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Answers to EPA HSRB Questions on Spatial Repellents
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These answers are given from the professional estimation of the author and do not intend to represent the position or policy of USDA.

Environmental Issues

1. What are the environmental (temperature, wind, time of day, humidity, proximity to water/plants, size and type of space) and human factors (height/weight; gender, age, ethnicity, density of humans in space) that can affect insect behavior and repellent efficacy relevant to space treatment studies?

Environmental factors have a strong effect on avidity of biting arthropods. The effects will depend greatly on the species of arthropod. For the best known arthropods, the parameters of maximum avidity are known and could be replicated in the lab or sought in the field. Also, use of controls will assure adequate avidity has been achieved. One environmental factor that is particularly important to the function of most spatial repellents is wind. Even light air movement will affect performance of products that are based on dispersal of a chemical in a large volume of air.

Humans vary a great deal in attractiveness to biting arthropods. This variation is affected by gender and diet, but these relationships are not well understood. Conservatively, the amount of variation is four-fold (attracts four time more mosquitoes) and translates to approximately 25% decrease in duration of DEET topical repellent. Presumably, this could affect performance of behavior-altering active ingredients but not that of lethal active ingredients.

2. What factors need to be considered for test spaces with respect to size of area in which the test is conducted? How is the most appropriate test area determined?

The test area should exceed the label claim for area protected. An ideal test would evaluate the area protected by measuring the occurrence of pests across a transect.

3. Does the number of human subjects within testing environments of different sizes affect insect activity? Does the number of subjects in a given area affect product efficacy or the measurement of product efficacy?

In general, results from field tests are unaffected by the number of subjects as long as the density of pests is sufficient. Often, the local population of pests is so high that additional traps or subjects do not affect results from each device or person. In the laboratory or large cage trials,

where populations are limited and controlled, additional traps or human bait subjects might reduce the number collected by each trap or person.

4. Are there any other special considerations regarding insect behavior in such studies that require inclusion in protocols?

If the product affects a specific behavior or a particular odorant receptor, then the behavioral status of the pest may affect performance. For example, if a chemical blocks a carbon dioxide receptor but the pest is not seeking a blood meal, then the product may appear to fail (e.g., pest goes through the repellent to a light trap) even though it would work well for its purpose (to prevent bites).

Statistics and Study Design

1. How is the location of open spaces typically selected? How many different or similar types of sites are appropriate to assess generalizability?

Locations are usually selected for the species and abundance of the pest. There is a danger that sites with low numbers of pests will be selected intentionally to make the product look more effective. In practice, there is probably a greater tendency to choose sites with a large number of pests in order to reduce the number of trials required in order to attain statistical significance.

The location should also be typical of the intended use site. For example, a mosquito coil or candle would be appropriately tested on an actual patio representing typical use site. If the product is registered for outdoor use only, it should be tested outdoors in a field or semi-field (large cage and/or intentional release of pests) situation.

The number of different sites is not as important as the number of kinds of pests in order to achieve generalizable results. These kinds of products are also very sensitive to wind, which might influence the number of trials required and selection of weather conditions.

2. What are common spatial dispensing devices? How are they related to the nature of the product dispensed (e.g., gas, suspended liquid, smoke)? What are design or measurement challenges for different dispensing devices and products?

Current products registered in the US include candles, mosquito coils, heated paper mat devices, reservoirs of active ingredient with a fan above them, granular products impregnated with active ingredients, liquid sprays, and no-pest strips. All attempt to suspend an active ingredient in the air, creating a gaseous or fine particle barrier between the human and the pests, or to create a bubble of active ingredient around the human. Only the no-pest strip is currently registered for indoor use. The product is designed to distribute the active ingredient at an even rate over a defined period of time. Heated devices probably deliver the most constant rate, since they are independent of ambient temperature. Passive systems like granules, sprays, and no-pest strips would release more active ingredient during higher temperatures and when there is more wind. These factors will make evaluation of duration more difficult.

3. What type of dosimetry data is required to determine amount of product application used in testing? How is discharge time determined? What are the relative design merits of the experimenter or subject discharging the repellent?

For the most part, the rate of release from spatial repellent products is not adjustable. Where the amount applied can be adjusted or the number of devices used simultaneously can be changed, the tests should follow proposed label rates. Discharge time is not a factor for currently designed devices. Installation and discharge by the experimenter may have a small advantage over that by the subject in order to reduce trial to trial variation.

4. How are outcomes measured in these studies? How are insect knockdown and mortality effects measured? Are both knockdown and landings/bites usually measured in the same study? What is the difference in knockdowns vs. bites in terms of information regarding product efficacy/effectiveness?

