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: Laznicka, E. (2015) Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic – Project Number 866E1. Unpublished document prepared by S. C. Johnson & Son, Inc., 1525 Howe Street Racine, WI 53403, October 21, 2015. 149 p. (MRID 49766001) (D430168)

ACTION REQUESTED

Conduct a science review of a completed field study testing the efficacy of an insect repellent formulated as a pressurized aerosol spray product containing diethyl toluamide (DEET) against mosquitoes. 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. 4822-380, Mark-4 OFF! Active Insect Repellent I (Unscented OFF! Insect Repellent) (15% DEET). The protocol used to conduct this study was previously reviewed and accepted by EPA and the HSRB on April 23, 2015. The protocol used in this study was amended to incorporate 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 EPA Human Studies Review Board. The study (MRID 49766001) was conducted in accordance with Good Laboratory Practices as described in 40 CFR §160 (with one minor GLP exception as described in Protocol Deviation #4, Appendix B, p. 116 of 149), 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 the complete protection time (CPT) of MARK-4 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 149).

Identification of the test system: In this study, landings of wild mosquitoes on replicate human subjects were used to evaluate the repellency of a pressurized aerosol insect repellent product (15% DEET) applied to human skin. Mosquitoes were used because they are one of the insect pest groups targeted by the product and one of the insect groups represented on the insect repellency 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*, *Culex*, *Mansonia*, and *Anopheles* (Tables 3 and 4; p. 11 of 149).

Table 3. Wisconsin Site Mosquito Species Collected - August 5, 2015

Species	Number Collected	% of Total
Coquillettidia peturbans	1	2%
Psorophora ferox	1	2%
Aedes vexans	5	9%
Aedes trivittatus	48	87%
Total	55	100%

Table 4. Florida Site Mosquito Species Collected – September 22, 2015

Species	Number Collected	% of Total

Anopheles crucians	1	2.3%
Aedes atlanticus	14	31.8%
Aedes infirmatus	17	38.6%
Aedes taeniorhynchus	6	13.7%
Culex erraticus	2	4.5%
Mansonia dyari	1	2.3%
Mansonia titillans	1	2.3%
Psorophora ferox	2	4.5%
Total	44	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 5, 2015, and one in Collier County, Florida on September 22, 2015. At the Wisconsin site, the experimental treatment group consisted of 10 different treated subjects while at the Florida site the treatment group consisted of 8 subjects (see Deviation #2, Appendix B, p. 114 of 149). The untreated control group at both sites consisted of two untreated subjects. Subjects at each site were selected from different candidate pools (Table 7). In Wisconsin and Florida testing was conducted during the course of a single day (§13.1, p. 20 of 149).

Table 7. Summary of Test Subject Participation in GLP866E1 – Mark -4 - EPA Reg. No. 4822-380. (This is the revised version of Table 7 appearing on p. 14 of 149 sent to EPA by S. C. Johnson & Son, Inc.)

GLP 866E1 - 4822-380	Wisconsin	Florida	Total
Number of Subjects Enrolled	24	17	41
Number of No-Shows to training and/or testing	No-shows for training: 5 No-shows for testing: 2	No-shows for training: 7 No-shows for testing: 0	14 (Note: SC Johnson had a typo of "13" in the original chart. It should be 14 including 12 no shows for training, 2 no shows for testing)
Number Assigned as Test Subjects	12	10	22
Number Assigned as Alternates or Extras	4 alternates (2 were used) 3 extras	0 alternates 0 extras	7 (Note: SC Johnson had a typo of 6 in the original chart.)
Number of Test Subjects Withdrawn Voluntarily	0	0	0
Number of Test Subjects Withdrawn Involuntarily	0	0	0
Number of Test Subjects Completed Research	12	10	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 19 trained participants.	In Florida: Only 10 trained participants were available on this test day. 8 treated test subjects and 2 controls were selected as described in the report out of the pool of 10 trained participants.	

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, p.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. 21 of 149). The two subjects most proficient at aspirating mosquitoes from the pool of subjects were used at each site as untreated controls to determine mosquito landing rate. The untreated control subjects chose which arm to expose to mosquitoes so the dominant hand could be used to aspirate mosquitoes.

Test preparation. 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 (§13.4 -13.5, p. 21 of 149).

