EPA-HSRB-21-4

Dr. Wayne Cascio

Acting Principal Deputy Assistant Administrator for Science

Performing Delegated Duties of Assistant Administrator

1200 Pennsylvania Avenue, NW

Washington, DC 20460

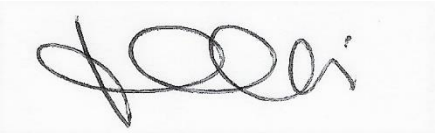
Subject: October 21, 2021 EPA Human Studies Review Board Meeting Report

Dear Dr. Cascio,

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a completed study involving human participants. On October 21, 2021, the HSRB considered a human exposure immersion/dip/soak (IDS) study submitted by the Antimicrobial Exposure Assessment Task Force II (AEATF II). Briefly, the goal of the completed study was to provide dermal and inhalation exposure data to develop unit exposure for people who perform sanitization of hard surfaces using antimicrobial products through immersing, dipping and/or soaking activities. Subjects were monitored while using a rag or sponge with a bucket to wipe surfaces, cleaning cooking equipment using a 3-compartment sink, and using cleaning-out-of-place tanks to clean food processing equipment.

The HSRB's responses to the charge questions presented at the meeting on October 21, 2021 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 October 21, 2021, 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 from the Antimicrobial Exposure Assessment Task Force II (AEATF II) AEA12: "A Study for Measurement of Potential Dermal and Inhalation Exposure During Antimicrobial Applications Involving Immersion, Dip, and Soak." In accordance with 40 CFR 26.1603 and 26.1604, the EPA sought HSRB review of the protocol for this research on October 23, 2018 and for the completed study at this meeting.

REVIEW PROCESS

The Board conducted a public meeting on October 21, 2021. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-10017-40-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 research.

The Agency staff presented their review of the scientific and ethical aspects of the completed 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 https://www.epa.gov/osa/october-19-21-2021-meeting-human-studies-review-board.

A Study for Measurement of Potential Dermal and Inhalation Exposure During Antimicrobial Applications Involving Immersion, Dip, and Soak.

Charge to the Board- Science:

Did the research in study AEA12 generate scientifically reliable data, useful for assessing the exposure of individuals who perform immersing, dipping, and/or soaking activities to sanitize hard surfaces?

HSRB Response:

The HSRB concludes that the research summarized in the study AEA12: "A Study for Measurement of Potential Dermal and Inhalation Exposure During Antimicrobial Applications Involving Immersion, Dip, and Soak" provides scientifically reliable data, useful for assessing the exposure of individuals who perform immersing, dipping, and/or soaking activities to sanitize hard surfaces.

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

Science review

Briefly, this study is being used to develop unit exposures for people who immerse, dip and/or soak restaurant equipment and utensils, or who use rags and/or sponges immersed in a bucket to wipe surfaces with an antimicrobial product (i.e., treatment solution). The protocol for this study was previously reviewed and approved by EPA and HSRB with some requested modifications, all of which were adequately addressed prior to conducting the study.

In this study, inhalation and dermal exposure was monitored in 54 different test subjects, in three different scenarios with 18 subjects each: 1) bucket and rag/sponge (bucket), 2) 3-compartment sink (sink), and 3) clean-out-of-place (COP) tank.

In the bucket scenario, 18 subjects with prior experience within the janitorial industry were recruited. The subjects dipped a rag or sponge into a diluted sanitizing solution in a bucket, wrung out the rag/sponge, and wiped vertical and horizontal surfaces. Three indoor sites in Orlando, Florida were used (two churches and an Elks Lodge). Subjects wiped horizontal and vertical surfaces (e.g., countertops, refrigerators, tables, etc.) at each site. Half of the subjects were monitored for 20 minutes and half of the subjects were monitored for 60 minutes.

In the sink scenario, subjects washed kitchenware at the same three sites used and described for the bucket scenario. Each of the three sinks had capacities of 22.4, 20.8, and 34.3 gallons at Sites 1, 2, and 3. Subjects filled each sink to the level they would usually fill it to. Half of the subjects were monitored for 60 minutes and half of the subjects were monitored for 120 minutes.

