UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

DATE: March 23, 2018

SUBJECT: Science Review of the AEATF II Determination of Removal Efficiency of 1,2-Benzisothiazol-3(2H)-one (BIT) from Hand Surfaces Using an Isopropyl Alcohol/Water Wipe and Wash Procedure (AEATF II Study Number: AEA08; MRID 50521601).

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This memorandum presents the EPA/OPP Antimicrobials Division (AD) science review of the human exposure hand wash removal efficiency study submitted by the Antimicrobial Exposure Assessment Task Force II (AEATF II). The removal efficiency data as represented in this review are acceptable and are recommended for use to correct/adjust the hand residue data collected in the AEATF II brush/roller paint study (MRID 50521701) and upcoming airless paint sprayer study.

EXECUTIVE SUMMARY

This document represents the USEPA, Office of Pesticides Program, Antimicrobials Division (AD) review of the Antimicrobial Exposure Assessment Task Force II (AEATF II) hand wash removal efficiency study. The AEATF II designed the study to develop a hand wash removal efficiency correction factor to use in their painting exposure studies (i.e., brush/roller and airless paint sprayer studies). The results of the hand wash removal efficiency study are reported herein. The protocol for this completed study was previously reviewed by the EPA and the Human Studies Review Board (HSRB) for ethical and scientific design. Both EPA and HSRB approved the protocol and provided recommendations for modifications (discussed within this memo). This memo contains the scientific review, recommended correction factors, and study limitations to be considered by users. The ethics review is contained in a separate memo. Both reviews are to be presented to the HSRB on April 25, 2018.

The study investigators monitored the removal of BIT (1,2-benzisothiazoline-3-one) treated paint that was intentionally placed onto the palms of test subject's hands. The hand wash procedure used in this study was the same procedure used by the AEATF II in their brush/roller study (and will be the same procedure in their upcoming airless paint sprayer study). All the test subjects were recruited from the general population. A total of 20 subjects and two concentrations of BIT-treated paint were used; 10 subjects had each hand exposed to 50 uL of paint containing 154 ppm of BIT (the two hands were combined as a single sample) and 10 subjects had each hand exposed to 50 uL of paint containing 547 ppm of BIT (combined as a single sample). The paint was allowed to dry on the subject's hands for 45 minutes prior to the hand wash procedure. The hand wash procedure included both a wash and a wipe and combined the left and right hand together for a single sample. The reader is referred to Section 3.0 for a discussion on the data limitations.

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. Therefore, the results will be used at the low and high concentrations (not combined) to correct the hand exposure data for the completed brush/roller and upcoming airless paint sprayer studies. However, it is important to note that the low and high level fortification levels used in this study are relative. The use of these removal efficiency data based on fortification levels can be adjusted as need be based on study specific paint concentrations. The results of the low level fortification (154 ppm BIT) indicate a 73.3% removal efficiency and the results of the high level fortification (547 ppm BIT) indicate a 60.3% removal efficiency.

In the brush/roller study, three concentrations of paint were used, a low concentration of between 141 and 147 ppm BIT, a mid-level concentration of between 368 and 382 ppm BIT, and a high level concentration of between 595 and 649 ppm BIT. It is reasonable to match the low concentration in the brush roller study with the very similar low concentration in the hand wash removal efficiency study and apply a correction factor of 73.3%. It is reasonable to match the high concentration in the brush roller study with the very similar high concentration in the hand wash removal efficiency study and apply a correction factor of 60.3%. Although the hand wash removal efficiency study did not measure the removal efficiency at the mid-level concentrations of BIT, a reasonable approach is to assume that the average removal efficiency is approximately linear in the BIT concentration, so that

the estimated arithmetic mean correction factor of 66.8% can be applied to the mid-level concentration data in the brush and roller study.

EPA intends to use this AEATF II hand wash removal efficiency study to correct the hand wash residue data collected using the same hand wash procedure in the AEATF II brush/roller exposure study as well as the upcoming airless paint sprayer exposure study.

