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: Palm, J. (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of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic – Unpublished document prepared by S. C. Johnson & Son, Inc., 1525 Howe Street Racine, WI 53403. September 24, 2015. 149 p. (MRID 49750101) (D430002).

ACTION REQUESTED

Conduct a science review of a completed field study testing the efficacy against mosquitoes of an insect repellent formulated as a pump spray 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 the EPA Repellency Awareness Graphic on the label of EPA Reg. No. 4822-276, Mark-3, Maximum

Strength Pump Spray Deep Woods Off! (98.25% DEET). The protocol used to conduct this study was previously reviewed and accepted by EPA and the HSRB with comment on April 23, 2014. 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. Study MRID 49750101 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-3 in the field against populations of wild mosquitoes using human volunteer subjects. This is a guideline study designed to fulfill the requirements of 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 149).

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 (98.25% 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 149).

Table 1. Wisconsin Site Mosquito Species collected - July 29, 2015 (revised table, §10.1, p. 11 of 149)

Species	Number Collected	% of Total
Coquillettidia perturbans	1	1.4%
Psorophora ferox	3	4.2%
Aedes vexans	2	3%
Aedes trivittatus	63	90%
Aedes stimulans	1	1.4%
Total	70	100%

Table 2. Florida Site Mosquito Species collected - September 10, 2015 (revised table, §10.1, p. 11 of 149)

Species	Number Collecte	% of Total
Anopheles atropos	9	3%
Anopheles crucians	4	1.3%
Aedes atlanticus	95	31.4%
Aedes infirmatus	137	45.4%
Aedes taeniorhynchus	7	2.3%
Coquillettidia perturbans	2	0.7%
Culex erraticus	4	1.3%
Mansonia dyari	4	1.3%
Mansonia titillans	4	1.3%
Psorophora ferox	30	10%
Wyeomyia mitchellae	6	2%
Total	302	100%

Experimental design: This is a field study conducted with human subjects at two geographically and ecologically distinct field sites, one in Kenosha County, Wisconsin on July 29, 2015, and one in Collier County, Florida on September 10, 2015. At each site, the experimental treatment groups of 10 different subjects 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 149). At each site, testing was conducted during the course of a single day (§13.1, p. 20 of 149).

Table 3. Summary of Test Subject Participation in GLP 865E1 (Revision of Table 7 in the Study §12.4, pp. 15-16 of 149).

GLP 865E1 - 4822-276	**Wisconsin**	**Florida**	**Total**
Number of Subjects Enrolled	22	22	44
Number of No-Shows to training and/or testing	No-shows for training: 0 No-shows for testing: 1	No-shows for training: 7 No-shows for testing: 0	8 *(Note to HSRB: In the study, the table cited 7)
Number Assigned as Test Subjects	12	12	24
Number Assigned as Alternates or Extras	4 alternates (1 was used) 5 extras	3 alternates 0 extras	12 (Note to HSRB: in the study, the table cited 13)

Number of Test Subjects Withdrawn Voluntarily	1 on training day, 2 on test day (post-treatment)	1 on test day (post-treatment)	4 (1 during training, 3 on test day) (Note to HSRB: in the study, the table cited 2)
Number of Test Subjects Withdrawn Involuntarily	0	0	0
Number of Test Subjects Completed Research	10	11	22
	In Wisconsin: 12 test subjects (10 treated test subjects and 2 controls) and 4 alternates were randomly selected as described in the report out of the pool of 22 trained participants.	In Florida: Only 15 trained participants were available on this test day. 12 test subjects (10 treated and 2 controls) and 3 alternates were selected as described in the report out of the pool of 15 trained participants.	
*No-show from WI test date was not previously included in the total			

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 149. 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 149). Two untreated control subjects were used at each site to determine 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. 24 of 149).

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 149).

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, and the untreated control subjects used to confirm that mosquito populations were adequate to test the repellency of the product were paired together. Pairs were separated from each other by at least twenty feet. Starting three hours after application of the repellent product, subjects observed their limbs for five minutes for landing mosquitoes. 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 in 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. 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 time to reach five landings on the untreated control subjects was also recorded by study staff for each five-minute exposure period.