In the COP scenario, a single manufacturing facility in Madison, Wisconsin was the site of the study. The three different sized stainless-steel COP tanks were used with all three tanks set up in the performance testing room where the monitoring took place. Six monitoring events were conducted with each tank in order to have variety in the equipment used. The subjects wore long-sleeved shirts, long pants, and chemical-resistant gloves. There were one-hour and two-hour scenarios, where the one-hour scenarios varied from 66 to 75 minutes and the two-hour scenarios varied from 126 to 153 minutes. During the one-hour scenario the subject did one cleaning process and during the two-hour scenario the subject did two cleaning processes.

Subjects wore inner and outer dosimeters and an air sampling pump. For the bucket and sink scenarios, the inner and outer dosimeter tops were short-sleeved because the tasks involved immersing the hands and forearms into the liquid. Dosimeter tops were long-sleeved for the COP scenario. Dermal exposure of the face and neck was measured by hand washes and face/neck wipes. Forearm washes were also used to measure dermal exposure in the bucket and sink scenarios. The EPA-registered quaternary ammonia product used as the test substance was Oasis® 146 Multi-Quat Sanitizer (Ecolab), which is registered for industrial and commercial uses. Chemicals monitored were C14 analog of alkyl dimethyl benzyl ammonium chloride (ADBAC) for dermal exposure and didecyl dimethyl ammonium chloride (DDAC) for inhalation exposure.

There were 4 protocol amendments: 1) increase in compensation for subjects in 2 scenarios, 2) change in the heat index for the stop point, 3) increase in compensation for the remaining scenario, and 4) corrected reporting procedure for sending protocol deviations to the IRB. There were also an additional 2 laboratory, 17 protocol, and 4 standard operating procedures deviations. EPA stated that these deviations do not affect the findings and value of the study, and the HSRB agreed. Deviations included for example, different size buckets being used, lack of airflow measurements in all cases, changes in LOQ for face and neck wipes due to sample interference with the blanks and use of DDAC in the inhalation measurements.

Summarized results were reported for dermal exposures and inhalation exposures based on a dose amount and an 8-hr time-weighted average (TWA), where arithmetic mean and 95% percentiles were calculated. In addition, exposure amounts are normalized by amount of product handled and the time spent (minutes converted to hours) engaged in the event. Inhalation doses are calculated using the inhalation exposures (measured using personal pumps) along with

breathing rates. The bucket scenario resulted in a mean dermal exposure of 0.096 (mg/(ppm a.i. x hours)) for long pants/short sleeves and no gloves scenario, with a mean inhalation dose of 1.98E-6 (mg/(ppm a.i. x hours)) (Table 1; EPA Review). For the bucket scenario the surface area wiped ranged from 107 to 846 ft^2 during the 20-minute exposure group to 569 to 1,635 ft^2 during the 60-minute exposure group. For the sink scenario the mean dermal exposure of 0.00371 (mg/(ppm a.i. x hours)) for long pants/short sleeves and no gloves scenario (this is the corrected value presented to the HSRB), with an inhalation dose of 3.88 E-6 (mg/(ppm a.i. x hours)). For the COP scenario the mean dermal exposure was 0.000734 (mg/(ppm a.i. x hours)) for long pants/long sleeves and gloves scenario, with a mean inhalation dose of 5.73 E-5 (mg/(ppm a.i. x hours)) (Table 1; EPA Review). In addition to the inhalation exposure reported as a dose, Table 1 of EPA Review also provides inhalation concentrations as 8-hr TWAs. Dermal and inhalation results are corrected by laboratory recoveries where the laboratory recoveries were below 100%; no corrections for field recoveries were applicable as all field recoveries were slightly above 100%. As expected, 95^{th} percentiles are higher in all cases. Hand exposures represent most (close to 100%) of the dermal exposures. Dermal exposure for COP was negligible. Results are also calculated by EPA, where they are not normalized by ppm or by time, or only by ppm.

These unit exposures (UE) will be used to look at risks to restaurant workers based on chemical specific inputs and default units. Calculation of other clothing scenarios are possible given the use of inner and outer dosimeters in this study. Dermal exposure also includes face/neck wipes and forehead wipes, and previous studies on hand wash removal efficiencies were used to adjust any skin wash measurements. Environmental conditions are reported, with great variability in the temperature and especially in the humidity measures. Air flow rates were also measured for bucket and rag and sponge and sink scenarios. The COP had no air flow

measurements due to lack of vents or doors. Due to problems with control samples for ADBAC, only the inhalation DDAC results are reported. Outer dosimeters for ADBAC also showed great background interference and the LOQ for the matrix was increased from 3ug to 10ug per sample.