1.0 Background

The AEATF II is developing a database representing inhalation and dermal exposure during many antimicrobial handler scenarios. Two of the scenarios measure exposure to subjects while painting (brush/roller and airless paint spraying). The dermal monitoring of the subjects during these two painting studies include hand wash procedures to measure hand exposures. The AEATF II has conducted this hand wash removal efficiency study as part of the method validation of the hand wash sampling procedure. To determine the hand wash removal efficiency, the AEATF II recruited test subjects from the general population, exposed the palms of their hands to paint fortified with BIT, allowed the paint to dry for 45 minutes, and then performed a hand wash procedure to determine the efficiency in which the BIT-treated paint is removed. The results of this study are being used in the AEATF II's brush/roller study (MRID 50521701) to adjust the hand exposure for incomplete removal of the BIT-treated paint from the hand wash procedure. Additionally, they also plan to use the results in the upcoming airless paint sprayer study. Prior to conducting intentional exposure studies in humans, the protocols are reviewed by the Human Studies Review Board (HSRB). The HSRB reviewed this hand wash removal efficiency study protocol in April 2014.

1.1 Hand Wash Removal Efficiency Defined

The hand wash removal efficiency in this study is defined as… "*The removal efficiency of BIT from the skin using the isopropyl alcohol/water wash and wipe procedure was determined by calculating the amount of BIT removed from the hands of each subject. … The removal efficiency was calculated using the following equation.*

$$\textit{Removal Efficiency (\%)} = \frac{\textit{Amount of BIT Removed from Hands (µg)}}{\textit{Amount of BIT Applied to Hands (µg)}} \times 100$$

The removal efficiency of the isopropyl alcohol/water wash and wipe procedure was calculated by averaging the removal efficiency determined for each individual subject." (V1:32)

1.2 Study Objective

The AEATF II's stated in their study protocol that their objective is to "*The primary objective of this study is to determine the removal efficiency of BIT in latex paint … from human hands.*" (AEATF 2014). 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.

1.3 Protocol Modifications, Amendments, and Deviations

1.3.1 Protocol Modifications Based on EPA and HSRB Reviews

EPA and the HSRB provided science-based changes to the hand wash removal efficiency study protocol during the review (EPA 2014 and HSRB 2014). The review comments and AEATF II responses are summarized in Table 1.

Table 1. EPA/HSRB Review and AEATF II Responses.

Issue Raised (Agency)	Proposed Response	Options/Comments
Researchers should consider video recording the procedure to use for training purposes for future studies. **(EPA)**	All the studies done for AEATF II include video recording. This study will include video.	Efforts will be made to get sufficient footage for training.
Researchers should consider whether glass capillary tubes might break or cause injury when used to spread test material. **(HSRB)**	The protocol will be modified to use a different tool to spread the test material which has less potential to break (e.g. solid glass rod).	Although these tubes have been used successfully on multiple previous rat and human studies there is some risk of breakage. An alternative spreader will be used.
The application of 500 µL of paint to the palm may be excessive. Researchers should consider using less paint. **(HSRB)**	Guidance is needed from EPA as paint volume was increased from 100 µL to 500 µL at EPA request from earlier review.	
The IPA application of 100 µL to the palm may be excessive. **(HSRB)**	The IPA group is to be eliminated from the study in order to increase the sample size of the paint group.	Multiple prior studies have applied 100 µL of IPA to the palm or similar area of the forearm without dripping.
The palm of the hand is known to have lower dermal permeability than other areas. Researchers should consider applying paint to the entire hand. **(HSRB)**	No change to protocol is planned.	The most common site of paint exposure during the study will likely be to the palm from holding painting equipment. Also, applying paint to the entire hand will make it very difficult for subjects to avoid touching treated areas to surfaces or themselves. Treating the palm will allow subjects to sit with their hands facing up on a padded surface during the study period.