Measuring repellency. The unit of measure for assessing repellent effects in this experiment was mosquito landings, similar to previous skin applied repellent evaluations where the "Landings" measure is used and repellency is measured as CPT. To determine CPT for each subject, 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 each subject or their partner, and if necessary, headlamps were used at night so subjects could see to aspirate mosquitoes. For this study, headlamps were not required. After the five-minute exposure period, subjects reported the number of mosquito landings on them to study staff who recorded the number for each subject. Aspirated mosquitoes were labeled and kept to be identified. The five-minute exposures were conducted every thirty minutes until repellent failure occurred except for exposure period 4 (3.5 hrs. post-treatment) due to a rain event as described in Protocol Deviation #3, Appendix B, p. 115 of 149.

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, pp. 23-24 of 149].

Subject specific Complete Protection Time (CPT) was calculated as time from application of the test substance to a subject and the First Confirmed Landing on that subject. A landing occurred when a mosquito alighted on the treated skin of a subject. A First Confirmed Landing is that which is followed by another landing within a 5 minute exposure period or, when one land occurs in such an exposure period and another landing occurs in the next exposure period (30 minutes later) (§3.1, p. 8 of 149). Subjects with repellent failures were removed from the field test when a First Confirmed Landing occurred. The test was terminated as determined by the study director.

The duration of repellency time 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 149).

Test substance application rate and treatment: The target application rate was 1.0 g per 600 $cm^2 \pm 10\%$. 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 (§14.6, p.24 of 149). The target amount and the amount applied to each subject is found in Table 8 (p. 25 of 149). The mean amount applied was slightly above the target amount (102% of target) with a range of 95-114% of the target amount. No deviation was reported for Florida subject ID #336 where the target amount was exceeded by 14% (> 10% as described in the protocol).

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

Table 8. Amount of Test Substance Applied.

Site	Test Subject No.	Date	Limb Treated	Target Amount [1] (g)	Actual Amount (g)	% of Target
Wisconsin	98	8/5/15	Right Arm	0.75	0.73	98
Wisconsin	99	8/5/15	Left Arm	0.73	0.72	99
Wisconsin	100	8/5/15	Right Arm	0.75	0.79	106
Wisconsin	101	8/5/15	Left Arm	0.82	0.84	103
Wisconsin	103	8/5/15	Left Arm	1.02	1.02	100
Wisconsin	112	8/5/15	Right Arm	1.03	1.03	100
Wisconsin	113	8/5/15	Left Arm	0.84	0.89	106
Wisconsin	115	8/5/15	Left Arm	0.96	0.97	101
Wisconsin	116	8/5/15	Right Arm	0.87	0.90	104
Wisconsin	120	8/5/15	Right Arm	0.86	0.87	101
Florida	319	9/22/15	Left Arm	1.0	0.98	98
Florida	321	9/22/15	Left Arm	1.25	1.29	103
Florida	329	9/22/15	Left Arm	1.09	1.10	101
Florida	330	9/22/15	Right Arm	0.76	0.76	100
Florida	332	9/22/15	Right Arm	0.75	0.79	105
Florida	333	9/22/15	Left Arm	0.97	1.01	104
Florida	334	9/22/15	Right Arm	0.88	0.83	95
Florida	336	9/22/15	Right Arm	0.93	1.06	114

[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 was one amendment to the protocol to change study directors (Appendix A, pp. 111-112 of 149). Four protocol deviations occurred during the study (Appendix B, p. 113 -116 of 149). Deviations #1 and #4 did not have a negative impact on the study. Deviation #2 reported the use of 8 subjects – consisting of 6 females and 2 males – instead of 10 subjects with an equal number of males and females. The study director conducted an analysis to show that the CPT for males and females were not significantly different (Appendix B, p. 117 of 149) and that the deviation had no impact on the study outcome. Deviation #3 (p. 115 of 149) reported that exposure period 4 was cancelled due to rain and exposure period 5 began 5 minutes early, also due to rain. The deviation made reference to subject ID #321 leaving the study at exposure period 5 yet he stayed in the study for two more exposure periods [confirmed according to Table 13 (p. 30 of 149)] but the CPT was moved back to 3.5 hrs. as explained in §13.6.7 (p. 23 of 149). This deviation lowered the CPT for the Florida site.

Results and analyses:

Test systems: Test subjects collected mosquitoes representing six genera (*Aedes, Anopheles, Coquillettidia, Culex, Mansonia, and Psorophora*) in this study across both field sites. Three genera including four species were present on the day of testing in Wisconsin while five genera including nine species were collected in Florida for a total of six genera including 11 species

across the two sites. *Aedes* spp. mosquitoes, a mosquito genus containing numerous important vectors of human diseases, represented 96% of the mosquitoes collected in Wisconsin with *Coquillettidia peturbans*, a vector of Eastern Equine Encephalomyelitis, accounting for 2% of the collection. Major disease vectors did not predominate the collections in Florida (Tables 3 and 4; §10.1, p. 11 of 149).