There is no standard method of measuring outcome. Mosquito coils were most commonly evaluated by measuring knockdown in cages, but this is probably inappropriate. The purpose of spatial repellents is to interrupt the pest on its way toward a host and that is what should be measured. A test of movement toward a host or attractant should be sufficient, eliminating the need to expose subjects to biting. An example would be current trials at USDA ARS Gainesville testing efficacy of proprietary devices inside a tent; about 18 small traps inside the tent to detect distribution under treated and untreated conditions; each trial is conducted with tent inside large cage with known number of mosquitoes.

5. What is the difference with respect to measurement in assessing efficacy of the active ingredient and effectiveness of the formulation?

The purpose of these tests is to compare or document products rather than active ingredients. What is more, formulation is likely to have a large influence on effectiveness. Therefore, tests of non-formulated active ingredients would be largely irrelevant.

Sample Size and Statistics

1. Depending on the outcome measure, what are best practices with respect to human sample size? What is the sample size norm in the field? How is determination of sample size related to square feet of test area? What is the best way to determine power for these studies?

Spatial repellents by their nature can protect one or many people simultaneously. However, if humans are used as bait, it is important to test a number of people of both genders to get representative results. Using more than one person at once would not accomplish this. No best practices have been established. For examples, please refer to the book chapter; best studies were against sand flies with ThermoCell, and two papers with Wirtz as author using Mosquito Beater:

B. Alten et al., Field evaluation of an area repellent system (Thermacell) against *Phlebotomus papatasi* (Diptera: Psychodidae) and *Ochlerotatus caspius* (Diptera: Culicidae) in Sanliurfa Province, Turkey, *J. Med. Entomol.*, 40, 930, 2003.

R. A. Wirtz, J. D. Turrentine, Jr., and L. C. Rutledge, Mosquito area repellents: Laboratory testing of candidate materials against *Aedes aegypti*, *Mosq. News*, 40, 432, 1980.

R. A. Wirtz, J. D. Turrentine, Jr., and R. C. Fox, Area repellents for mosquitoes (Diptera: Culicidae): identification of the active ingredients in a petroleum oil fraction, *J. Med. Entomol.*, 18, 126, 1981.

2. What are best practices with respect to statistical analysis? How is censored data handled?

I am not aware of any best practices. Please refer to the book chapter.

3. What are the pros and cons of various endpoints (e.g. ending the study after a set number of hours, waiting until the first landing/bite, other) to assess product efficacy (e.g. to meet assumptions for appropriate statistical analyses)?

Since human exposure to bites is not necessary, there is no reason not to test products to failure in order to develop a complete chronological curve of effectiveness. Many of these products do not achieve complete protection, implying that the best measure is a profile of percentage repellency over the period of time to complete failure.

Human Subjects

1. Why are human subjects necessary for such studies if the outcome measures are knockdowns or mortality?

Human subjects are not necessary unless the product is designed to disrupt particular insect behaviors. If humans are needed as bait, they do not need to be exposed to any bites. Knockdown and mortality are not the best measures of effectiveness. Even with a lethal ingredient like transfluthrin or allethrin, a certain number of mosquitoes at low dosages will probably be stopped from approaching a host or trap even though they are not killed (true repellency); therefore measuring only mortality underestimates benefit.

2. What are the potential risks to treated subjects (e.g. inhalation, dermal effects)? What are exclusion criteria in subject selection to avoid such risks? How is the degree of risk related to dosage, ingredient, formulations, and aerosol pressure?

The risk of the product itself to the subject should be minimal and acceptable based on toxicological evaluations by EPA. Very basically, safety is determined by studies of exposure and risk. Risk is determined by a large battery of acute and chronic tox tests required by EPA. EPA makes a judgment whether the intended use exposes the public to a significant risk, with appropriate safety factors. These evaluations are the foundation of safe use of pesticides in the US.

3. What is the methodological rationale for continuous versus intermittent exposure? How does human risk differ for these types of exposures? Will exposure start at the beginning of the test period immediately after release of the product?

Where humans are used as bait, there should be no additional risk from continuous exposure. The most accurate results would be obtained from studies with continuous exposure because the products are designed for continuous use during the protection time claimed. These studies would require continuous or interval counts of pests as they fly into a screen trap surrounding the human bait. These approaches do not work for topical repellents because the distances over which they work are so small; whereas, spatial repellents are intended to work over a large area.

4. If the test agent has properties to repel or destroy an insect, what is the relationship (if any) to a related mechanism of action to humans?

Most “safe” toxicants and repellents do not affect humans significantly at the dosages used in the product because humans are either capable of detoxifying the chemical metabolically or they do not share the same physiological target with insects.