The amount of active ingredient may be excessive along with the amount of paint. Researchers should consider reducing amount of active ingredient. **(HSRB)**	No change to protocol is planned.	The concentration of active ingredient in the paint is the same as what will be used in the brush and roller study. This will provide the most direct comparison when correcting recoveries in that study.
Researchers should consider whether it is practical for subjects to avoid use of their hands for 45 minutes. Researchers should consider a small pilot test with non-toxic household item to test. **(HSRB)**	No change to protocol is planned.	Multiple human studies (pre-HSRB) have been conducted with 30 minute exposure times without incident. Subjects will be allowed to place hands face up on a padded surface and will be provided with TV during the 45 minute period. Any subject who has difficulty can ask to end their participation early.
The use of individual hands in this removal efficiency study may result in a removal efficiency that is different from the two hand procedure planned for the brush and roller study. Researchers should consider using the same technique for both. Researchers should also consider whether this change will require a change in number of subjects per group to provide adequate statistics. **(HSRB)**	The protocol will be modified to consider two hands as one sample and use the same wash procedure as the brush and roller study. The IPA dose groups will be eliminated in order to keep the total number of subjects the same and still have the same replicate number of samples per group.	We felt that the one hand procedure would be a good approximation of the two hand procedure, and any bias would be conservative (less removed with one hand wash procedure). The one hand procedure was chosen to increase sample size with the minimum number of subjects. After reflection we agree with modifying the protocol to be identical with brush and roller.
Researchers should consider whether removal from the dominant hand might be different than the non-dominant hand. **(HSRB)**	The protocol will be modified as stated above.	Both dominant and non-dominant hands will be tested together from each subject.

1.3.2 Protocol Amendments

The study report (page 37) lists 2 protocol amendments. The amendments included (1) reducing the volume of the paint to be applied to the subject's two hands from 500 uL to 100 uL (i.e., 50 uL per hand) as suggested by the HSRB; changed the inclusion criteria of residency for recruitment to surrounding areas of Fresno County; and clarified the numbering of subjects, and (2) corrected the analytical method number and title.

1.3.3 Protocol, Method, and SOP Deviations

Three protocol and four SOP deviations were noted in the study (study report page 37). The protocol deviations included not using the *California Advocate* during the advertisement for study recruitment; a different wash solvent used to prepare BIT solution for dosing the paint; and a single instead of duplicate field controls were used. The four SOP deviations included the temperature of the refrigerator storing the reference calibration standard dropped below the set temperature; minimum and maximum temperatures were not recorded for 3 weeks where the internal standard and reference substance were being stored; "*temperature of the freezer that stored the internal standard raised above the allowed temperature of ≤ -10 °C reaching -7 °C and then -4 °C a week later"*; and *"An audit report was not addressed by the Study Director in a timely manner*." EPA accepts the study author's conclusion that these deviations did not adversely affect the outcome of the study.

1.4 Material & Methods

The following is a summary of the key field aspects of the study.

- ***Study Location:*** The hand wash removal efficiency study was conducted at the Golden Pacific Laboratories (GPL), LLC in Fresno, CA. The monitoring took place on April 7 and 9, 2015.
- ***Substance Tested:*** The test substance monitored was 1,2-Benzisothiazolin-3-one (BIT) as the active ingredient; CAS number 2634-33-5.
- ***Test System:*** The test subjects had a total of 100 uL of BIT-treated paint applied to both the right and left palmer surface areas (i.e., 50 uL per hand). The washes from the two hands were combined as a single sample. The residence time for the paint on the subject's hands was 45 minutes. Two paint concentrations were used for two separate groups of subjects. One set of subjects received paint with a concentration of 154 ppm of BIT and the other 547 ppm of BIT. The test subjects and setup at the testing facility was described in the study report as follows:
    - *"The subject numbers were randomized using a research randomizer program accessible at the following internet website: http://www.randomizer.org. During the enrollment period, a total of 40 subjects were enrolled to participate in the study. The first 28 numbers in the generated randomized list determined the initial group of participating subjects."*
    - *"On each day of the study, the conference room at GPL was used as the test site. The conference room at GPL consists of a table with six chairs around it… [T]he conference table was set for five subjects at a time… At each seat, an X-large towel was folded and used to create a comfortable surface for the subjects to rest their arms on during the testing period. … a narrow table was set up for conducting the removal of the paint from the subject's hands at the appropriate time. The narrow table was covered with bench paper and on top of the bench paper were absorbent pads, which were changed between each subject. The bench paper was changed between each group session on each day of the study. A large deep sided metal mixing bowl for collecting the wash, a package of dressing*

sponges, and a 1-liter glass jar pre-labeled for the subject were set up on the table."

