March 30, 2016

EPA-HSRB-16-1

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Subject: January 12-13, 2016 EPA Human Studies Review Board Meeting Report

Dear Dr. Burke,

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics reviews of several items, which were reviewed at a meeting of the Board on January 12-13, 2016.

The first item reviewed was a published study entitled Assessing Intermittent Pesticide Exposure From Flea Control Collars Containing the Organophosphorus Insecticide Tetrachlorvinphos (TCVP), by M. Keith Davis, J. Scott Boone, John E. Moran, John W. Tyler and Janice E. Chambers. *Journal of Exposure Science and Environmental Epidemiology* (2008) 18, 564–570. The HSRB was requested to review the scientific and ethical aspects of this published study because of the EPA's interest in relying on some of the data in this publication for agency decision-making.

The other items under review consisted of a series of five completed studies conducted under a common protocol design for field testing of skin-applied mosquito repellent products to support the use of the EPA Insect Repellency Awareness Graphic on product labels for these products.

The HSRB reviewed the scientific and ethical aspects of these five studies, which were conducted by the SC Johnson Company and reported to the Agency in five separate reports.

The Board's key responses to the charge questions are detailed in the enclosed final report of the meeting.

Signed,

Liza Dawson, PhD
Chair
EPA Human Studies Review Board

INTRODUCTION

On January 12-13, 2016, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to several items:

- A published study entitled Assessing Intermittent Pesticide Exposure From Flea Control Collars Containing the Organophosphorus Insecticide Tetrachlorvinphos (TCVP), by M. Keith Davis, J. Scott Boone, John E. Moran, John W. Tyler and Janice E. Chambers. *Journal of Exposure Science and Environmental Epidemiology* (2008) 18, 564–570.

- Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic, GLP Study Number 865E1, J. Palm, September 24, 2015. Test Substance: MARK-3 OFF! Deep Woods Sportsmen Insect Repellent I (Maximum Strength Pump Spray Deep Woods OFF! EPA Reg. No. 4822-276)

- Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic, GLP Study Number 873E1, C. Talbert, October 21, 2015. Test Substance: MARK-8 OFF! Deep Woods Insect Repellent V (OFF! Insect Repellent Formula, EPA Reg. No. 4822-167)

- Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic, GLP Study Number 866E1, E. Laznicka, October 21, 2015. Test Substance: MARK-4 OFF! Active Insect Repellent I (Unscented OFF! Insect Repellent, EPA Reg. No. 4822-380)

- Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic, GLP Study Number 864E1, J. Palm,

September 24, 2015. Test Substance: MARK-2 OFF! Deep Woods Sportsmen Insect Repellent II (UNSCENTED DEEP WOODS OFF! EPA Reg. No. 4822-397)

- Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support Their Use of the EPA Repellency Awareness Graphic, GLP Study Number 867E1, E. Laznicka, October 21, 2015. Test Substance: MARK-5 OFF! Family Care Insect Repellent IV (Unscented) (UNSCENTED OFF! SKINTASTIC SPRAY INSECT REPELLENT, EPA Reg. No. 4822-395)

REVIEW PROCESS

The Board conducted a public meeting on January 12-13, 2016. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA-HQ-ORD-2015-0588).

This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale and consensus in response to each charge question for each of these items.

For each agenda item, Agency staff first presented their review of the science and the Board asked the Agency presenters clarifying questions. The staff then described their review of the ethical aspects and the Board asked clarifying questions with regard to the ethical review. The HSRB solicited public comments and next proceeded to address the charge questions, first discussing scientific review and then ethical review, for each study. The Chair called for a vote to confirm concurrence on a summary statement in response to each charge question.

For their evaluation and discussion, the Board considered materials presented at the meeting, study reports, related materials and documents provided by the study sponsors, the Agency's science and ethics reviews of the studies, as well as oral responses from the study sponsor and protocol teams at the HSRB meeting. A comprehensive list of background documents is available online at http://www.epa.gov/osa/human-studies-review-board.

The HSRB review of each of the six reported studies (one published study and five field testing studies related to the Repellency Awareness Graphic) is presented below. This report presents the board's finding for each study in the order in which the studies were reviewed at the meeting. Because some of the same scientific concerns were raised for several studies over the course of the deliberations, the report makes note of the common issues raised but does not duplicate the discussion of issues when identical concerns were raised several times.

Assessing Intermittent Pesticide Exposure From Flea Control Collars Containing the Organophosphorus Insecticide Tetrachlorvinphos (TCVP), by M. Keith Davis, J. Scott Boone, John E. Moran, John W. Tyler and Janice E. Chambers. *Journal of Exposure Science and Environmental Epidemiology* (2008) 18, 564–570.

Science Review

Charge to the Board

Is this research scientifically sound, providing reliable pet fur transferable residue data for use in evaluating potential exposures of adults and children from contact with pets treated with tetrachlorvinphos containing pet collars?

Board Response

The research is scientifically sound and, if used appropriately, the pet fur transferable residue data from the rubbing protocol used in the study can provide useful information for evaluating potential exposures of adults and children from contact with dogs treated with tetrachlorvinphos containing pet collars.

HSRB Detailed Recommendations and Rationale

The Agency is proposing to use data from Davis et.al, (2008) on the levels of transferable tetrachlorvinphos (TCVP) residue from the fur of dogs treated with flea collars containing 14.55% (4.8 g) of TCVP. The subject of this HSRB review is a published paper from research funded by a USEPA Science to Achieve Results (STAR) grant.[i] The research included children but the Agency is not seeking to use the data collected on children's exposure in this case. Rather, the Agency is interested in using data from assessment of pesticide residues on the dogs' fur that was carried out by adults who were research staff. Therefore, the subject of this HSRB review is limited to a part of the project that measured residue transfers from treated dogs to gloved hand during scripted petting activity by research staff.

