UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

December 15, 2015

MEMORANDUM

SUBJECT: Science Review of Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic.

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REF: Talbert, C. (2015) Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic – Unpublished document prepared by S. C. Johnson & Son, Inc., 1525 Howe Street Racine, WI 53403. October 21, 2015. 154 p. (MRID 49761601)(D430082)

ACTION REQUESTED

Conduct a science review of a completed field study testing the efficacy against mosquitoes of an insect repellent formulated as a pressurized aerosol product containing diethyl toluamide (DEET). Determine the adequacy of the methods employed and the scientific validity of the reported data. These data are required to establish the median complete protection time (CPT) against mosquitoes for use in the EPA Repellency Awareness Graphic on the label of EPA Reg.

No. 4822-167, Mark-8 OFF! Insect Repellent Formula V (25% DEET). The protocol used to conduct this study was previously reviewed and accepted by EPA and the HSRB with comment on April 23, 2015. The protocol used in this study was amended to address EPA and HSRB recommendations.

CONCLUSIONS

The EPA assessed the scientific aspects of the research in relation to the recommendations of the EPA §810.3700 product performance testing guideline and the Human Studies Review Board. The study (MRID 49761601) was conducted in accordance with Good Laboratory Practices as described in 40 CFR §160 (with one minor exception), and provides scientific data that are acceptable. The Human Studies Review Board will be asked to comment on this study.

SCIENCE REVIEW

Study objective: The objective of this study is to establish, for the EPA Repellency Awareness Graphic, the median complete protection time of MARK-8 in the field against populations of wild mosquitoes using human volunteer subjects. This is a guideline study designed to fulfill the requirements in OPPTS Series §810.3700 product performance guideline, Insect Repellents to be Applied to Human Skin. This study was conducted in accordance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 (October 1989). (p. 3 of 154).

Identification of the test system: In this study, landings of wild mosquitoes on replicate human subjects were used to evaluate the repellency of an insect repellent product (25% DEET) applied to human skin. Mosquitoes were used because they are one of the insect pest groups repelled by the product and one of the insect groups represented on the EPA Repellency Awareness Graphic. The registrant conducted tests in two field locations, one in Florida and one in Wisconsin. The following mosquito genera were collected and identified, *Coquillettidia*, *Psorophora*, *Aedes*, *Mansonia*, and *Wyeomyia* (Tables 1, 2; §10.1, p. 11 of 154).

Table 1. Wisconsin Site Mosquito Species collected - August 10, 2015 (§10.1, p. 11 of 154)

Species	Number Collected	% of Total
Coquillettidia perturbans	4	3
Psorophora ferox	2	2
Aedes vexans	3	3
Aedes trivittatus	109	92
Total	118	100

Table 2. Florida Site Mosquito Species collected - August 25, 2015 (§10.1, p. 11 of 154)

Species	Number Collected	% of Total

Aedes atlanticus	51	28.7%
Aedes infirmatus	107	60.1%
Aedes taeniorhynchus	3	1.7%
Mansonia dyari	1	0.6%
Mansonia titillans	12	6.7%
Psorophora ferox	2	1.1%
Wyeomyia spp.	2	1.1%
Total	178	100.0%

Experimental design: This field study was conducted with human subjects at two geographically and ecologically distinct field sites, one in Kenosha County, Wisconsin on August 10, 2015, and one in Collier County, Florida on August 25, 2015. At each site, the experimental treatment groups consisted of 10 different treated subjects and the untreated control group consisted of two untreated subjects. Subjects at each site were selected from a pool of trained participants (Table 3; §12.4, pp. 15-16 of 154). At each site, testing was conducted during the course of a single day (§13.1, p. 20 of 154).

Table 3. Summary of Test Subject Participation in GLP 873E1 (Revision of Table 7 in the study §12.4, pp. 15-16 of 154).