- *"On the first day, ten subjects (five in the morning and five in the afternoon) had a 100 µL aliquot of paint containing 154 ppm of BIT applied to their hands. On the second day, ten subjects (five in the morning and five in the afternoon) had a 100 µL of paint containing 547 ppm of BIT applied to their hands."*
- *"The paint was applied to the first subject's hand using a positive displacement micropipette containing approximately 100 µL of paint containing BIT. The research associate applied the paint as evenly as possible between the two hands and the Study Director/PI followed behind spreading the paint across the palms using a glass stir rod with rounded annealed ends. The goal was to distribute the paint consistently over the palmar surface. Once the paint was distributed, the Study Director/PI started a timer unique to each subject and the time the paint was applied was recorded. Ten minutes (± 1 minute) later, paint was applied to the palms of the next subject in the same manner. The glass rods were unique to each subject and were retained as a sample in properly labeled glass test tubes for analysis. This process was continued until all five subjects had paint on their palms. During the drying period, subjects sat with their palms facing up and hands open."* Note: The BIT residues were extracted from the glass rods using methanol/water (10:90, v/v) and the resulting residues were used to subtract from the amount of BIT pipetted onto the subject's hands to account for BIT-treated paint not applied to the palms.
- After 45 minutes the hand wash procedure commenced as follows: "*Over the bowl, a small amount (~50 mL) of the premeasured 500 mL of isopropyl alcohol/water (50:50, v/v) sample was poured over one of the gauze wipes (BAND-AID® Johnson & Johnson Large Mirasorb® Gauze Sponges, 4 in. x 4 in.) and the subject's hands to moisten the dry paint. With the wet gauze wipe, the Study Director scrubbed one hand, loosening and removing the paint. The second gauze wipe was wet with some fresh isopropyl alcohol/water (50:50, v/v) and used to scrub the second hand, loosening and removing the paint. The two gauze wipes were added to the collection bowl. The Study Director then slowly poured more of the isopropyl alcohol/water (50:50, v/v) over the subject's hands while they rubbed and washed their hands together like one would when washing under a faucet. The subject was instructed to rub and scrub their hands together. The remainder of the premeasured 500 mL of isopropyl alcohol/water (50:50, v/v) was slowly poured over the subject's hands while the Study Director directed them to rub and rinse their hands without touching the grey water in the bowl for a final clean rinse. Once the entire 500 mL of isopropyl alcohol/water (50:50, v/v) was poured over the hands, the subjects were instructed to let the solution drip off, then gently shake and flick their fingers slightly in order to collect as much as possible.*" The duration of the hand wash procedure itself was approximately 3 to 5 minutes.
- Figure 1 illustrates photos of the 100 uL pipetted onto the subject's hands (i.e., 50 uL/hand); the paint spread on the palm; subjects seated around the table, palms up; the wash procedure; and post-wash with paint removed.

Figure 1. Photos of the hand fortification and wash procedure.

- ***Sample Size:*** The study consisted of 20 subjects (12 males and 8 females) that participated in the sampling. Each subject had their right and left hand (palm) fortified with the BIT-treated paint and the two hand samples combined for one sampling result. Therefore, the number of samples is 20 (n=20).

- ***Duration:*** The residence time for the paint on the subject's hands is 45 minutes. The duration of the brush/roller exposure study monitoring events averaged 113 minutes (ranged from 48 to 173 minutes).

- ***Amount of BIT:*** The amount of BIT fortified on the subject's hands (L+R) at the lower BIT concentration was ~22 ug/both hands and ~76 ug/both hands at the higher concentration of BIT. In the brush/roller study, the BIT residues collected on the test subject's hands (uncorrected residues) ranged from 37.7 to 2424 ug/both hands (averaged 461 ug/both hands). The hand residues in the brush/roller study, when corrected for the hand wash removal efficiency results in this study, range from 64 to 4045 ug/both hands (averaged 770 ug/both hands).

- ***Environmental Conditions*:** Environmental conditions (humidity and indoor temperatures) are reported for each of the two days of monitoring, morning and afternoon. on page 46 of the AEATF II study report. Indoor temperatures ranged from 69.6 to 71.8 F. The humidity indoors ranged from 36.2 to 48.3%.

2.0 Results

2.1 QA/QC

Controls. The non-fortified laboratory and field control samples (blanks) were all non-detect. The limit of quantitation (LOQ) for the hand wash was 1 ng/mL (hand wash samples were 500 mL per sample).