Statistical Review

Bucket scenario

For the bucket scenario, dermal unit exposures are based on the subject wearing a short sleeve shirt, long pants, and no gloves. The hand exposure represents nearly 100% of the exposure. Total dermal exposure is calculated by summing exposure across all body parts for each individual; for the bucket scenario, the inner upper arms, the inner front and inner rear torso, and the inner lower and inner upper legs were summed along with the hands, face and neck, and forearm.

The geometric mean, arithmetic mean and 95th percentile were based alternatively on empirical estimates and assuming a lognormal distribution and a simple random sample. The 95% confidence limits were obtained using 10,000 parametric bootstrap samples from the fitted lognormal distribution. The fold relative accuracy for each was determined as the 95th percentile of the maximum of the two ratios of the sample statistic to the parameter, after the parameter is replaced by its estimated value.

The geometric mean, arithmetic mean, and 95th percentile were shown to be accurate within 3-fold with 95% confidence for the bucket scenario. The inhalation unit exposure did not meet the 95th percentile objective for the bucket scenario for the empirical simple random sampling model. EPA states that no additional monitoring for the scenario is required.

Several statistical models were explored, including a linear regression model for the log exposure versus the log of the ppm x duration (predictor), a quadratic model regressing log exposure (outcome) against log ppm x duration and log ppm x duration squared, a log-log-logistic model, a three-parameter logistic model, and a gamma regression model. The linear model was selected based on the AIC criterion and ease of implementation.

The log-log-linear model was used with log exposure as the response and log of the ppm x duration as the predictor. If the log-log-linear model has a slope of 1, then the arithmetic mean exposure will be a multiple of the ppm x duration. The statistical test is used to test if the slope is different from 1 (indicating 'proportionality') or different from 0 (indicating complete independence between exposure and ppm x duration). If the slope is positive, but not zero and not 1, then the arithmetic mean exposure tends to increase with the ppm x duration but not proportionally. If the confidence interval for slope excludes both 1 and 0 but the slope is positive, then proportionality and independence are both rejected and exposure tends to increase with ppm x duration but not proportionally.

There is evidence consistent with log-log-linearity with a slope of 1 between dermal exposure and the treatment solution concentration and exposure duration. The confidence intervals for the slope exclude 0 and include 1 for the dermal and the inhalation 8-hour TWA. The 'unit exposure' approach for both the dermal and inhalation for the 8-hour TWA is a reasonable approximation.

A secondary study objective for EPA is to meet 80% power for detecting log-log-linearity with a slope of 1. This objective is met if the widths of the confidence intervals for the slopes are <= 1.4.

3-compartment sink scenario

For the sink scenario, dermal unit exposures are based on the subject wearing a short sleeve shirt, long pants, and no gloves. The hand exposure represents nearly 100% of the exposure. Total dermal exposure is calculated by summing exposure across all body parts for each individual; for the sink scenario, the inner upper arms, the inner front and inner rear torso, and the inner lower and inner upper legs, along with hands, face and neck, and forearm were summed.

Various alternative statistical models were explored for estimating the exposure from the ppm x duration. The major model is a linear regression model for log exposure against the log of the ppm x duration. The HSRB-recommended quadratic regression model regressing log exposure against log (ppm x duration) and log (ppm x duration) squared is also included. Quantile and regression plots are used to evaluate the linear regression model. Additional models considered are log-log-logistic, three-parameter logistic, and gamma regression models recommended by the HSRB. The best-fitting models for most exposure routes are either the linear or gamma models, based on the AIC statistical criterion. Since the gamma model's AIC scores were not significantly different from the linear models and the linear models are much easier to implement, the linear models were selected.

The benchmark objective for AEATF II scenarios is for the geometric mean (GM), the arithmetic mean (AM), and the 95th percentile (P95) to be accurate within 3-fold with 95% confidence. EPA has used diverse statistical techniques to evaluate this benchmark. To characterize the unit exposures, normal and lognormal quantile plots of dermal and inhalation UEs are provided. The lognormal distribution is a better fit than the normal distribution for the normalized exposure.