The study was designed and conducted in a way that could provide scientifically sufficient data. The paper includes two studies, with the first study using twenty three dogs and the second study using an additional twenty two dogs. The dogs included a variety of breeds (long and short-hair) and sizes (8 – 85 pounds). The first study collected data on residue transfers at nine time points over 112 days (including one pre-application) and the second study measured residue transfers at three time points over 12 days (including one pre-application). Justification by the authors for measuring the F_{ar} at fewer time points during the second study was that the maximum transfer was reached within 12 days of application during the first study. In addition to measuring F_{ar}, both studies included measurement of cholinesterase inhibition in the pets and the second study included measurements of TCVP residue transfer to t-shirts worn by children and metabolites in urine of adults and children. The EPA is only considering use of the data on transferable residue to gloves as a source of "transferable pet fur residue data" (no t-shirt data or biomarker data is to be used).

The experimental methods for determining residue transfer using the scripted petting procedure with gloved hands was well established and consistent with other studies conducted for other active ingredients. The sampling and analysis methods for TCVP were fully described and sufficiently verified. The measurements of the amount of TCVP transferred from treated dogs to gloved hands is reliable but the question is whether the measurements are appropriate for the use that is being proposed by the Agency.

The Agency is proposing to use the average mass of TCVP transferred to gloved hands over a period of four months or 12 days following application as the transferrable residue. The average transferable residue will be divided by the initial mass applied in the collar (4,800 mg) to calculate the available fraction of applied active ingredient (F_{ar}) as described in the EPA Standard Operating Procedures (SOP) for Residential Pesticide Exposure Assessment (October 2012).[ii]

To put the parameter into context, the SOP describes methods for estimating exposure (dermal, inhalation and hand-to-mouth) to pesticides applied to pets, including flea collars (ready-to-use liquid). The relevant equation given in the SOP for post-application exposure assessment is

$$E = TC*TR*ET$$

Where *E* is exposure (mg/day), *TC* is the transfer coefficient (cm^2/h), *TR* is transferable residue (mg/cm^2) and *ET* is exposure time (hours/day). The *TR* parameter gives the amount of active ingredient on the surface of the animal that is available for transfer when the animal is touched.

TR is further defined in the SOP as

$$TR = AR*F_{ar}/SA$$

Where *AR* is the amount of active ingredient applied to the dog (mg), F_{ar} is the fraction of applied active ingredient that is available as transferable residue (unitless) and *SA* is the surface area of the dog (cm^2). The F_{ar} is the specific parameter that the Agency is proposing to estimate using data from Davis et.al. (2008).

Transferable residues were measured using research staff who were students from the college of Veterinary Medicine, Mississippi State University. The students stroked/pet/rubbed the dogs using gloved hands (pre-cleaned white cotton gloves) in a designated area (4 inches by 10 inches) following a predefined motion on the neck (with and without collar present) and on the back near the base of the tail at each sampling time point. Results are reported graphically as the average mass (micrograms) of TCVP transferred to glove during petting for each location and time point.

Flea collars for the Davis et.al (2008) study were purchased new over the counter with all collars from the same lot. The TCVP collars were designed to be used four months per manufacturer recommendations. It is assumed that the collars were used as received (i.e., one size used for all dogs and not cut to fit) so that the application of active ingredients was assumed to be the same for all dogs at 4.8 g per dog. Instructions for flea collars typically requires that the collar be fit to the dog and then the excess collar be trimmed off to get the appropriate application rate for the sized dog. Assuming that the same mass of TCVP is applied can potentially bias the F_{ar} upwards because the circumference and area of the neck of dogs ranging in size from 8 to 85 pounds will vary significantly resulting in a higher mass per area for smaller dogs than larger dogs. It is unclear how this potential difference in loading on the dog's neck will impact the amount or residue transferred to the glove during simulated petting events.

The Agency is proposing to use the data from each study that was reported as average residue on the gloves over all dogs and all time points over the total study period, in the first study for the

period 12 days, and for the second study, 4 months. EPA calculated the average residue by summing the amount measured on the neck with collar and on the back then dividing by the original mass applied. This results in values for F_{ar} of 0.3% and 0.4% for studies 1 and 2, respectively. The amount of TCVP transferred to gloves from the back of the dogs was insignificant (0.01 mg and 0.08 mg) compared to the neck (14.3 mg and 19.0 mg) for studies 1 and 2, respectively so can essentially be ignored in the calculation. Averaging over all the time points in each study biases the F_{ar} value down because the transferable residue is at its maximum around day 7 then declines dramatically over the course of the study. It is likely that the decline in observed transferable residue is due to a decline in the amount of TCVP on the dog over time.

The measurement that is most relevant to the F_{ar} (i.e., the amount transferred relative to the amount applied) would be closer to the initial application date of the collar when the amount applied is still present on the dog. For example, the maximum measured transferable residue was collected on approximately day 7 of the study. It is easier to assume that the total applied amount of TCVP (4,800 mg) is still on the collar and the dog at the time when the maximum transferable residue is measured. Given that the average (across all dogs) mass of TCVP on the gloves at day 7 and day 5 is 24,000 and 22,000 for study 1 and 2, respectively (values estimated from the figures in the paper) and assuming that the total applied mass is still on the dogs at that time, then the resulting F_{ar} would be 0.5%.