Method Validation. The results of the pre-study method validation (MRID 50549401) for the sponge wipes averaged 99.9±3.23% and for the hand wash solution 96.8±3.04%. The dressing sponges were fortified at 100 ng/sample, 10 ug/sample, and 100 ug/sample. The hand wash solutions were fortified at 1 ng/mL, 100 ng/mL, and 1 ug/mL. Seven samples per fortification were prepared and analyzed.

Laboratory Recoveries. The concurrent laboratory recovery values for the hand wash solution with two gauze sponges averaged 94.2±5.47 % (n=4). Samples of the wash solution with the two gauze sponges were fortified at the LOQ and 160x the LOQ. The hand wash removal efficiency samples were not corrected for concurrent laboratory results.

Field Recoveries. The field recovery values for the hand wash solution with two gauze sponges averaged 102±4.90 % (n=8). Samples were fortified at 44x the LOQ and 150x the LOQ. The hand wash removal efficiency samples were not corrected for field recovery results.

2.2 Calculating Hand Wash Removal Efficiency

The hand wash removal efficiency was determined using the following equations:

Removal Efficiency (%) =
(Amount of BIT Removed from Hands (μg) ÷ Amount of BIT on Hands (μg)) x 100

Where:

Amount of BIT on Hands (μg) =
Amount of BIT Applied (μg) – BIT Left on Glass Rod (μg)

Where:

Amount of BIT Applied (μg) =
Concentration of BIT in Paint (μg/g) x Mass of Paint Applied (g)

2.3 Hand Wash Removal Efficiency Results

A summary of the individual and mean hand wash removal efficiency results is presented in Table 2. The results of this hand wash removal efficiency study indicate an average 73.3 and 60.3 percent removal efficiency at BIT concentrations of 154 and 547 ppm, respectively. Appendix A, includes various analyses of the removal efficiency data which are summarized

here. The distributions of the hand wash removal percentages for each concentration were examined. The percentages, their reciprocals, and the logarithms of the percentages were each consistent with a normal distribution (based on the Kolmogorov-Smirnov test, other normality tests, and on quantile-quantile plots). The fit is better at the higher concentration. Based on a normal distribution, the 95% confidence intervals for the arithmetic means at the Low and High concentrations were 73.3 (64.9 – 81.6) % and 60.3 (53.2 – 67.4) %, respectively. The observed precision is within the range of the estimated precision from the protocol review that was based on previous studies. T tests showed that the means of the Low and High concentration removal efficiencies were statistically significantly different at the 5% level. This is also true for the mean reciprocal and the mean logarithm. Thus, the removal efficiencies for the two concentrations should not be combined. Instead, the low and high BIT concentration removal efficiencies should be applied to the paint data at the same, or nearly the same BIT concentration. As discussed above, for mid-level BIT concentrations a simple and reasonable approach is to apply the average of removal efficiencies, making the approximation that the removal efficiency is linear in the BIT concentration.

Table 2. Summary of Hand Wash Removal Efficiencies.

BIT Level	*Monitoring Event (ME)*	*BIT Applied (µg)*	*BIT Left on Glass Rod (µg)*	*BIT on Hands (µg)*	*Removal Efficiency (%)*
Low	1	21.791	0.033	21.759	66.2
Low	2	21.806	0.120	21.686	58.1
Low	3	21.606	0.317	21.289	90.2
Low	4	22.361	0.036	22.325	86.5
Low	5	22.238	0.041	22.198	80.2
Low	6	21.683	0.071	21.612	65.7
Low	7	22.777	0.115	22.662	66.2
Low	8	23.008	0.044	22.964	71.4
Low	9	21.760	0.119	21.641	61.5
Low	10	22.684	0.108	22.576	86.8
High	11	76.854	0.370	76.484	54.1
High	12	77.619	0.282	77.337	62.8
High	13	76.361	0.055	76.306	43.6
High	14	75.595	0.236	75.359	64.1
High	15	74.720	0.305	74.415	59.8
High	16	76.252	0.077	76.175	58.3
High	17	77.510	0.174	77.336	55.2
High	18	76.033	0.189	75.844	72.6
High	19	77.182	0.996	76.186	53.8
High	20	76.197	0.254	75.943	78.6
Low	Empirical Mean	22.171	0.100	22.071	73.3
Low	Empirical SD	0.514	0.085	0.551	11.7
Low	Lognormal SRS Mean	22.172	0.101	22.072	73.4
Low	Lognormal SRS SD	0.512	0.084	0.550	11.6
High	Empirical Mean	76.432	0.294	76.139	60.3
High	Empirical SD	0.894	0.265	0.864	10.0
High	Lognormal SRS Mean	76.433	0.304	76.139	60.4
High	Lognormal SRS SD	0.896	0.292	0.866	10.1
All	Empirical Mean	49.302	0.197	49.105	66.8
All	Empirical SD	27.844	0.216	27.745	12.5
All	Lognormal SRS Mean	50.360	0.199	50.162	66.8
All	Lognormal SRS SD	35.505	0.229	35.392	12.6