EPA calculated estimates of the GM, AM, and P95 based on two different calculation methods (i.e., empirical estimates; and assuming a lognormal distribution and a simple random sample). The 95% confidence limits for these estimates were obtained by generating 10,000 parametric bootstrap samples from the fitted lognormal distribution. Then, the fRA for each was determined as the 95th percentile of the maximum of the two ratios of the sample statistic to the parameter after the parameter is replaced by its estimated value. Appendix A shows fRA values calculated using a non-parametric bootstrap approach, with generally similar results.

For the sink scenario, the confidence intervals for the slope exclude 0 and include 1 for dermal. Thus, the assumption of independence was rejected, and the assumption of log-log-linearity with slope 1 was supported. Therefore, the "unit exposure" approach for the dermal is reasonable. However, the slope is negative for inhalation 8-hr TWA exposure, and the confidence intervals include 0 but not 1. Therefore, the assumption of independence was supported, and the assumption of log-log-linearity with slope 1 was rejected. The results for inhalation exposure are counterintuitive.

A secondary objective is to meet 80% power for detecting log-log-linearity with a slope of 1. This objective is met if the widths of the confidence intervals for the slopes are at most 1.4. This secondary objective was met for the sink scenario, and the statistical power is greater than 80.

Clean-Out-of-Place Scenario (COP)

For the COP scenario, dermal unit exposures are based on the subject wearing a long sleeve shirt, long pants, and gloves. Total dermal exposure is calculated by summing exposure across all body parts for each individual; for the COP scenario, the inner upper arms, the inner

lower arms, the inner front and inner rear torso, and the inner lower and inner upper legs were summed along with the hands and face and neck.

Various statistical models were explored for estimating the exposure from the ppm x duration. The major model is a linear regression model for log exposure against the log of the ppm x duration, though other models were considered. There was very little dermal exposure because of the long sleeves and gloves and no model was needed to describe exposure as a function of ppm x time. Dermal exposure was declared to be negligible. There was more inhalation of the compounds for this scenario because of the jets in the tanks causes the chemical to circulate creating a turbulence causing particles to go into the air. These particles were then available for inhalation. The log exposure regressed on log of ppm x time provided to be a useful model for predicting exposure.

Recommendations:

The HSRB has the following set of recommendations and minor points to consider:

- For the sink scenario, subjects were instructed to work for either 1- or 2-hour durations. In addition, the number of items cleaned over the work period was recorded. In some cases, subjects were instructed to rewash items to meet the desired ME duration. In the EPA scientific review, there is mention of work efficiency, based on the number of items cleaned, which was confusing the reader. We suggest that EPA further clarify this section to indicate that the sampling was time-based and this time-based unit was related to exposure units, not work efficiency.
- The sanitizer used contained ADBAC and DDAC, which are both low volatility antimicrobial products. It is unclear whether the UE derived from the IDS scenarios

within this study would be applicable to higher volatility antimicrobial agents. This is especially the case for inhalation exposures. EPA in any risk assessments may need to consider other means (i.e., studies or modeling) to predict exposures for higher volatility compounds, especially given the flat line regression for exposure related to time in this study.

- The current dermal exposure assessment techniques (i.e., wash and wipe sampling), while the best available, may not fully capture the chemicals absorbed through the skin during IDS activities. For example, exposure deposited to the skin may be subsequently wiped away when using a cleaning rag or dipping a hand within a bucket. In addition to highlighting this uncertainty, the HSRB urges EPA to continually evaluate new dermal exposure assessment techniques.
- It was noted that items needed to be cleaned prior to sanitizing and workers may use sanitizer rather than cleaners during the cleaning process. This puts workers at risk for additional exposures which may include mixtures of antimicrobial products. In using the data for risk assessment, we urge the EPA to consider risk posed when workers are exposed to mixtures.

- It was the consensus of the HSRB that power analyses should only be used for study design and sample size considerations. This is supported through the scientific literature. We recommend that future studies refrain from analyzing the secondary objective of computing post-hoc power.

- The statistical analyses should consider a model that has a possible concentration by time exposure interaction. The slopes of the regression lines may be different for each

concentration (three regression lines) or may be different for each time (two regression lines). The models could have different slopes for each concentration by time combination (six regression lines), but there are only three observations at each combination which provides little power for detecting differences.