GLP 873E1 - 4822-167	**Wisconsin**	**Florida**	**Total**
Number of Subjects Enrolled	22	35	57
Number of No-Shows to training and/or testing	No-shows for training: 2 No-shows for testing: 0	No-shows for training: 18 No-shows for testing: 0	20
Number Assigned as Test Subjects	12	12	24
Number Assigned as Alternates or Extras	4 alternates 4 extras	4 alternates 0 extras	12 *(Note to HSRB: In the study, the table cited 11.)
Number of Test Subjects Withdrawn Voluntarily	1 on test day (post-treatment)	1 on training day	2 **(Note to HSRB: In the study, the table cited 1*. However, it should be 2, 1 on training day and 1 post-treatment on test day.)
Number of Test Subjects Withdrawn Involuntarily	0	0	0
Number of Test Subjects Completed Research	11	12	23 **(Note to HSRB: In the study, the table cited 24**.)
	In Wisconsin: 12 test subjects (10 treated and 2 controls) and 4 alternates were randomly selected as described in the report	In Florida: Only 16 trained participants were available on this test day. 12 test subjects (10 treated and 2 controls) and 4 alternates were selected as described	

	out of the pool of 20 trained participants.	in the report out of the pool of 16 trained participants.	
*typo			
**post-treatment withdrawal was previously considered as 'completing research' instead of 'withdrawn'			

The experiment was partially randomized, the test subjects were randomly selected from a pool of potential subjects that met the inclusion criteria found in §12.3, pp. 14-15 of 154. Using an allocation table, treatments were assigned to the limbs of each subject. If the subject was assigned an odd number, the left limb was treated and if assigned an even number the right arm was treated. Treated subjects were blinded to the identity of the test substance (§13.3, p. 20 of 154). Two untreated control subjects were used at each site to determine mosquito landing rate and aspirate mosquitoes. The control subjects chose which arm to expose to mosquitoes so the dominant hand could be used to aspirate mosquitoes (§13.7, p. 23 of 154).

At the testing sites, study staff prepared subjects for testing in a tent enclosure to protect them from mosquitoes during preparation. Subjects washed their limbs before dressing in bug suit pants and jackets, and gloves to protect untreated body parts from mosquito bites. To treat the forearm, the selected forearm of the bug suit was rolled up and the arm treated as discussed in the Test Substance Application Rate and Treatment section below (§13.2-13.5, pp. 20-21 of 154).

The unit of measure for determining repellent effects (Complete Protection Time) in this experiment was mosquito landings, similar to previous skin applied repellent evaluations where the “Landings” measure is used and efficacy is measured as CPT. To assess CPT, subjects were grouped into pairs. Pairs were separated from each other by at least twenty feet. Starting two hours after application of the repellent product, subjects observed their limbs for five minutes for landing mosquitoes. Mosquitoes that landed were aspirated by the subjects or their partner, and if necessary, headlamps were used at night so subjects could see to aspirate mosquitoes. After the five-minute exposure period, subjects reported the number of mosquito landings to study staff who recorded the number for each subject, and aspirated mosquitoes were labeled and kept to be identified. The five-minute exposures were conducted every thirty minutes until repellent failure occurred. Repellent failure (i.e., first confirmed landing) is the exposure period in which two or more mosquito landings occurred, or when one land occurred in an exposure period and another landing occurred in the subsequent exposure period. A rain event occurred at the Florida site causing exposure periods 6 and 7 to be skipped. The protocol did not address how to determine repellent failure in the event of a rain delay; therefore, the study staff treated landings in the first exposure period after the rain delay as the first confirmed landing and exposure period 6 was used for repellent failure (Deviation 3). If the repellent failed on a subject, the subject was removed from the test. However, the repellent did not fail on all subjects, thus at the discretion of the study direction the study at the site in Wisconsin was ended after 17 exposure periods (10 h post treatment), and the study in Florida was ended after 14 exposure periods (8.5 h post-treatment) [§13.6, pp. 21-23 of 154].

Two untreated control subjects were paired together and used to confirm that mosquito populations were adequate to test the repellency of the product. Untreated control subjects were exposed to mosquitoes for a five-minute period every thirty minutes as described above for treated subjects. However, to reduce the exposure of untreated control subjects to mosquitoes, untreated subjects covered their exposed limb after five landings in the five-minute exposure period. Five landings on each untreated subject during the five-minute exposure period were considered the minimum necessary to ensure the mosquito population was large enough to determine repellency of the tested product. The time to reach five landings on the untreated control subjects was recorded for each five-minute exposure period [§13.7, p. 23 of 154].