Let X_i be the i^{th} AaiH or unit exposure value and let $Y_i = \ln(X_i)$.

Empirical Mean = $\overline{X} = \sum_{i=1}^{18} X_i / 18$

Empirical SD = $S_X = \sqrt{\sum_{i=1}^{18} \left(X_i - \overline{X}\right)^2 / 17}$. Suppose X is lognormally distributed, so that Y = ln(X) is normally distributed with a population mean μ and a population variance σ^2.

Lognormal Simple Random Sample (SRS) Mean = Estimated population mean of X = Estimate of $\exp(\mu + \frac{1}{2}\sigma^2) = \exp(\overline{Y} + \frac{1}{2} S_Y^2)$

where $\overline{Y} = \sum_{i=1}^{18} Y_i / 18$ and $S_Y = \sqrt{\sum_{i=1}^{18} \left(Y_i - \overline{Y}\right)^2 / 17}$.

Lognormal Simple Random Sample (SRS) SD = Estimated population standard deviation of X = Estimate of

$\exp(\mu + \frac{1}{2}\sigma^2) \sqrt{\exp(\sigma^2) - 1} = \exp(\overline{Y} + \frac{1}{2} S_Y^2) \sqrt{\exp(S_Y^2) - 1}$.

3.0 Discussion and Limitations

The need for account for the removal efficiency from a hand wash method has been discussed previously (SAP 2007). The study protocol for this hand wash removal efficiency study was previously reviewed by the EPA and HSRB (EPA 2014 and HSRB 2014). Suggestions and recommendations were made during the review, not all of which were concise nor a consensus. Many of the suggestions were easily incorporated by the AEATF II and are summarized in Table 1 above. The scientific design of this study, with caveats noted, is sufficient for the results to be used to correct the hand exposure for method efficiency in the completed brush/roller study and upcoming airless paint sprayer study. Additional research to better characterize the uncertainties noted in the protocol review, mainly fortifying the palm versus the entire hand, does not outweigh the timely conduct of the study, and was satisfied by having the researchers video tape the hand wash procedures in the brush/roller study to visually assure removal of paint from the entire hand as was done in this hand wash removal efficiency study (see discussion below and photos in Figure 2). The following items are provided to potential users of these data to characterize the results of this sampling effort:

- The HSRB was concerned that the glass capillary tubes were likely to break while applying the paint to the palms of the subjects. This comment was made under both the science and ethics portion of their review. The researchers switched the glass capillary tubes for glass stir rods to apply the paint to the subject's hands (based on personal communication, none of the stir rods broke during the study).
- The HSRB suggested using an estimate of 24 or 25 cm^2 as the palmar surface area (single hand) when the paint is applied up to 2 cm from edge of palm. EPA deferred to the HSRB and the AEATF II used the 25 cm^2 estimate of the area of palm to determine the lower amount of paint to be used to fortify the subject's hands. Note: This area proposed by the HSRB during the protocol review was only an estimate to determine the volume of paint to use for fortification of the palms of the subjects; there were no proposals to make hand measurements and no measurements were taken.
- During the protocol review, the HSRB was concerned that using 500 uL of paint to fortify the palms would yield a paint thickness of ~2 mm which was excessive (and believed would result in an over-estimate of the recovery at lower loading levels anticipated in the brush/roller study). The HSRB further stated that a 0.04 mm thick layer (4 uL/cm^2) would result if the proposed 500 uL was reduced to 100 uL and this thickness would be less of concern. The AEATF II reduced the volume of paint applied to 50 uL per hand, yielding 0.02 mm thickness (2 uL/cm^2). The appropriateness of the paint loading is dependent upon the results of the completed brush/roller study (Catch-22). However, the AEATF has responded to the suggestions provided by the HSRB and used a thinner paint thickness to fortify paint on the subject's hands. Note: The actual loading on the hands from the now completed brush/roller study cannot be accurately determined from a hand wash as paint exposure to the hands is not uniformly distributed on the hands.
- The same wash and wipe procedure using the same isopropyl alcohol/water concentration and volume were used in both the removal efficiency study and brush/roller study.
- The HSRB stated that the palm has the lowest dermal permeability of any body part and was smooth and easy to clean which will bias recovery results upwards. The HSRB recommended to dose all or most of the hand. The AEATF II only dosed the palmar surface area of the hand. To overcome this short coming, EPA recommended that the AEATF II video tape the hand wash procedure in the brush/roller study to ensure the