- For clarity, within the EPA Scientific Report, we suggest labeling the first three rows with 'dermal' in Table 8.

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?

Response: The available information supports that study AEA12 was conducted in substantial compliance with the requirements of 40 CFR part 26, subpart Q.

Ethics review:

AEATF II IDS Study (AEA12) is a three-arm study (bucket, sink, and COP) to determine potential dermal and inhalation exposure to consumers and/or professional workers using an antimicrobial to sanitize hard surfaces in each setting. An excellent summary of the trial history, design and conduct has been provided in the study report executive summary, as well as in the EPA science and ethics reviews. This review focuses on the relevant ethics questions/topics.

Assessment of ethics per EPA regulations (40 CFR part 26, subpart Q):

Under Subpart Q:

§26.1703 (a): Prohibits reliance on scientifically invalid research

As stated above, the HSRB concluded that the research is scientifically valid and substantially compliant.

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

The protocol excluded participants less than 18 years of age and the actual participants in the study were 19 years of age or older. The protocol also excluded participants who were pregnant, or nursing based on self-report at the time of enrollment, or who tested positive on urine home pregnancy tests self-administered on each study day. The pregnancy testing was done in private and shared with a female study staff for confirmation and recording. No pregnant participants took part in the study. The research is thus in substantial compliance with §26.1703.

40 CFR §26.1705 requires that the EPA have "adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part." Within this range, only subparts K and L are directly applicable to the conduct of third-party research such as this.

Subpart K corresponds to subpart A of 45 CFR 46, the Common Rule. With respect to this subpart:

- Prior study initiation, the protocol and consent documents, as well as recruitment materials were reviewed and approved by the Advarra IRB, an IRB registered with the Office of Human Research Protections (OHRP). In addition, the protocol and consent documents were reviewed and recommendations made by the EPA and the HSRB. Those recommendations were adequately addressed in the IRB-approved protocol.

- The protocol included a number of provisions to minimize risks to study participants, including (but not limited to):
    - Exclusion of pregnant or lactating study participants or study participants under 18 years of age
    - Exclusion of certain medical conditions based on self-report
    - The use of Oasis 146 Multi-Quat Sanitizer as the test agent which, after dilution, did not require the use of gloves or personal protective equipment
    - Eye protection for all participants
    - Education of study participants about risks of heat intolerance and how to manage
    - Use of English and Spanish consents and bilingual study personnel to improve communications
- Four protocol amendments and, when appropriate, updated consents were reviewed and approved by the Advarra IRB. Amendments 1 and 3 increased compensations to study participants due to longer travel times to participate in the research than had previously been anticipated. Amendment 2 corrected a typographical error regarding a stopping rule. Amendment 4 changed the criteria for reporting deviations to Advarra IRB so that only deviations that affect participant safety or the consent process are required to be reported to the IRB. None of the amendments were due to unexpected or new safety concerns regarding study participants, and none of the amendments adversely impacted the safety of study participants.
- Study recruitment, consent, and conduct was largely carried out consistent with the protocol. Efforts were made to recruit a diverse group of study participants who were representative of the population engaged in the tasks being studied. Several participants

who preferred Spanish consents/communications were enrolled in and completed the study. Understanding of the consent materials was checked via a number of questions, and important elements of the consent form, including the right to withdraw from the study at any time, were repeated at several points during the study, including on the day of participation in the study.

- 17 protocol deviations were reported. Many of the deviations were administrative or related to sample collections and/or analysis. There were 3 deviations from the AEATF II SOPs. SOP deviation 2 involved the questions administered as part of the consent process to check study participant understanding. For one subject the form with the questions was retained, but not completed. For a second subject the form with the questions was completed, but not retained. In neither case did the deviation affect the health and welfare of the study participants. Overall, none of the deviations adversely affected the study's conduct or the study participants health and welfare.

Overall, the research was conducted in substantial compliance with Subpart K.

Subpart L prohibits third party research that involves intentional exposure to a study participant who is pregnant, nursing or lactating or who is a child. As noted in the discussion regarding §26.1703 above, all these groups were excluded from the study, so the study is compliant with Subpart L.

Taken together, compliance with subparts K and L means that this study is compliant with 40 CFR §26.1705.