There are a number of limitations related to the studies published in Davis et.al. (2008) that are mostly related to a lack of information and details which are not available to the Agency. The limitations identified by the HSRB are listed at the end of this section. However, despite these limitations, the data as presented are sufficiently sound to support an estimate of the F_{ar} if the maximum transferable residue is used. The data is less satisfying for averaging over the duration of each study but can still support screening assessments.

Statistics

The following statistical review assess the adequacy of the methods employed and the scientific validity of the reported data in Davis et al., 2008. Study authors used SAS procedure for general linear models (*PROC GLM*) for glove and t-shirt residues analysis hypothesis testing, mean and confidence interval estimates and mean comparisons based on One-way analysis of variance

(*ANOVA*) of randomized complete block designs (*RCBD*). The *GLM* analysis is appropriate for fixed effects hypothesis testing and mean comparisons. However, *GLM* is not appropriate for estimating residue means and standard errors where random blocks were involved. To estimate correct standard errors in the presence of random blocks, SAS mixed linear models procedure *(PROC MIXED)* should have been used with random blocks. Therefore the reported confidence intervals based on GLM underestimate TCVP residue estimates. However, the Agency is relying solely on the mean estimates.

Urinary TCVP data were not being used by the Agency and so are not addressed here.

In the absence of performing data analysis on the transformed data, the error distribution is assumed to have a normal distribution. This assumption was not validated in this research study. Although the data from T-shirts is not going to be used by the Agency, the presence of extreme large outliers (The higher mean TCVP tee shirt residues on days 9 and 11 result from one very-high residue (17.9 and 17.3 mg/g shirt) obtained from one tee shirt on each of these two sampling days) may suggest the residue distribution may not have a normal distribution. If this assumption is correct then the reported point estimates (treatment means and standard error [SE]) are underestimates and any recommendation based on this study estimate may be incorrect. Since the Agency is using data only from the gloves, and it is unclear whether the presence of outliers in the T-shirt data has implications for the other data that were collected.

The error bars in Figure 1 and 2 are very uniform and consistent across all data points which raises the question as to whether they are overall SE or if they are SE at the given time point across all dogs. It would be more informative to have the error (standard deviation) at each point so data quality decisions can be made particularly if the maximum value will be used rather than the overall average.

Additional notes on scientific issues identified in review of the Davis et.al. (2008) paper:

1. The application rate of active ingredient is assumed to be the same for all dogs regardless of size/weight (i.e., 4.8 grams per dog). The Agency requested information from the principal investigator of the study, but the information is not available.
2. Assuming that the application rate was the same on all dogs, it is unclear how the long tails on the collars were handled on the small dogs and whether the long tail on the collar

could be contacted by the glove during petting. If the collar was tucked in behind and wrapped multiple times around the neck then it would potentially bias the transferable residue upwards.

3. The original data from the study are not available. A lack of information about dog size and individual glove data prevents any statistical assessment of the results beyond what is already reported (which is minimal with respect to the residue transfer data)
4. It is assumed that the five minute petting events remove all of the transferable residue without the gloves becoming saturated and without significant leaching through to the skin of the research staff undertaking the data collection.
5. Information is lacking to assess the relationship between transferable residue and dog size, type, hair which would be important to estimate worst case F_{ar} values.
6. It is unknown whether there is any loss of active ingredient through the cotton gloves to the technicians' hands. This loss would reduce the value of transferable residue and in turn reduce the resulting F_{ar}.

Ethics Review

Charge to the Board

- Does the HSRB have any comments on EPA's determination that the samplers were not human subjects?
- Does the HSRB have any comments on the ethical conduct of the research?

Board Response

With regard to the first charge question, questions were raised by several committee members about the PI's and IRB's determinations that the samplers were not human subjects in the study; rather, they were viewed as study staff. Some members of the board asserted that the student/technicians, by virtue of being potentially exposed to the pesticide as part of the conduct of the study, should have been considered human subjects. Furthermore, if they had been treated as subjects, they might have been considered "vulnerable" due to their status as students. It was noted that the flea control collars were commercially available at the time, and that the potential exposure to the pesticide residues through petting the dogs for five minute periods wearing

cotton gloves was likely much less than average exposure of a pet owner. There is no information available about whether there was any "bleed through" of pesticide from cotton gloves to the skin of the samplers and therefore the actual exposure is unknown. Considering all of these factors, the committee felt that the risks of exposure were not greater than those experienced in everyday life. Thus, even if the determination regarding the status of the samples as study staff rather than subjects was mistaken, the committee did not believe this resulted in any material harms and so this question should not prevent the EPA from using the pet fur transferable residue data derived from the study for making a decision to impose a more stringent regulatory restriction than could be justified without the data.

With regard to the second charge question, Board members observed that the records from correspondence with EPA staff regarding the study suggest the consent form was amended to include disclosure to parents about the risks of pesticide exposure, although the final approved consent form was not provided. A question was raised about the decision made to provide incomplete assent to the minor subjects following parental permission. Study documents suggest this was an intentional choice ("We will not explain the connection to the pesticide residues on the dog…"), which was made, according to study documents, in order to avoid confounding the results by causing alterations in the children's behavior around their dogs. Board members noted that the amount and type of information provided to children in an assent process will vary depending on the age of the child; the children participating in the study were between the ages of 3 and 11 years old and therefore would have had varying levels of capacity to process the information about the study. It was noted that the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which existed at the time of these studies, states that it's unlawful to use any pesticide in tests on humans unless they are fully informed of the nature and purposes of the test. Although some board members viewed the assent as incomplete in this case, because parents are presumed to have given fully-informed permission, and given that the flea control collars were commercially available at the time and already in use in the households recruited to the study, the committee felt that the risks of exposure were not greater than those experienced in everyday life. Thus, the committee did not believe this resulted in any material harms and so this question should not prevent the EPA from using the pet fur transferable residue data derived

from the study for making a decision to impose a more stringent regulatory restriction than could be justified without the data.