The duration of repellency to appear on the Repellency Awareness Graphic on the product label will be based on the EPA Repellency Awareness Guidance for Skin-Applied Insect Repellent Products, which states, "The mosquito claim should be calculated using the most conservative (i.e., lowest) CPT from all available studies (In this study - Florida compared to Wisconsin)." "And "The number of hours of protection appearing on the repellency awareness graphic should be expressed as a whole number. If the most conservative calculated CPT is not a whole number, EPA recommends rounding down to the nearest whole number."(§4.1, p. 8 of 154).

Test Substance Application Rate and Treatment: The target application rate was 1.0 g per 600 cm^2. Formula 1 (below) was used to calculate the amount of the test substance to apply to each subject. Prior to applying the test substance, each subject washed the limb to be treated with water and unscented soap, dried the limb, and then wiped the limb in a 70% solution of isopropanol and allowed the limb to dry. After drying, the area to be treated was marked with a felt-tipped pen. Adhesive surgical tape and adhesive bandage were wrapped around the area to be treated so the area to be treated was the only exposed skin. The test substance was placed on the balance and the balance tared. The test substance was applied directly from the aerosol can onto the skin. After spraying, the sample was returned to the balance and the amount sprayed onto the skin was determined. If the amount sprayed was below the target weight, more was sprayed onto the forearm and the can weighed again (§7.1.8.2, pp. 68-69 of 154). The amount applied to each subject is found in Table 4 (§13.7, p. 23 of 154).

Formula 1. Weight of test substance to apply (g) = [Area of limb cm^2/600 cm^2] * 1.0 g

Table 4. Amount of Test Substance Applied (§13.7, p. 23 of 154).

Site	Test Subject No.	Date	Limb Treated	Target Amount [1] (g)	Actual Amount (g)	% of Target
Wisconsin	121	8/10/15	Left Arm	0.72	0.73	101
Wisconsin	122	8/10/15	Right Arm	0.68	0.68	100
Wisconsin	123	8/10/15	Left Arm	0.72	0.73	101
Wisconsin	124	8/10/15	Right Arm	0.76	0.73	96
Wisconsin	125	8/10115	Left Arm	0.64	0.69	108

Site	Test Subject No.	Date	Limb Treated	Target Amount [1] (g)	Actual Amount (g)	% of Target
Wisconsin	134	8/10/15	Right Arm	1.06	1.09	103
Wisconsin	135	8/10/15	Left Arm	1.02	1.08	106
Wisconsin	136	8/10/15	Right Arm	1.01	1.05	104
Wisconsin	139	8/10/15	Left Arm	0.91	0.9	99
Wisconsin	143	8/10/15	Left Arm	0.76	0.76	100
Florida	194	8/25/15	Right Arm	1.12	1.2	107
Florida	199	8/25/15	Left Arm	1.03	l.09	106
Florida	205	8/25/15	Left Arm	0.76	0.7	92
Florida	207	8/25/15	Left Arm	0.78	0.88	113
Florida	209	8/25/15	Left Arm	0.97	1.04	107
Florida	210	8/25/15	Right Arm	0.85	0.84	99
Florida	219	8/25/15	Left Arm	1.07	1.01	94
Florida	220	8/25/15	Right Arm	1.01	1.01	100
Florida	221	8/25/15	Left Arm	1.21	1.3	107
Florida	229	8/25/15	Left Arm	0.99	0.99	100

[1]Target amount was based upon forearm surface area calculation (Formula 1).

Protocol amendments and deviations: The approved protocol was dated June 26, 2015. **There were no amendments to the protocol** (§20.2 p. 30 of 154).

Six protocol deviations occurred during the study (Appendix B, p. 112 – 117 of 154). Four of the six deviations did not affect the study. Deviation 4 (Appendix B, p. 115 of 154) documents that the test product was applied to subject 207 at a slightly higher volume; however, because lower doses of the repellent on other subjects also lasted the full duration of the study, this minimal increase in application volume did not affect the study. Deviation 3 (Appendix B, p. 114 of 154), which did affect the study, resulted in the cancellation of two exposure periods because of heavy rain and lightning, the effect of this deviation on the study is discussed in the experimental design section above and the results section below. In future draft protocols for field studies testing insect repellents, EPA should ensure that the protocol addresses how to determine repellent breakdown points in the event of a weather delay.