Landing frequency and 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 Wisconsin site, the time to count five mosquito landings on control subjects was less than 60 seconds for all exposure periods. At the Florida site, the time to count five mosquito landings on control subjects was less than 120 seconds (Table 11, pp. 27-28 of 149). A rain event occurred at the Florida site causing exposure period 4 to be skipped (Protocol Deviation #3, p. 115 of 149). The protocol did not address how to determine repellent failure in the event of a rain delay but landings received in exposure period 3 did not result in a measured FCL in exposure period 5. However, Subject ID #321, received a landing in exposure period 5 and the study director made a conservative assumption that the landing could have resulted in a FCL if period 4 had been available and the CPT was moved back to 3.5 hours (exposure period 4) (§13.6.7, p. 23 of 149). The repellent failed on all subjects except: Subject ID #112 and 116 at the Wisconsin site but the study director decided to stop testing at 10.0 hours post-treatment. Subject ID #332 at the Florida site did not fail but the study director stopped testing at 5.5 hours post-treatment [Tables 12 and 13, pp. 29-30 of 149].

Median Complete Protection Times: Median complete protection times with 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 (CPT) for the test substance (Mark-4) was 7.5 hours in Wisconsin and 5.0 hours in Florida (Tables 1 and 2, pp. 8-9 of 149). The reduced sample size at the Florida site from 10 subjects to only 8 was not addressed in the analysis.

Table 1. MARK-4 (15% DEET aerosol) Repellency Duration Results Summary, Hours, Sample size = 10 per site (p. 8 of 149).

Measure	Wisconsin Site	Florida Site
Median	7.5	5.0
95% LCL	4.0	2.5
95% UCL	9.0	5.5
Range	4.0 - 10.0	2.5 – 5.5

Table 2. MARK-4 (15% DEET aerosol) Repellency Duration Results, Hours (p. 9 of 149)

Wisconsin Site	Florida Site

Subject No.	Repellency Duration	Subject No.	Repellency Duration
98	4.0	319	5.5
99	7.0	321	3.5
100	5.5	329	3.5
101	8.5	330	5.0
103	5.0	332	5.5
112	10.0	333	5.0
113	8.0	334	2.5
115	9.0	336	5.5
116	10.0		
120	7.0		

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 5.0 hours against mosquitoes for the EPA Repellency Awareness Graphic on the label of EPA Reg. No. 4822-380, Mark-4 OFF! Active Insect Repellent I (Unscented OFF! Insect Repellent).

Note:

- The study director should have reported a protocol deviation for the amount of repellent applied to Florida subject ID #336 because it was 14% greater than the target dose.
- The study also lacked alternates and in this instance an alternate could have been used at the Florida site when one subject received >10% of the target amount and one only eight subjects were present for testing.

HSRB Comments and Science Recommendations 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 (study Section 14.6, p. 24 of 149) and results reported in Table 8 (p. 25 of 149). The mean amount applied was equal to 102% of the target amount with a range of 95-114% 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.

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 they occurred in less than five minutes and these results were reported in the study (Table 11, p. 28 of 149). Because all studies were performed at the same two sites, landing pressure appeared to be fairly consistent across sites, therefore normalization was 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 as directed by the protocol because the test substance contained 15% DEET as the active ingredient. Subjects completed at least three exposure cycles before getting a confirmed landing.

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 greater generalization of results to a 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. That being stated, this study was conducted in one day with 10 subjects at the Wisconsin site and on one day with only 8 subjects at the Florida site. However, this approach was not planned and was described as Protocol Deviation #2 (p. 114 of 149). The data were not analyzed differently and the effect of less than 10 subjects was not discussed.

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 regarding sample size presented in the protocol for testing repellents against ticks to the Human Studies Review Board at the October meeting would apply to these studies.

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 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: Protocol Deviation #2 reported the use of 10 subjects at the Wisconsin site and only 8 subjects at the Florida site. There were no alternates in this study. The data analysis was conducted as directed by the protocol but no discussion of the effect of only 8 subjects on the Median CPT was included.

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 §14.6 (p. 24 of 149). However, one of the applications to a subject exceeded the target amount by >10%.

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 CPT and 95% 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.