3b if some subjects are in both scenarios.

These statistical procedures were deemed appropriate. The EPA recommendations are reasonable, appropriate, and pragmatic.

Recommendations:

- The Board recommends that an assessment that the assumptions of ANOVA are met be conducted:
    - The dependent variable should be approximately normally distributed for each category of the independent variable, which can be checked using the skewness and kurtosis;
    - The homogeneity of variances is satisfied, which can be checked by using the Levene test. If the p value is greater than 0.05, this assumption is validated;
    - There should be no substantial outliers.
- The Board recommends that an assessment that the assumptions of linear regression are met be conducted:
    - There needs to be a linear relationship between the dependent and independent variables, which can be checked using a scatterplot;
    - There should be no substantial outliers;
    - The data should be approximately normally distributed, which can be checked using the skewness and kurtosis and a normal probability plot (i.e., a Normal P-P Plot);
    - The data should show homoscedasticity, which can be checked by inspection of a plot of the unstandardized or standardized residual values against the unstandardized or standardized predicted values.

- On page 6 of the EPA review, the EPA states that "…the adequacy of the sample sizes of completed studies will be revisited," indicating that a post-hoc power analysis will be done. Post-hoc power analysis should be done to inform future studies. This should be clarified in the EPA Science Review.
- On page 15 of the EPA review, the EPA states that "…the results of those analyses will not be stratified by group…. unless useful patterns are found." An example should be added to clarify that follow-up comparisons may be considered if differences are found in the scenarios. Alternatively, the statement "unless useful patterns are found" can be stricken to not infer that additional analyses will be conducted until something statistically significant arises (p-hacking).

Ethics Review

Charge to the Board – Ethics:

Is the research proposed in the protocol "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products", the "Study Addendum: Addition of Electrostatic Sprayers" and related documents likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Response

The research proposed in the protocol "A Study for Measurement of Potential Dermal and Inhalation Exposure During Pressurized Hand-Wand Spraying of Antimicrobial Products", the "Study Addendum: Addition of Electrostatic Sprayers" and related documents is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L, given the recommendations of the EPA and HSRB are adequately addressed.

Applicable Requirements of 40 CFR Part 26:

The Board concurs with the EPA Science and Ethics Review of AEATF II Pressurized Hand-Wand and Electrostatic Spraying Scenarios Design and Protocol for Exposure Monitoring that reaches the following conclusions:

> An IRB-approved protocol addressing all of the necessary elements in 40 CFR 26, Subpart K (see Attachments 2-6) has been submitted to EPA for review, along with an additional scenario that will be incorporated into the protocol and reviewed by the IRB prior to implementation. EPA has reviewed the protocol and all associated documents, and is presenting the documents and EPA's review to the HSRB. All subjects enrolled in this study will give voluntary, informed consent and be notified about the pesticide to which they will be exposed.

In addition, 40 CFR 26 Subpart L, at §26.1703, as amended effective September 23, 2019, provides in pertinent part:

> 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.

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

Recommendations

Addressing risk from SARS- CoV-2, the virus that causes COVID-19

The Board discussed how the COVID-19 virus and transmission of SARS-CoV-2 in conducting the research study could be addressed. The EPA recommends that protocol be revised to acknowledge and address the risks associated with COVID-19, and adhere to national, state and local guidance, which the Board agreed. There was much discussion with regard to how best protect human subjects from risk associated with COVID-19 virus, with respect to the study. The Board was not in agreement of the best approach. One Board member recognized that the risk of COVID-19 is not unique to the study, but a general community concern. While other Board

members believed that a more protective approach is needed to prevent risk of transmission to and from study participants. Recommendations include:

- Study participants who have signs or symptoms related to COVID-19, have tested positive for COVID-19 within the last 14 days, or have had contact (15 minutes, 6 feet or closer) with someone who has tested positive for COVID-19 in the last 14 days should be excluded from the study. Screening and exclusion criteria should be updated.
- Study protocols should use remote (phone or video) screening. Initial phone screens should occur in close proximity to the monitoring day. When possible, participants and study staff should wear masks and maintain 6 feet of social distancing.
- Study staff should maintain a protocol for monitoring staff signs, symptoms and exposure to COVID-19 to reduce study participant exposure to sick individuals.
- A contact tracing protocol should be developed in the event that an exposure occurs.

Experience level for electrostatic sprayer

The Board discussed the experience required for the participants using the electrostatic sprayer. We recognize the complexity of determining the appropriate experience level given that the use is steadily increasing as a result of the COVID-19 pandemic. The Board suggests that an effort should be made to recruit participants with familiarity with the electrostatic sprayer. The Board recommends that the level of participant's 'experience' be noted and quantified to potentially consider as a covariate in the analyses.