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. If the repellent failed on a subject, the test was finished for the subject. However, the repellent did not fail on all subjects. In the event subjects withdrew from the study, they were not replaced with an alternate subject, instead the exposure period at which the subjects withdrew was used as their CPT endpoint. If landing pressure on the untreated control was lower than five landings per exposure period during 10% of the exposure periods, the study was ended because landing rates were not high enough to assess efficacy. At the Wisconsin site, the study was ended after 20 exposure periods (12.5 h post treatment) because landings on an untreated control were lower than five landings in five minutes on one subject following an exposure period during which neither untreated control subject observed five landings on their exposed arm. At the discretion of the study director, the study was ended at the Florida site after 19 exposure periods (12 h post-treatment) because the median CPT of 12 was the same as the median CPT from the Wisconsin site (§13.6 – 13.7, pp. 21-23 of 149).

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 was the same as 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 149).

Test Substance Application Rate and Treatment: The target application rate was 0.5 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 treated area was marked with a felt-tipped pen. Adhesive surgical tape and adhesive bandage were wrapped around the treated area so the treated area was the only exposed skin. Study staff weighed the test substance container, tared the balance, and then removed the target amount of the test substance from the container using a pipette and distributed the tested substance onto the forearm of the treated subject. After the test substance was placed on the forearm, to ensure complete coverage, study staff spread the repellent around the forearm of the subject using two fingers of a gloved hand. The amount applied to each subject is found in Table 4 (§13.7, p. 23 of 149).

Formula 1. Weight of test substance to apply (g) = [Area of limb cm^2/600 cm^2] * 0.5 g

Table 4. Amount of Test Substance Applied (§13.7, p. 23 of 149).

Site	Test Subject No.	Date	Limb Treated	Target Amount [1] (g)	Actual Amount (g)	% of Target
Wisconsin	26	7/29/15	Right Arm	0.50	0.51	102%
Wisconsin	27	7/29/15	Left Arm	0.40	0.41	103%
Wisconsin	28	7/29/15	Right Arm	0.38	0.37	97%
Wisconsin	29	7/29/15	Left Arm	0.45	0.45	100%
Wisconsin	32	7/29/15	Right Arm	0.45	0.45	100%
Wisconsin	37	7/29/15	Left Arm	0.45	0.45	100%
Wisconsin	38	7/29/15	Right Arm	0.54	0.56	104%
Wisconsin	41	7/29/15	Left Arm	0.39	0.39	100%
Wisconsin	43	7/29/15	Left Arm	0.49	0.49	100%
Wisconsin	45	7/29/15	Left Arm	0.54	0.55	102%
Florida	241	9/10/15	Left Arm	0.46	0.47	102%
Florida	242	9/10/15	Right Arm	0.48	0.50	104%
Florida	243	9/10/15	Left Arm	0.51	0.51	100%
Florida	244	9/10/15	Right Arm	0.48	0.49	102%
Florida	245	9/10/15	Left Arm	0.57	0.58	102%
Florida	253	9/10/15	Left Arm	0.39	0.40	103%
Florida	254	9/10/15	Right Arm	0.42	0.41	98%
Florida	255	9/10/15	Left Arm	0.34	0.35	103%
Florida	256	9/10/15	Right Arm	0.55	0.56	102%
Florida	257	9/10/15	Left Arm	0.40	0.40	100%
					Average	101%
					Min	97%
					Max	104%

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

Protocol amendments and deviations: The approved protocol was dated June 26, 2015. **There were no amendments to the protocol** (§20.2 p. 31 of 149).

Two protocol deviations occurred during the study (Appendix B, p. 112 – 114 of 149). Neither of the deviations affected the study.

Results and analyses:

Test Systems: Test subjects collected mosquitoes representing three genera (*Coquillettidia*, *Psorophora*, and *Aedes*) and five species in Wisconsin, and seven genera (*Coquillettidia*, *Mansonia*, *Psorophora*, *Wyeomyia*, *Anopheles*, *Culex*, and *Aedes*) and eleven species in Florida for a total of seven genera and 14 species across the two sites. *Aedes* spp. mosquitoes, a mosquito

genus containing numerous important vectors of human diseases, represented 94% of the mosquitoes collected in Wisconsin, and 77% of the mosquitoes collected in Florida (Tables 1, 2; §10.1 p. 11 of 149).