HSRB review of five completed studies of insect repellent to support the use of the EPA Repellency Awareness Graphic.

The five completed S.C. Johnson studies each tested a single insect repellent against mosquitoes in the field to determine the median complete protection time (CPT) of five of their skin applied repellent products for the purpose of consumer labelling. The labelling will be done through the EPA Repellency Awareness Graphic,[iii] an EPA program to raise public awareness of the health protectiveness (efficacy) of mosquito and tick repellents applied to the skin, shows the typical length of time the product repels mosquitoes or ticks. The purpose is to increase EPA and consumer confidence in the efficacy claims on labels and improve consumer protection against vector borne diseases, such as West Nile virus and Lyme disease.

Each of the five studies was evaluated individually by the Board and the scientific and ethical aspects of each were assessed. The Board concluded that in each case, data from the studies is sufficiently scientifically sound to be used for the repellency awareness graphic. Conduct of the studies was in accordance with the study protocol in each case. The studies met ethical and regulatory requirements and were conducted under appropriate IRB oversight. However, some deficiencies in design and statistical analysis were noted in scientific review. These were not serious enough to constitute a barrier to the use of data for the repellency graphic, but important enough that the Board included comments in discussion and in this report for the purpose of better design of future studies of this type.

Detailed discussion and consensus points are described for the studies below, but in brief, the main areas of concern were the following:

- No discussion of, or justification for, the lack of a positive control substance in the tests and the lack of an inert substance negative control, in the studies. The issue of positive controls had not been raised during previous HSRB review of these protocols, but had been more recently raised by the HSRB for other repellency awareness studies.

- No discussion of whether additional sites (beyond two sites per study) might be needed to capture the full range of likely outcomes for repellency of the products, for example, the need to measure repellency under different conditions or in the presence of different mosquito species;
- Lack of sample size calculations and lack of scientific justification for the sample size chosen;
- No use of confidence intervals or other mechanism for expressing the degree of variability in the data;
- No analysis of the data collected on variables that may affect study outcomes, such as demographic variables and environmental conditions at the time of testing;
- No use of small sample size tests for measuring secondary questions of interest, for example, whether there is a significant difference in repellency for women versus men using the products;

Field Testing of SC Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic, GLP Study Number 865E1, J. Palm, September 24, 2015. Test Substance: MARK-3 OFF! Deep Woods Sportsmen Insect Repellent I (Maximum Strength Pump Spray Deep Woods OFF! EPA Reg. No. 4822-276)

Science Review

Charge to the Board

Is the study sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

Board Response

The study is sufficiently sound, from a scientific perspective, to be used to estimate the minimum median complete protection time against mosquitoes provided by the tested repellent in support of the EPA Repellency Awareness Graphic.

HSRB Detailed Recommendations and Rationale

The HSRB was charged to conduct a science review of a study testing the efficacy of a 98.25% DEET (diethyl toluamide) formulated as a pump spray against mosquitoes (Mark 3). The board's review focused on the methodology and validity of the data for use to establish median complete protection time (CPT), the unit of measure. The protocol was accepted by EPA on April 23, 2015. Landings of wild mosquitoes on replicate subjects at two different locations were used to evaluate repellency. The experimental design consisted of ten different treated subjects and two untreated controls grouped into pairs and partially randomized based on random selection from a pool of subjects and assigned based on numbers.

The HSRB concluded that the Mark 3 study was sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent. However, several weaknesses in experimental design were identified that detract from the overall quality of the study. The collection of mosquitoes to identify the populations represented in the tested sample was remarkably different between Wisconsin and Florida, with 70 versus 302 mosquitoes collected at the two sites, respectively. Based on feedback provided by the study sponsor, SC Johnson, the mosquitoes collected for sampling were collected by study staff at random and not necessarily by the experimental subjects. As such, no direct correlations could be established between the numbers of species identified in the sample pool relative to the species actually sampled in repellency testing. This weakness leaves open the question that repellency measures are only applicable to selected species and not generalizable to the entire populations present in the sample and challenges statements made in the report suggesting that collected mosquitoes represented those aspirated after the five-minute testing period.

While experimental design elements, such as the number of landings in the five-minute exposure period considered to be the minimum necessary to ensure the mosquito population was large enough to determine repellency or sample size calculations based on studies with different insects, were said to have been derived from historical values and past experience, the lack of

adequate justification and experimental details provided in the report detract from the overall quality of the reporting effort and hamper the ability of external reviewers to complete an independent rigorous evaluation of the experimental approach. In this regard, it should be noted that power calculations using ticks as experimental insects may or may not be applicable to mosquitoes and this area needs to be more rigorously investigated. Another area of concern was that clear distinctions were not made in the report to calculate target concentrations of product from target rates of application, which clearly represent different experimental variables. It should also be noted that while some amendments were made to the study protocol to incorporate EPA's and HSRB's previous recommendations, not all recommendations made were integrated into the final protocol. Also relevant to the present set of studies are concerns about lack of positive controls. Comments on positive controls were made previously by the HSRB on a different protocol addressing ticks (October 2015). For future studies of this type, the HSRB recommends additional efforts to integrate positive and negative inert substance controls into the experimental design or else provide clear scientific justification for their absence. Furthermore, SC Johnson's handling of response to feedback provided regarding measurement of landing pressures and generalizability of environmental conditions was not optimal.