Results and analyses:

Test Systems: Test subjects collected mosquitoes representing three genera (*Coquillettidia*, *Psorophora*, and *Aedes*) and four species in Wisconsin, and four genera (*Mansonia*, *Psorophora*, *Wyeomyia*, and *Aedes*) and seven species in Florida for a total of five genera and 10 species across the two sites. *Aedes* spp. mosquitoes, a mosquito genus containing numerous important vectors of human diseases, represented 95% of the mosquitoes collected in Wisconsin, and 90% of the mosquitoes collected in Florida (Tables 1, 2; §10.1, p. 11 of 154).

Duration of Repellency: At both the Florida and Wisconsin sites, at least 5 mosquitoes landed on the untreated control in all of the five-minute exposure periods. At the Florida site, the time to count five mosquito landings on control subjects ranged from 11 to 97 seconds. At the Wisconsin site, the time to count five mosquito landings on control subjects ranged from 15 seconds to 3 minutes. One of the untreated control subjects withdrew after the fifth exposure period at the Wisconsin site and the subject that withdrew was not replaced with an alternate subject. However, landings on the remaining untreated control subject were adequate to confirm a mosquito population high enough to test for repellency throughout the study.

Median complete protection time and 95% confidence limits (lower confidence limit [LCL] and upper confidence limit [UCL]) were calculated by Kaplan Meier analysis using PROC LIFETEST in SAS, which employs a generalization of the Brookmeyer and Crowley (1982) method under a log-log transformation. The median duration of repellency for the test substance was 8.25 hours in Wisconsin, and 8.0 hours in Florida (Tables 5, 6; §4.1, pp. 8-9 of 154). In Florida, because of a rain event the first confirmed landing on three treated subjects (Subject Nos. 199, 210, 229) occurred in the first exposure period immediately after two skipped exposure periods, the repellency duration for these three subjects is recorded as 4.5 hours (Deviation 3).

Table 5. MARK-8 (25% DEET aerosol) Repellency Duration Results Summary, Hours, Sample size = 10 Wisconsin site, Sample size = 10 Florida Site (§4.1, p. 8 of 154).

Measure	Wisconsin Site	Florida Site
Median	8.25	8.0
95% LCL	6.0	3.5
95% UCL	10.0	8.5
Range	6.0 - 10.0	3.5 - 8.5

Table 6. MARK-8 (25% DEET aerosol) Repellency Duration Results, Hours (§4.1, p. 9 of 154).

Wisconsin Site		Florida Site	
Subject No.	Repellency Duration	Subject No.	Repellency Duration
121	10.0	194	8.5
122	8.0	199	4.5
123	10.0	205	8.5
124	8.5	207	8.5
125	9.0	209	7.5
134	7.5	210	4.5
135	6.5	219	8.5
136	8.0	220	8.5

139	6.0	221	3.5
143	8.5	229	4.5

Conclusions:

The methods used in this study were adequate to produce scientifically reliable results. The methods were based on the protocol reviewed and accepted by the EPA and HSRB on April 23, 2015 as amended to incorporate EPA and HSRB recommendations before testing began. The data in the study are acceptable to support a median CPT of 8.0 hours against mosquitoes for the EPA Repellency Awareness Graphic on the label for EPA Reg. No. 4822-167, Mark-8 OFF! Insect Repellent Formula V (25% DEET).

Note:
The limb measurement forms should have been included with the raw data.

HSRB Comments and Science Recommendations on the Protocol and S.C. Johnson Responses from the April 2015 Meeting Report Dated June 23, 2015:

Product application rate: The Board understands that the data from this protocol will be used to calculate median CPT values across all subjects from each of two sites rounded down to the nearest integer and the lowest value will be used for the product graphic. (p. 11 of 39, Sweeney and Sherman). Given the stated use of the data, the Board agrees with the use of a standardized dose of 1 gram product per 600 cm^2 treated skin. However, we strongly suggest changing the language from standardized *dose* to standardized *application* rate and reserve "dose" to describe how much active ingredient is applied.