visual removal of the paint. The photos in Figure 2, pulled from the video tape, illustrate that the paint was removed during the scrubbing portion of the hand wash procedure (the post scrubbing in this photo was followed up by the final rinse). Similar to the rat dermal absorption study (MRID 46327901), BIT remained on the skin of the palms and not easily washed off as evident in the recoveries of 60.3% and 70.3% in this study.

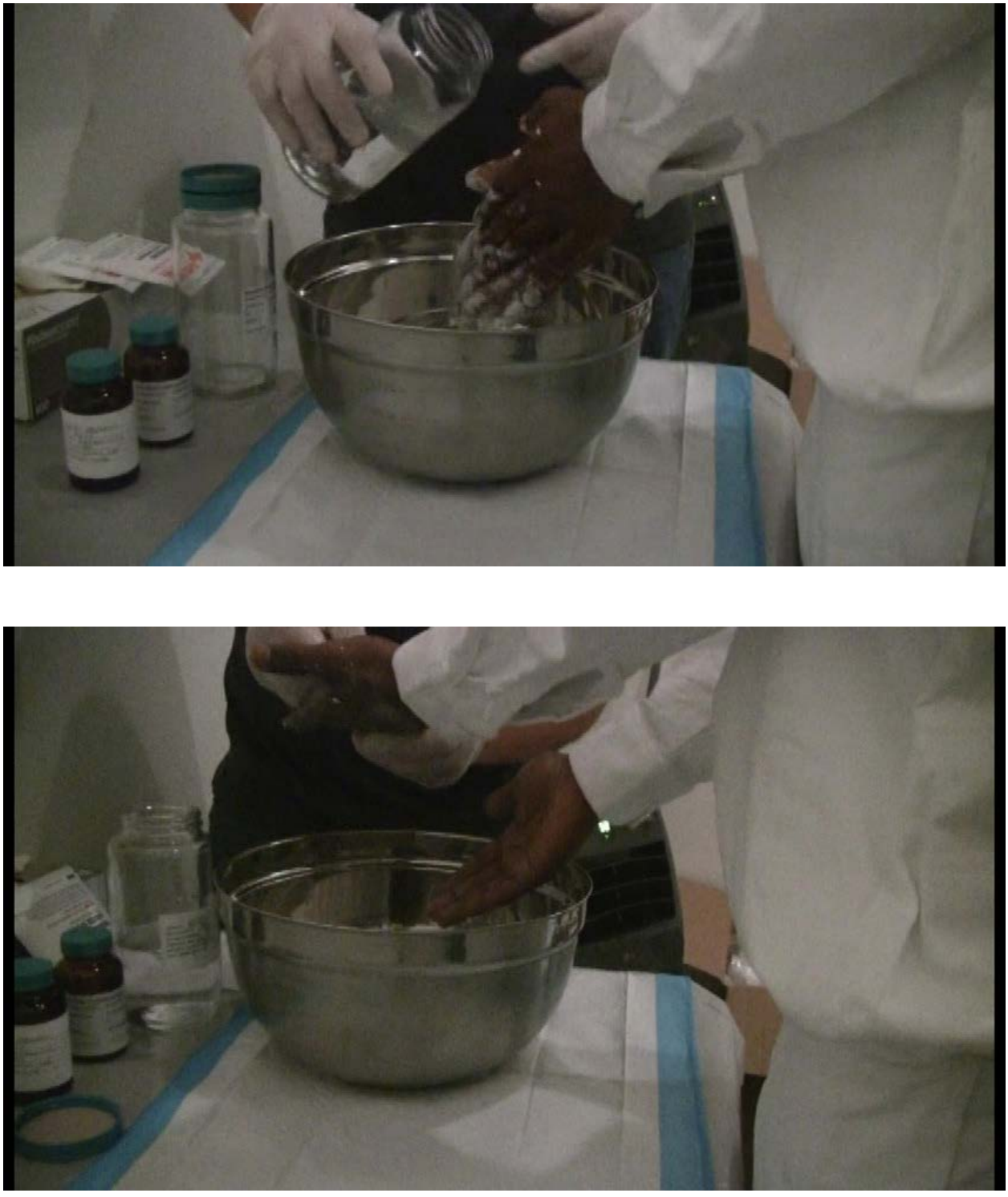

Figure 2. Start and Post-Scrubbing Portion of Hand Wash Procedure During Brush/Roller Study.

4.0 Conclusions

EPA has reviewed the AEATF II hand wash removal efficiency study and concludes that the AEATF II made the appropriate changes to the protocol proposed by the EPA and HSRB and has successfully executed the study. The protocol deviations that occurred and were reported have not adversely impacted the reliability of these data. The EPA recommends that the hand wash removal efficiency correction factors generated in this study be used to correct the hand exposure residues from the AEATF II painting studies (i.e., brush/roller and airless paint sprayer). The following is a summary of our conclusions:

- T tests showed that the means of the removal efficiencies were statistically significantly different at the 5% level. This is also true for the mean reciprocal and the mean logarithm. Thus, the removal efficiencies for the two concentrations should not be combined when being applied to similar paint concentrations. Therefore, the results from this study correspond to the low and high concentrations (not combined) to correct the hand exposure data collected in the brush/roller and upcoming airless paint sprayer studies.
- Low level fortifications (154 ppm BIT) are represented by a 73.3% removal efficiency.
- High level fortifications (547 ppm BIT) are represented by a 60.3% removal efficiency.