Statistics

The purpose of the proposed study was to determine the complete protection time of up to 18 EPA-registered S.C. Johnson skin-applied repellent products. The resulting data are intended to support the products' use of EPA's repellency awareness graphic on the product labels.

The statistical issues listed above as general issues for all five studies were raised for this product.

SC Johnson responded to the statistical issues raised in previous reviews including experimental design, randomization, sample size calculation, sources of variation, and data compiling/processing. The responses are deemed to be adequate for the purpose of using the data for the repellency graphic, although as noted above, there some areas of study design and analysis that could be improved for increased scientific rigor.

Ethics

Charge to the Board

Does available information support a determination that the study was conducted in substantial compliance with 40 CFR Part 26, subparts K and L?

Board Response

The information provided supports a determination that the studies were conducted in substantial compliance with subparts K and L of 40 CFR Part 26.

HSRB Detailed Recommendations and Rationale

40 CFR 26 subpart K requires that studies initiated on or after April 7, 2006 involving intentional exposure of human subjects to a pesticide be reviewed and approved by an institutional review board (IRB) that meets the membership and review criteria listed in that subpart. The study listed above was approved by Schulman Associates Institutional Review Board (SAIRB) on 7/7/15 after the protocol was revised per EPA and HSRB recommendations. The study team maintained SAIRB approval throughout the study.

40 CFR 26 subpart K mandates studies minimize risk to subjects, equitably select subjects, seek and appropriately document informed consent, make adequate provisions to ensure safety of subjects, and protect the privacy of subjects and confidentiality of data. The study listed above minimized risk to subjects by adequately training subject to remove mosquitos before biting occurred and either using lab-reared mosquitos (at the Wisconsin site) or monitoring appropriate reporting agencies to determine that mosquito-borne diseases have not been reported by health authorities in the previous month (at the Florida site). Subjects were equitably selected from maintained list of recruiting firms in Wisconsin (J. Reckner Associates, Inc) and Florida (Herron Associates, Inc) to represent the demographics of US repellent users. The Study Director or Principal Investigator explained the study to the subjects at the training session, at which time subject's questions were answered and consent was documented. Provisions to ensure safety and

comfort included use of bug suits to prevent mosquito biting, monitoring of the Florida site for other environmental nuisances, food and beverages, and seating. Privacy and confidentiality were maintained through the use of assigned code numbers, maintenance of records on a password-protected computer server, and limiting access to records.

40 CFR 26 subpart L prohibits the EPA from relying on third-party research involving intentional exposure to a pesticide of human subjects who are children or pregnant or nursing women. Materials submitted to EPA and HSRB indicate that no children or pregnant or nursing women participated in this study. The study's inclusion criteria required that subjects be 18-55 years of age and able to provide valid proof of their age (such as a driver's license or passport). Female subjects completed pregnancy tests in a private bathroom on the training day, which was within 48 hours of the study day. Women who chose not to participate in the study after taking the pregnancy test were not asked to provide a reason for their decision not to participate. However, if the subject remained interested in study participation after taking the pregnancy test, a female study team member verified the negative pregnancy test. The pregnancy test results were not recorded and remained confidential. As a result, the study was conducted in substantial compliance with 40 CFR part 26, subpart L.

Field Testing of SC Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic, GLP Study Number 873E1, C. Talbert, October 21, 2015. Test Substance: MARK-8 OFF! Deep Woods Insect Repellent V (OFF! Insect Repellent Formula, EPA Reg. No. 4822-167)

Science Review

Charge to the Board

Is the study sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

Board Response

The study is sufficiently sound, from a scientific perspective, to be used to estimate the minimum median complete protection time against mosquitoes provided by the tested repellent in support of the EPA Repellency Awareness Graphic.

HSRB Detailed Recommendations and Rationale

The HSRB was charged to conduct a science review of a study testing the efficacy of a 25% DEET (diethyl toluamide) formulated as pressurized aerosol against mosquitoes (Mark 8). The board's review focused on the methodology and validity of the data for use to establish median complete protection time (CPT), the unit of measure. The protocol was accepted on April 23, 2015. Landings of wild mosquitoes on replicate subjects at two different locations were used to evaluate repellency. The experimental design consisted of ten different treated subjects and two untreated controls grouped into pairs and partially randomized based on random selection from a pool of subjects and assigned based on numbers.

The strengths and weaknesses in experimental design identified for the Mark 3 study are applicable for the present study. Of note, as stated in the review of the Mark 3 study, are concerns related to the absence of sample size calculations and robustness of the scientific information provided in the report. In this study, there were also questions about the effect of a rain delay on variability of the findings reported. At one site, rainy weather led to the cancellation of two exposure periods on a test day. The resulting calculations of CPT for that day entailed estimating time to first confirmed landing based on the timing of the first cancelled exposure period. The net effect of this estimation on the variability in the data is unclear.

Statistics

SCJ responded to some of the statistical design and analysis issues raised by the Board in its previous review but their response is not clear on some issues. For example, SCJ did discuss sample size calculations from studies in the literature (pages 94-97 of the Final Study Report).[iv]

However, those reported studies were reporting means and not medians, which for skewed distributions can be quite different. The final sample size requirement in the present study was apparently chosen based at least partially on ethical considerations—that is, with the objective of minimizing the number of subjects exposed to mosquitoes. A complete scientific rationale for sample size was not provided.