The protocol specifies that the actual amount of product applied will be recorded. The percent active ingredient in each product is also available so the Agency can in fact assess the relationship between dose (active ingredient) and efficacy (or CPT) as a quality assurance check of the data. An assessment of the relationship between CPT and dose (mass active ingredient per treated area) can provide an indication of the quality of the data or point out data that might be suspect because it does not fall along the expected dose-response trend. The Board recommends that the data analysis include a dose-response comparison for all products where multiple concentrations are available (e.g., DEET content in the different products ranges from 5% to 98.25%) to help assess data quality. In addition, the Agency might consider normalizing the CPT results to better represent the expected application rates derived from earlier dosimetry studies when calculating the final graphic number.

S.C. Johnson Response: The word "*dose*" was changed to "*application rate*" throughout. A dose-response comparison was not included in the data analysis because each study only tested a single product.

Product application method: The protocol proposes to use a variety of application methods including using pipettes for transferring liquid contents from pump sprays and spatulas for lotions, while aerosol sprays are applied directly. In each case, the product is applied and spread

on the subject's skin by a staff member. The Board was concerned that these application methods were not representative of actual application methods but ultimately concluded that the need for consistency outweighs the need for the protocol to be representative of consumer behavior. However, the Board stresses the importance of accurately reporting the application rate (mass of product per area of skin) for each subject.

A particular concern with the aerosol application method was that the iterative procedure leaves open the possibility to repeatedly apply more than the target 1 gram. Some means should be integrated within the protocol to limit or preclude the potential to bias the average dose upward. One (and perhaps not the best) way to preclude such a bias would be to place some upper bound on the highest level above 1 gram that would be allowed to proceed to the field testing phase. Such an upper bound (or similar restriction) could be applied to all application methods, but over-exposures seem most likely to occur with the aerosol application method.

S.C. Johnson Response: The test substance (aerosol formulation) was applied as directed by the revised protocol (Section 7.1.8.2, p. 68-69 of 154) and results reported in Table 8 (p. 23 of 154). Note the study incorrectly lists the application method as the method for pump sprays. The mean amount applied was equal to the target amount with a range of 92 - 113% of the target amount applied.

Use of sites outside the U.S: The protocol specifies the use of two established and ecologically distinct field sites in the United States for testing, and the Board agrees that this will provide sufficient representation for determining CPT, but the Board shares the concerns expressed by the Agency about using sites located outside of the U.S. The study sponsor has described that in addition to the two established sites in the U.S., there is at least one established site in Australia that could be used. The protocol needs to provide more information on what constitutes an "established site" either in the U.S. or another country (*i.e.*, climate, mosquito species present, other hazards such as other mosquito borne diseases, presence of cell phone service, representativeness of local demographics) and more importantly should describe how the data collected at the alternate sites outside the U.S. will be related to the U.S. consumer demographics and the expected mosquito populations in the U.S.

S.C. Johnson Response: The protocol only states that sites will be qualified by confirming mosquito populations are adequate to achieve the minimum landing pressure (5 mosquitoes on an untreated control in five minutes) required to evaluate efficacy. In addition, the protocol was revised to include the requested information (e.g., mosquito species present, climate, etc.) for the proposed site in Australia. However, testing was not conducted outside of the U.S., so this information is not relevant to the submitted study.

Potential for cross contamination: There are a number of places in the protocol that provide opportunities for insecticide to be inadvertently either lost or gained from/to the treated area on subjects. Simple precautions can be taken to alleviate this issue, but the protocol should specify steps that will be taken to insure that the treated area on subjects is not impacted by activities that

take place before or during the experiment (*i.e.*, rubbing sleeve or pant leg across the treated area).

S.C. Johnson Response: Subjects were not transported using a vehicle after the test substance was applied, and subjects were reminded not to touch or contact the treated skin in any manner. Any inadvertent contact with the treated area was reported to the study staff and documented in the raw data.

Potential for "carryover": No justification was provided for the adequacy of separating multiple participations by any test subject by a minimum of one day (Science Response #6 in S.C. Johnson letter of 17 April 2015). It is important to verify that no carryover effect is present on subjects used on multiple days. The protocol suggests that a day between treatments will be sufficient when the same subject is used a second time, but justification or references are needed to support this. If a subject is treated with 98.25% DEET, is there any residual effect after 24 hours that might affect a low dose treatment (application of 5.6% DEET wipe)?