Duration of Repellency: At the Wisconsin site, at least five mosquitoes landed on the untreated control subjects in all but one exposure period (exposure period 14 on subject 47) through the 18th exposure period. Five mosquito landings did not occur on either untreated control subject during exposure period 19, and five landings only occurred on one untreated control subject during the 20th exposure period. For exposure periods in Wisconsin where five landings were observed on the untreated control subjects, the time to count five mosquito landings on control subjects ranged from 17 seconds to 3 minutes and 45 seconds.

At the Florida site at least 5 mosquitoes landed on the untreated control subjects in all of the five-minute exposure periods, and the time to count five mosquito landings on control subjects ranged from 18 seconds to 4 minutes and 57 seconds.

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 tested product was 12.0 hours in Wisconsin and Florida (Table 5, 6; §4.1, pp. 8-9 of 149).

At the Wisconsin site, two treated subjects withdrew before the end of the test; test subject 29 withdrew after exposure period 19 and subject 38 withdrew after exposure period 4 (Table 6; §4.1, p. 9 of 149). At the Florida site, one treated subject withdrew before the decision was made to end the test; subject 243 withdrew after exposure period 19 which was the last exposure period for testing (Table 6; §4.1, p. 9 of 149). At the Florida site, subject 258, one of the untreated control subjects also withdrew after exposure period 19, but this did not affect the study because the study ended after exposure period 19.

Table 5. MARK-3 (98.25% DEET pump spray) Repellency Duration Results Summary, Hours, Sample size = 10 Wisconsin site, Sample size = 10 Florida Site (§4.1, p. 8 of 149).

Measure	Wisconsin Site	Florida Site
Median	12.0	12.0
95% LCL	6.0	8.5
95% UCL	12.0	12.0
Range	4.5 - 12.0	8.5 - 12.0

Table 6. MARK-3 (98.25% DEET pump spray) Repellency Duration Results, Hours (Revised table, §4.1, p. 9 of 149).

Wisconsin Site	Florida Site

Subject No.	Repellency Duration	Subject No.	Repellency Duration
26	12.5	241	12.0
27	12.5	242	12.0
28	6.0	243	12.0[2]
29	12.0[3]	244	12.0
32	7.0	245	12.0
37	11.0	253	9.0
38	4.5[1]	254	8.5
41	12.5	255	8.5
43	12.5	256	12.0
45	8.0	257	11.0

[1]Subject withdrew at 4.5 hrs post treatment.
[2]Subject withdrew at 12.0 hrs post treatment.
[3]Subject withdrew at 12.0 hrs post treatment.

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 12.0 hours against mosquitoes for the EPA Repellency Awareness Graphic on the label for EPA Reg. No. 4822-276, Mark-3, Maximum Strength Pump Spray Deep Woods Off! (98.25% DEET).

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

HSRB Comments and Science Recommendations on the Protocol with S.C. Johnson's 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 (pump spray formulation) was applied using a pipette, and S.C. Johnson reported the exact amount of the test substance applied to each subject. The limit on the upper dose was not implemented because the test substance was applied by pipette.

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 three hours after application. The test substance contained 98.25% 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 with S.C. Johnson's 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 except in §16.0 p. 27 of 149 and on the data sheet for subject 28, where the term "bite" was used. S.C. Johnson confirmed these are written/typographical errors and landings were evaluated.

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: At the Wisconsin site, two treated subjects withdrew before the end of the test; test subject 29 withdrew after exposure period 19 and subject 38 withdrew after exposure period 4. Exposure period 20 was not included in the statistical analysis. At the Florida site, one treated subject withdrew before the decision was made to end the test; subject 243 withdrew after exposure period 19 which was the last exposure period for testing. For subjects that withdrew before receiving their first confirmed landing, their CPT was considered to be the time at which they withdrew. These "censored" subjects are indicated by a + on the survival curve graphs. At the Florida site, subject 243, one of the untreated control subjects also withdrew after exposure period 19, but this did not affect the study because the study ended after exposure period 19.

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 the Wisconsin site was below the minimum landing pressure required during the last two exposure periods (#19, 20); therefore, the study was ended after exposure period 20. Exposure period 20 was not included in the Kaplan Meier analysis for this site. Landing pressure at the Florida site reached the minimum landing pressure for all exposure periods therefore this comment does not apply to this site.

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: After weighing, study staff pipetted the test substance onto the exposed skin and spread the substance evenly over the treatment area using a gloved hand.

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.