Other recommendations raised in the Board's previous review, such as analyzing the effect of demographic characteristics and environmental conditions, were not taken up. These variables (demographics, environmental conditions) were collected and reported but did not appear to be utilized in the analysis in any way.

SCJ reported estimates of the smallest median CPT at each site as well as the overall smallest median CPT. For this product, choosing the smaller of the two site smallest median CPTs produced the same result as would be have been obtained as the smallest median CPT of the combined sample after rounding downward. It appears that the smaller of the two site median CPT values were used by SCJ (page 99 of the Final Report). SCJ confirmed this to be their approach.

Since the objective was to produce a single estimate without any indication of a standard error or confidence limits, the statistical analyses reported by SCJ would be deemed to be adequate.

Ethics Review

Charge to the Board

Does the available information support a determination that the research was conducted in substantial compliance with 40 CFR part 26, subparts K and L?

Board Response

Available information indicates the research was conducted in substantial compliance with applicable provisions of 40 CFR part 26 subparts K and L.

HSRB Detailed Recommendations and Rationale

Study maintained approval from an IRB operating in accordance with 40CFR26. Because this study was initiated after April 7, 2006, submission of the protocol and supporting materials to EPA prior to conducting the study was required and appropriately completed.

The study excluded children. No pregnant or nursing female subjects participated in the study.

Field Testing of SC Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic, GLP Study Number 864E1, J. Palm, September 24, 2015. Test Substance: MARK-2 OFF! Deep Woods Sportsmen Insect Repellent II (UNSCENTED DEEP WOODS OFF! EPA Reg. No. 4822-397)

Science Review

Charge to the Board

Is the study sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

Board Response

The study is sufficiently sound, from a scientific perspective, to be used to estimate the minimum median complete protection time against mosquitoes provided by the tested repellent in support of the EPA Repellency Awareness Graphic.

HSRB Detailed Recommendations and Rationale

The HSRB was charged to conduct a science review of a study testing the efficacy of a 30% DEET (diethyl toluamide) formulated as pressurized aerosol against mosquitoes (Mark 2). The board's review focused on the methodology and validity of the data for use to establish median complete protection time (CPT), the unit of measure. The protocol was accepted by EPA on April 23, 2015. Landings of wild mosquitoes on replicate subjects at two different locations were

used to evaluate repellency. The experimental design consisted of ten different treated subjects and two untreated controls grouped into pairs and partially randomized based on random selection from a pool of subjects and assigned based on numbers.

The strengths and weaknesses in experimental design identified for the Mark 3 and Mark 8 studies are applicable for the present study. Of note are concerns related to the absence of sample size calculations and robustness of the scientific information provided in the report. Given that the Mark 8 and Mark 2 studies both examined pressurized aerosols at different concentrations, comparisons between the two studies should be made to gain additional insight into the validity and comparability of findings.

Statistics

The purpose of the proposed study is to determine the complete protection time of up to 18 EPA-registered S.C. Johnson skin-applied repellent products in a laboratory setting. The resulting data are intended to support the products' use of EPA's repellency awareness graphic on the product labels.

Four statistical issues about the protocol were raised.

SC Johnson responded to the statistical issues raised in previous reviews including experimental design, randomization, sample size calculation, sources of variation, and data compiling/processing. The responses are deemed to be adequate for the purpose of using the data for the repellency graphic, although as noted above, there are some areas of study design and analysis that could be improved for increased scientific rigor.

Ethics review

Charge to the Board

Does available information support a determination that the study was conducted in substantial compliance with 40 CFR Part 26, subparts K and L?

Board Response

The information provided supports a determination that the studies were conducted in substantial compliance with subparts K and L of 40 CFR Part 26.

HSRB Detailed Recommendations and Rationale:

40 CFR 26 subpart K requires that studies initiated on or after April 7, 2006 involving intentional exposure of human subjects to a pesticide be reviewed and approved by an institutional review board (IRB) that meets the membership and review criteria listed in that subpart. The study listed above was approved by Schulman Associates Institutional Review Board (SAIRB) on 7/7/15 after the protocol was revised per EPA and HSRB recommendations. The study team maintained SAIRB approval throughout the study.

40 CFR 26 subpart K mandates studies minimize risk to subjects, equitably select subjects, seek and appropriately document informed consent, make adequate provisions to ensure safety of subjects, and protect the privacy of subjects and confidentiality of data. The study listed above minimized risk to subjects by adequately training subject to remove mosquitos before biting occurred and either using lab-reared mosquitos (at the Wisconsin site) or monitoring appropriate reporting agencies to determine whether any mosquito-borne disease had been reported in the previous month (at the Florida site). Subjects were equitably selected from maintained list of recruiting firms in Wisconsin (J. Reckner Associates, Inc) and Florida (Herron Associates, Inc) to represent the demographics of US repellent users. The Study Director or Principal Investigator explained the study to the subjects at the training session, at which time subject's questions were answered and consent was documented. Provisions to ensure safety and comfort included use of bug suits to prevent mosquito biting, monitoring of the Florida site for other environmental nuisances, food and beverages, and seating. Privacy and confidentiality were maintained through the use of assigned code numbers, maintenance of records on a password-protected computer server, and limiting access to records.