S.C. Johnson Response: Subjects washed before and after each test, and a minimum of two days was required to pass before subjects could participate in another test. There is no indication that test subjects participated in multiple tests so we assume all subjects did not previously participate in other tests and therefore there was no "carryover" effect.

Landing pressure: The protocol includes untreated control subjects with each test to insure that there is sufficient landing pressure to provide valid results. However, the landing pressure is not measured in quantitative terms, only whether it is sufficient or not (five landings in five minutes). Discussions during the meeting seemed to imply that landing pressure will influence the measured CPT. If in fact the landing pressure can influence the resulting median CPT and products tested on different days are subjected to different landing pressures, then it would be important to collect quantitative information on landing pressure that could be used to correct, normalize, or at least interpret the resulting CPT values. The Board recommends that the Agency and S.C. Johnson consider how a quantitative estimate of landing pressure can be determined without increasing the likelihood of bites if landing pressure is excessive (*e.g.*, recording the time of each landing, the time to reach 5 landings, or the total landings in 5 minutes) and how that information can be used to normalize or interpret CPTs measured under different landing pressure conditions.

S.C. Johnson Response: Study staff recorded the time to reach five landings if less than five minutes. Because all studies were performed at the same two sites, landing pressure appears to be fairly consistent across sites, therefore normalization is not required.

Delayed start: The Board recognizes the advantages of delaying the exposure to mosquitoes for subjects treated with products that are known from previous experience to last for a long time. However, the protocol needs to provide more information about the criteria used to determine how long to wait before starting the test cycles (5 minute exposure at 30 minute intervals).

Regardless of how long the subject's exposure is delayed, the protocol should require a minimum number of completed cycles to insure valid results. For example, following a delayed exposure, the subject should complete at least three exposure cycles before getting a confirmed landing.

S.C. Johnson Response: Exposures were delayed until two hours after application (Deviation 6). The test substance contained 25% DEET as the active ingredient and according to the protocol exposures were to be delayed by three hours for products containing more than 16% DEET. This deviation did not affect the study, and all subjects were exposed to mosquitoes for at least three exposure cycles before a confirmed landing was recorded. In addition, delaying exposure periods reduced the exposure of the subjects to bites from mosquitoes.

Experimental design: The design as presented tests all ten subjects assigned to a product on a single day. The downside of this design is that it does not allow results to be easily generalized to a range of environmental conditions that may affect the attractiveness of a subject (e.g., sweating due to temperature and humidity levels). An alternative would be to test each product on several days; e.g., five subjects on each of two days or three subjects on each of two days and the remaining four subjects on a third day. Each day would form a block for the analysis of that product's data. Such a design would allow testing of multiple products on a given day. If it were of interest to compare product formulations and/or application methods, the combined data could be analyzed as a block design with multiple replications of each product within each block (day).

These alternative designs that utilize blocking (*e.g.*, by individual test subject) could be considered to account for known sources of variation (*e.g.*, individual effects).

S.C. Johnson Response: The two different sites in different areas of the country should allow for generalizing results to a greater range of environmental conditions than a single site. Also, the main goal of the study is to determine the CPT for an individual product, not to compare multiple products.

Randomization: The randomization mechanism should be described in more detail and rationale should be given for any given choice of randomization within the protocol. For example, it is not clear whether/how cross-substance relations are to be evaluated in the data analysis and why randomization among test substances is needed. An explanation of this would be helpful. In addition, when the conditions support use of arm rather than leg for exposure, then it may be more important to consider handedness when selecting what arm to treat, rather than randomly assigning to left or right hand, so the subject can have their dominant hand to remove landing mosquitoes before they bite.

S.C. Johnson Response: One test substance was tested on each day, therefore randomizing the treatment was not necessary. The mechanism for randomizing the arm was not provided in the study; however, after discussions with S.C. Johnson, they indicated that the mechanism for randomizing the arm to be treated was based on the random selection of test ID numbers. Subjects assigned odd numbers had their left arm treated, and subjects with even numbers had

their right arms treated. In addition, the protocol notes that aspirating mosquitoes is not difficult even with a non-dominant hand. The untreated control subjects were allowed to choose which arm to expose.