- Mid level fortifications (350 ppm BIT) can be represented by a 66.8% removal efficiency, assuming a linear relationship.
- The "low" and "high" level fortification levels used in this study are relative. The use of these fortification-level based removal efficiency correction factors may need to be adjusted, on a case-by-case basis, for study-specific paint concentrations.

5.0 References

ACC. 2011. American Chemistry Council, Antimicrobial Exposure Assessment Task Force II (AEATF II) Governing Document for a Multi-year Antimicrobial Chemical Exposure Monitoring Program. Interim Draft Document. Version 3. July 8, 2011.

HSRB Report. 2014. EPA-HSRB-14-01, Subject: April 8-9, 2014 EPA Human Studies Review Board Meeting Report, from Rebecca T. Parkin, PhD, MPH, Chair EPA Human Studies Review Board to Robert Kavlock, Ph.D., Interim EPA Science Advisor, Office of the Science Advisor, 1200 Pennsylvania Avenue, NW, Washington, DC 20460, dated June 25, 2014.

MRID 50549401. Validation of Method GPL-MTH-081 and Freezer Storage Stability: Analytical Method for the Determination of Benzisothiazolinone (BIT) in Paint, Dressing Sponges, Hand Washes, Cotton Inner and Outer Dosimeters, Painter's Hats, Air Sampling Tubes and Fiberglass Filters. GPL Study No.: 130478. Study Completion: March 1, 2017.

SAP. 2007. Memorandum: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held January 9 – 12, 2007 on the Review of Worker Exposure Assessment Methods. U.S. Environmental Protection Agency.

USEPA. 2014. Science and Ethics Review of AEATF II Paint Hand Wash Removal Efficiency Protocol. Memorandum from Timothy Leighton, Kelly Sherman (USEPA) and Jonathan Cohen, PhD. (ICF International) to Steven Weiss (USEPA), dated March 18, 2014.

Appendix A

Statistical Review of the AEATF II Paint Hand Wash Removal Efficiency Study

(To be included as a separate electronic file)