40 CFR 26 subpart L prohibits the EPA from relying on third-party research involving intentional exposure to a pesticide of human subjects who are children or pregnant or nursing women. Materials submitted to EPA and HSRB indicate that no children or pregnant or nursing women participated in this study. The study's inclusion criteria required that subjects be 18-55 years of age and able to provide valid proof of their age (such as a driver's license or passport). Female subjects completed pregnancy tests in a private bathroom on the training day, which was within 48 hours of the study day. Women who chose not to participate in the study after taking the pregnancy test were not asked to provide a reason for their decision not to participate. However, if the subject remained interested in study participation after taking the pregnancy test, a female study team member verified the negative pregnancy test. The pregnancy test results were not recorded and remained confidential. As a result, the study was conducted in substantial compliance with 40 CFR part 26, subpart L.

Field Testing of SC Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic, GLP Study Number 866E1, E. Laznicka, October 21, 2015. Test Substance: MARK-4 OFF! Active Insect Repellent I (Unscented OFF! Insect Repellent, EPA Reg. No. 4822-380)

Science review

Charge to the Board

Is the study sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

Board Response

The study is sufficiently sound, from a scientific perspective, to be used to estimate the minimum median complete protection time against mosquitoes provided by the tested repellent in support of the EPA Repellency Awareness Graphic.

HSRB Detailed Recommendations and Rationale

The protocol for the field testing of Mark-4 OFF! Active Insect Repellent I (Unscented OFF! Insect Repellent, 15% DEET) was reviewed by HSRB at the April 2015 meeting. The Board concluded that the protocol titled "Field Testing of S.C. Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic", if modified according to Agency and HSRB recommendations, is likely to generate scientifically reliable data, useful for estimating the complete protection time as defined in the protocol, of various EPA-registered S.C. Johnson skin-applied mosquito repellents in the field against wild adult mosquito populations. The protocol was amended to incorporate EPA and HSRB recommendations. The study was completed following the approved protocol with one amendment and four reported deviations. One deviation listed in the study report was actually an IRB approved protocol amendment to change the study director for the study, and hence was not technically a deviation. Another deviation was the use of light meters and stopwatches that were not GLP-qualified. However the devices are NIST certified and so are suitable and adequate for study procedures. There were two deviations in data collection procedures: one site had fewer subjects than planned with an unbalanced (2 males and 6 females design; and one exposure period was skipped due to rain. An analysis was conducted to show that the deviations had no detectable impact on the outcome. As a result, the data produced are sufficiently sound, from a scientific perspective, to support a median CPT of 5 hours against mosquitoes for the EPA Repellency Awareness Graphic.

Statistics

SCJ responded to some of the statistical design and analysis issues raised by the Board in its previous review but their response is not clear on some issues. For example, as with the studies reviewed earlier in the meeting, SCJ did not carry out a formal sample size calculation but relied on the same justification based on the literature despite its questionable applicability (pages 95-96 of the Final Report). As before, demographic characteristics and environmental conditions were reported but did not appear to be utilized in any way.

A test for a difference in median CPT between males and females was conducted using a combined set of data from the five products in this review plus one additional product using the Wisconsin test site data (page 117 of the Final Report). The results indicated no significant difference. While useful, the analysis does not directly address differences for MARK-4 as a single product. Small sample size tables for the Mann-Whitney statistic are widely available and could have been utilized. In fact, a test of these data from MARK-4 using the Wilcoxon rank sum version of the Mann-Whitney finds a significant difference between males and females at the 5% level.

SCJ reported estimates of the smallest median CPT at each site as well as the overall smallest median CPT. For this product, choosing the smaller of the two site smallest median CPTs produced the same result as would be have been obtained as the smallest median CPT of the combined sample after rounding. SCJ indicated during the meeting the approach was to choose the smaller site median CPT.

Table 2 (page 9 of the Final Study Report) containing the individual subject durations indicates large site differences in the durations. While using the smaller site median as the final CPT is the conservative approach, the apparent large site to site variability would lead one to question whether or not sites with much smaller medians CPTs might exist.

Since the objective was to produce a single estimate without any indication of a standard error or confidence limits, the statistical analyses reported by SCJ would be deemed to be adequate for this purpose.

Ethics review

Charge to the Board

Does the available information support a determination that the research was conducted in substantial compliance with 40 CFR part 26, subparts K and L?

Board Response

The research was conducted in substantial compliance with applicable provisions of 40 CFR part 26, subparts K and L.

HSRB Detailed Recommendations and Rationale

40 CFR 26 subpart K requires that studies initiated on or after April 7, 2006 involving intentional exposure of human subjects to a pesticide be reviewed and approved by an institutional review board (IRB) that meets the membership and review criteria listed in that subpart. The study listed above was approved by the Schulman Associates convened IRB (SAIRB) on July 7, 2015 after the sponsor revised study materials in light of EPA and HSRB recommendations. The study team maintained SAIRB approval throughout the conduct of the study. The letter to subjects notifying about birds testing positive for West Nile Virus being detected in the county where the research took place also received SAIRB approval. SAIRB meets the appropriate membership criteria and followed review criteria listed in this subpart. As a result, the study was conducted in substantial compliance with 40 CFR part 26, subpart K.

40 CFR 26 subpart L prohibits the EPA from relying on third-party research involving intentional exposure to a pesticide of human subjects who are children or pregnant or nursing women. Materials submitted to EPA and HSRB indicate that no children or pregnant or nursing women participated in this study. The study's inclusion criteria required that subjects be 18-55 years of age and able to provide valid proof of their age (such as a driver's license or passport). Female subjects completed pregnancy tests in a private bathroom within 48 hours of the study training day. Women who chose not to participate in the study after taking the pregnancy test were not asked to provide a reason for their decision not to participate. However, if the subject remained interested in study participation after taking the pregnancy test, the negative pregnancy test was verified by a female study team member. The pregnancy test results were not recorded and remained confidential. As a result, the study was conducted in substantial compliance with 40 CFR part 26, subpart L.