Sample size determination: A sample size calculation would be useful here to inform the power of testing and the width of confidence intervals. Power and sample size calculation can be implemented using existing SAS procedures. Information about appropriate sample size calculations is included in the EPA document "Product performance Test Guidelines OPPTS810.3700: Insect Repellents to be Applied to Human Skin."

S.C. Johnson Response: After discussions with S.C. Johnson, they indicated that they did not conduct a sample size calculation or power analysis but the table which summarizes the effect of sample size on the width of the confidence interval for median CPT presented in the protocol for testing repellents against ticks to the Human Studies Review board at the October meeting would apply to these studies because both studies use 10 subjects.

Sources of variation: Multiple sources of variation including, for example, site selection, treatment dosage, application rate, mosquito type/age/condition, and landing pressure can impact the results. For the most part, however, they are not accounted for explicitly within the study, and when the source of variation is not controlled (e.g. as it is using a standard application rate) then the contribution to variance should be acknowledged or discussed. The protocol does not currently specify the conditions that might cause the CPT data from the two sites to differ; however, the researchers should consider collecting information to explain any large and potentially significant differences in the CPT values between otherwise matched studies conducted at two different sites.

S.C. Johnson Response: The researchers identified mosquitoes to species, recorded habitat characteristics, climatic conditions, and the time to five landings on the untreated control subjects, information which could explain large and significant differences in CPT. Note the CPTs at both sites were similar.

EPA Comments and Science Recommendations on the Protocol and S.C. Johnson Responses from the April 2015 Meeting Review Dated March 31, 2015:

EPA Comment: Change "mosquito biting pressure" to "mosquito landing rate" as subject bites are not counted or recorded in this study.

S.C. Johnson Response: The term "mosquito biting pressure" was changed to "mosquito landing rate" throughout the protocol.

EPA Comment: Describe how the data will be analyzed if the number of test subjects at the end of the test is less than ten. In other words, what if subjects withdraw? If alternates replace them, how will Johnson account for this change of subjects in the data analysis?

S.C. Johnson Response: None of the treated subjects withdrew from the study at either location. One of the untreated control subjects withdrew from the study, but the untreated control subjects were not used to calculate CPT.

EPA Comment: The protocol states that up to 10% of the exposure periods in a test may have less than the minimum landing (biting in the protocol) pressure of five mosquitoes landing in five minutes or less. Will treatment exposures occur during periods of insufficient landing pressure? If treatment data are collected during these periods, how will they be used in CPT calculation? If they are not used, how will the lack of data points be considered in the K-M analysis and calculation of Median CPT?

S.C. Johnson Response: Landing pressure at both locations reached the minimum biting pressure for all exposure periods therefore this comment does not apply to this study.

EPA Comment: State/justify why no positive control substance is to be used.

S.C. Johnson Response: A positive control was not used because the Agency did not provide information on how positive control data would be used to normalize the data across sites. Therefore exposing additional subjects to repellent products and mosquitoes was not justified.

EPA Comment: Product application is not fully described. After weighing the set dose, how is the product applied to the limb for pump sprays and lotions? For instance, is the required amount left in the container and the pump used to spray it on the limb? For lotions, the amount to be applied is removed with a spatula instead of a larger syringe so transfer to the subject might be easier? For aerosols, Johnson could estimate the delivery of the prescribed amount of product by counting the number seconds needed to deliver the dose to the limb and determine the amount applied per second of spraying to more closely estimate the application amount? How does this compare to the product's label directions? Will study staff be spreading the lotion with a gloved hand?

S.C. Johnson Response: The exact method of determining the amount applied to each subject in this study is described in §7.1.8.2 (pp. 68-69 of 154).

EPA Comment: Appendix III – Land Data Form. Identification of which limb was treated needs to be added to this data sheet.

S.C. Johnson Response: A line was added to the data form to identify the treated limb.

EPA Comment: Data compilation and processing. Little detail is provided in the protocol on how the data from these sheets will be compiled and processed before entry into Excel, JMP, or SAS, etc.

S.C. Johnson Response: Median confidence limits were calculated by Kaplan Meier analysis using PROC LIFETEST in SAS, which employs a generalization of the Brookmeyer and Crowley (1982) method under a log-log transformation.