11/25/15

Supplemental Information for Human Studies Review Board (HSRB)

For Discussion regarding i2LResearch Tick Protocol

EPA is providing this supplemental information to the Human Studies Review Board (HSRB) in preparation for its December 7, 2015 discussion. The October 20, 2015 HSRB meeting focused on the i2LResearch protocol for testing of S.C. Johnson's personal tick repellent products to support their use of the EPA Repellency Awareness Graphic. As you know, the proposed testing approach is to treat 10 subjects per test with the test substance; one arm from each subject will be treated with the repellent product to be tested and the other arm will serve as the untreated control. This document provides additional information to the HSRB regarding the basis for the proposed design of the i2LResearch tick protocol as it relates to use of the untreated control arm.

A. Negative and Positive Controls

As you know, the Office of Pesticide Programs (OPP) has relied on and followed advice from the HSRB for many years. During the October 2006 HSRB meeting, the HSRB approved revised tick and mosquito protocols which included the use of the untreated arm of each subject as a "negative control", an approach which has been effectively used for the last eight years. Subsequent to the October 2006 HSRB meeting, every insect repellent protocol brought before the HSRB that focused on a tick study in a laboratory or a mosquito or black fly study in the field used an untreated control to qualify ticks or monitor mosquito landing pressure.

Some history on the development of the approaches used today may be helpful to the HSRB. When EPA briefed the Scientific Advisory Panel (SAP) in 2000, the SAP recommended tick field studies with % repellency as the unit of measure. The USDA-Agricultural Research Service (ARS) developed a finger test in which a line is drawn at each joint of the index finger. The USDA finger test is referenced in the attached published article entitled, "*Activity of Repellents Applied to Skin for Protection against Amblyomma Americanum and Ixodes Scapularis Ticks (Acari: Ixodidae).*" The test involving Ixodes Scapularis ticks calls for the repellent to be applied to the first and third joint area. Ticks are placed in the middle of the two finger joints. If the ticks don't leave the untreated middle joint area they are considered repelled; if they do, they are not. With this test, most repellents fail in a short time. Likewise, the USDA test was adopted to evaluate tick behavior by having them crawling up the finger and crossing lines from untreated to treated areas of the finger, which is similar to what is being done in the i2LResearch tick protocol. It may take some time for a tick to be affected by a repellent because their exoskeletons are far less permeable and they detect host scents differently. If you use the length of a finger, you don't see the same response that you will observe over the greater length of the arm. USDA also developed a method in which a subject's foot is placed in a tub of 100 ticks and the number advancing up the leg is counted. Untreated and treated legs are compared. That method is referenced in the attached published study entitled, "*Formulations of Deet, Picaradin, and IR3535 Applied to Skin Repel Nymphs of the Lone Star Tick (Acari: Ixodidae) for 12 Hours.*" The purpose of the Lone Star tick study was to evaluate DEET alternatives and compare four products against the military repellent. The control regime was an untreated negative control.

OPP scientists think that the untreated arm fulfills the role of a negative control in a tick repellency study. The rationale for using untreated controls is to allow comparison of tick behavior in the presence and absence of the test substance, with all other factors being held constant to the greatest degree possible. The use of each test subject as their own control will allow a very tight comparison of tick activity on the untreated and treated skin, where there are no other differences between the treated and untreated forearms. As discussed in section 7.5 of the draft i2LResearch protocol and in EPA's associated science review, four

lines (a release line, boundary line, crossing line, and upper boundary line) will be drawn on both of the subject's forearms (the treated and untreated forearms) to assess the tick crossing. OPP continues to support the use of the untreated arm in fulfilling the role of a negative control.

In the aforementioned 2006 HSRB meeting, the Board also recommended against having a positive control and repellent studies since 2006 have not included a positive control. Positive controls typically are used to verify that the test system is capable of measuring a response. This has not been an issue for repellent efficacy studies. If the Agency used 20% DEET in ethanol as the positive control, how would EPA use with the resulting data to justify use of the EPA repellency awareness graphic on a specific product? If the Complete Protection Time was greater than another product, for example a 30% DEET product, that would not affect the scientific conclusions associated with the focus of the i2LResearch tick protocol or the content of the repellency awareness graphic for the 30% DEET product. What would we learn from the positive control as it relates to the repellency awareness graphic, which is the underlying reason for the study? The formulation of the insect repellent affects efficacy because it affects the release rate of the active ingredient when applied to the skin. Additionally, the researcher would not be able to test the same ticks in the negative, positive control and treatments. EPA needs the negative control to qualify the ticks. Tick behavior and response is a much different system than the other test systems with which the HSRB may be more familiar. The test system proposed has been used in a number of laboratories for decades to evaluate repellency against ticks.

Positive controls are also used to establish a benchmark for comparison of results from different studies. However, the i2LResearch protocol and proposed study will not directly compare products or be used to make comparative claims. The study is only evaluating the duration of efficacy for individual products and EPA is not comparing one product to another product.

With regard to studies in general, EPA recognizes that there may be variability from study to study. However, the i2LResearch study is not being conducted in the field. The same i2LResearch laboratory is being used for all of the studies being conducted under the proposed protocol. We do not anticipate that conditions in the laboratory will affect the duration of repellency.

In summary, given the purpose of the proposed studies, the history of this topic and EPA's experience with the proposed approach, OPP considers that a test in which each subject serves as his own control (using one arm for the untreated, negative control and one arm for the test) is an appropriate design that is statistically sound and will generate reliable results.

The only endpoint which EPA requires to be measured in the tick repellent study is the duration of efficacy. The unit of measure for determination of the repellent effects is Complete Protection Time (CPT). The CPT for each tick species will be calculated as the time from test substance application to the time of the First Confirmed Crossing. The time in hours for each individual test subject will be used to calculate the median protection time for each species separately. EPA's perspective is that there is no need to measure other endpoints given the underlying objective for the proposed study.

B. Timing of Pregnancy Testing

At the HSRB's April 22-23, 2015 meeting, the HSRB discussed S.C. Johnson's protocol for testing mosquito repellent products to support the use of EPA's repellency awareness graphic. During the April 2015 HSRB meeting, SC Johnson's proposal for pregnancy testing within 48 hours prior to the repellency test was approved by the HSRB without comment. S.C. Johnson is also the sponsor for the tick protocol brought before the board during the October 2015 meeting. EPA anticipates that both the Agency and sponsor will try to address the HSRB's new concerns discussed at the October 2015 HSRB meeting. OPP

does not foresee a problem with giving a female participant the option of taking a pregnancy test either 48 hours prior to the test or on the day of the test; the HSRB, OPP and the study sponsor all recognize that same-day pregnancy testing will increase the duration of an already lengthy test day.

OPP, however, does have concerns regarding the counseling suggestion during the last HSRB discussion. Regarding the pesticides which are the focus of the tick protocol, if EPA believed that the use of the pesticides according to their approved label directions presented a health concern to the U.S. population, including reproductive effects, EPA would not have registered the pesticides. The Reregistration Eligibility Decision for DEET and EPA fact sheets on picaridin and p-Menthan-3, 8 diol are all publicly available for reference by the HSRB. Given the minimal risk, EPA thinks there is no need to counsel female participants to avoid behaviors that might lead to pregnancy as a precaution before participating in the research. Instead, OPP believes that the study sponsor should notify interested participants of available options for taking a pregnancy test within the context of the study.

EPA is sharing this information with the HSRB in preparation for the HSRB's December 7, 2015 public discussion. We appreciate the HSRB reviewing this information prior to the next discussion.