Field Testing of SC Johnson Personal Mosquito Repellent Products to Support their Use of the EPA Repellency Awareness Graphic, GLP Study Number 867E1, E. Laznicka, October 21, 2015. Test Substance: MARK-5 OFF! Family Care Insect Repellent IV (Unscented) (UNSCENTED OFF! SKINTASTIC SPRAY INSECT REPELLENT, EPA Reg. No. 4822-395)

Science Review

Charge to the Board

Is the study sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

Board Response

The study is sufficiently sound, from a scientific perspective, to be used to estimate the minimum median complete protection time against mosquitoes provided by the tested repellent in support of the EPA Repellency Awareness Graphic.

HSRB Detailed Recommendations and Rationale

The protocol for the field testing of MARK-5 (Unscented OFF! Skintastic Spray Insect Repellent 7% DEET) was reviewed by HSRB, and the protocol team responded to EPA and HSRB recommendations. The study was completed following the EPA-approved protocol, and the data produced are sufficiently sound, from a scientific perspective, to support a median CPT of 2 hours against mosquitoes for the EPA Repellency Awareness Graphic.

Statistics

The stated study objective is to establish the median complete protection time (CPT) against mosquitoes for use in the EPA Repellency Awareness Graphic on the label of EPA Registered products. The study was completed at two locations a planned and the median CPTs with 95% confidence limits [Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL)] were calculated for each location by Kaplan Meier analysis using PROC LIFETEST in SAS, which employs a generalization of the Brookmeyer and Crowley (1982)[v] method under a log-log transformation. The statistical design used and analysis performed are adequate for the proposed study goal. However, the analysis can be further enhanced by performing stratified analysis by sites using the STRATA statement in SAS PROC LIFETEST. This stratified analysis will provide the separate median CPT and CI estimates for each site but then further statistically test

whether these CPT values are statistically similar to each other. If the CPT times are determined to be statistically similar then a pooled CPT estimates over the two sites can be derived providing a larger sample size and increased confidence.

Ethics Review

Charge to the Board

Does the available information support a determination that the research was conducted in substantial compliance with 40 CFR part 26, subparts K and L?

Board Response

The research was conducted in substantial compliance with applicable provisions of subparts K and L of 40 CFR 26, met all applicable ethical standards for the protection of human subjects of research, and satisfied the requirements for documentation of ethical conduct of research.

HSRB Detailed Recommendations and Rationale

The purpose of this study was to establish the complete protection time (CPT) of MARK-5 OFF! Testing took place at two field sites (Wisconsin and Florida) with 24 adult (ages 18-55) human subjects who completed the testing (12 at each site). The repellent test products are registered by EPA and have been found to present little or no risk when used as directed. The protocol for this study was approved by the Schulman Associates Institutional Review Board (SAIRB). The protocol was discussed in a public meeting by the Human Studies Review Board (HSRB) on April 22-23, 2015, which concluded that "the amended protocol … should meet all applicable ethical standards."

A third-party recruiting firm was used to identify potential subjects. Subjects who met the inclusion/ exclusion criteria and were available for both the training and test dates were selected for the studies (20 at Florida and 24 at Wisconsin). Pregnancy testing was performed by potential

female subjects alone in a private bathroom. The results were reported/verified by the subject only.

Consent was obtained before beginning the procedures training session. Subjects were paid $60 for participating in a 3-4 hour training session conducted prior to the field testing. For each field test day, subjects were paid $15 per hour for the test day. Subjects were advised on several occasions that they could withdraw from the study for any reason, without penalty. No subjects withdrew. Risks were appropriately identified and precautions were taken to mitigate identified hazards; e.g. the study was conducted in areas where the presence of mosquito-borne disease had not been detected by county or state health staff or mosquito abatement district staff within one month prior to the test date. Starting thirty minutes after the test substance application to the forearm, there were five minute exposures to mosquitoes in the field at 30 minute intervals.

Subpart K (Third-Party Human Research for Pesticides):

Study maintained approval from an IRB operating in accordance with 40CFR26. Because this study was initiated after April 7, 2006, submission of the protocol and supporting materials to EPA prior to conducting the study was required and appropriately completed.

Subpart L (Prohibitions):

Study excluded children. No pregnant or nursing female subjects participated in the study.

NOTICE

This report has been written as part of the activities of the EPA Human Studies Review Board, a Federal advisory committee providing advice, information and recommendations on issues related to scientific and ethical aspects of human subjects research. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the view and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does the mention of trade names or commercial products constitute a recommendation for use. You may obtain further information about the EPA Human Studies Review Board from its website at http://www.epa.gov/osa/hsrb. You may also contact the HSRB Designated Federal Officer, via e-mail at ord-osa-hsrb@epa.gov

In preparing this document, the Board carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented within the structure of the charge by the Agency.

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[i] EPA Grant Number: R828017; Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides from the Fur of Dogs;
https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/779/report/0

[ii] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide#sops

[iii] http://www.epa.gov/insect-repellents/repellency-awareness-graphic

[iv] Final study report, Mark 8

[v] Brookmeyer, R. and Crowley, J. (1982. A confidence interval for the median survival time. Biometrics 38: 29-41.