# U.S. EPA Office of Pesticide Programs
# Presentation of the Draft Insect Repellent Product Performance Testing Guideline 810.3700 to the Human Studies Review Board June 2006.

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## INTRODUCTION

Insect repellent products are used to protect humans from the bites of nuisance and disease-transmitting arthropods (insects and ticks). These products may be applied directly to human skin or to clothing, or may be designed to repel pests from an indoor or outdoor area. Since these products aid in protecting the public health, EPA has developed a guideline recommending specific methods for testing their performance. EPA consulted in 2000 with the FIFRA Scientific Advisory Panel (SAP) on scientific issues concerning the guideline. Since then EPA has edited the guideline for clarity, and narrowed its scope to cover only repellents applied directly to human skin.

Blood-seeking arthropods locate their hosts by smelling a complex set of olfactory cues (odors). They are very sensitive to the odor of carbon dioxide, and respond to as little as a few molecules traveling in thin plumes on the wind. The smell of carbon dioxide alerts the arthropod to the presence of animals that might provide their next bloodmeal. As host-seeking arthropods near a potential host, they begin to smell for clues to its identity. Every animal species emits a unique odor, and different species of arthropods often prefer a particular species or group of closely related species as hosts. For instance, even though birds and humans are both warm-blooded animals, ticks and mosquitoes evolve to prefer one or the other but rarely both. Effective insect repellents must prevent arthropods from smelling the characteristic odor emitted by humans. It is commonly known that some people are more attractive to pests than others, and repellents must be tested using designs that account for this variability in order to yield a repellent that is effective when used by all consumers.

Despite much research and experimentation, animal models or laboratory systems that mimic human odors and other cues important to host-seeking arthropods are not available as substitutes for human subjects. Therefore the scientific methods available for assessing repellent efficacy require the use of human research subjects.

## BACKGROUND

### Developing Effective Insect Repellents

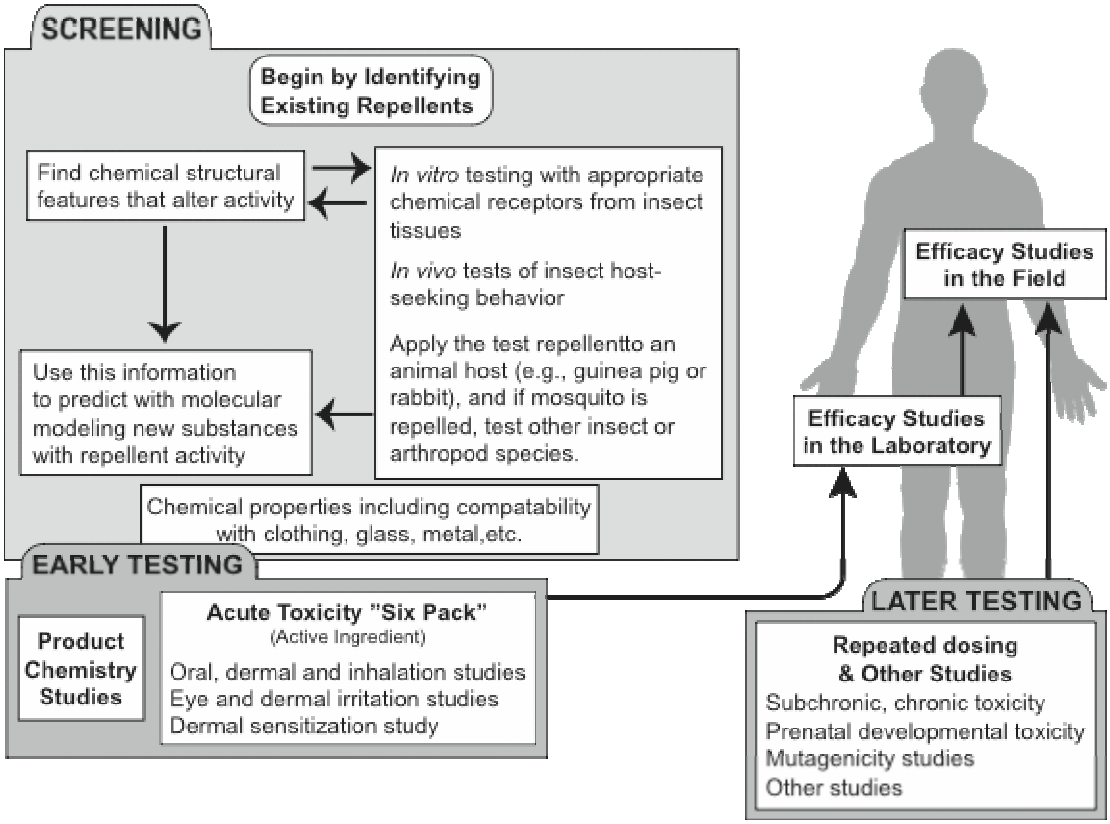
Since EPA does not directly regulate many aspects of the process by which insect repellents are developed, companies can follow a variety of processes for the development of repellent products. The following represents a typical sequence of research and testing steps a company might employ when attempting to identify a commercially promising new repellent or to improve the formulation of an existing repellent product. These steps would generally precede the preparation of an application for registration of the repellent product for submission to EPA.

Development of new repellents begins with knowledge of substances already observed to have such activity. Investigators may discover new repellents by chemically modifying the structure of an existing repellent in an attempt to enhance its activity, or by trying to extract repellents from natural sources such as plants. Substances are typically screened for activity in a series of bioassays that evaluate reactions of chemoreceptor cells from mosquito antennae, effects on host-seeking or locomotor behavior of various insects, or the capacity to repel insects after application to the skin of laboratory animals. Results from the bioassays may also be correlated with chemical structures in computer models designed to predict new repellent structures to be investigated.

After synthesis and preliminary screening the next step is to evaluate the physical/ chemical properties and potential toxicity of promising active ingredients. The substance's physical and chemical properties provide insights into its compatibility with clothing, metals, plastics, etc., and the first toxicity studies (usually acute oral, dermal, and inhalation toxicity, eye and skin irritation, and dermal sensitization studies) serve to characterize the substance's hazard potential (e.g., high, medium, or low toxicity) and to establish the basis for a dosage regimen in subsequent repeated-dose animal toxicity studies. The six basic acute toxicity studies may also provide limited information on absorption and the mode of toxic action of the repellent.

A promising candidate repellent with a low toxicity profile would be considered for further evaluation in laboratory efficacy trials with human subjects to establish formulations and concentrations for the active ingredient. A candidate with a higher acute toxicity profile would not be evaluated further with human subjects until additional animal studies such as subchronic toxicity, prenatal developmental toxicity, mutagenicity, and other studies were conducted and the safety of subjects in any subsequent human research could be better ensured.

In summary, a compound is typically selected for development based initially on in-vitro assays. An acute toxicity battery and physical/chemical properties studies are completed before initiating laboratory testing with human subjects. Subchronic animal toxicity testing may be completed before field efficacy testing with human subjects.




**Types of Potential Risks to Human Subjects in Repellent Efficacy Studies**

The testing procedures described in the draft guideline pose three different types of risks for research subjects: 1) risks resulting from exposure to the insect repellent itself; 2) risks associated with exposure to biting arthropods during laboratory or field testing; and 3) risks associated with possible exposure to vectors of arthropod-borne diseases in field testing.

Exposure to the insect repellent itself.

Testing may be performed with repellents applied directly to the skin surface to be protected in order to prevent bites. As a result, human subjects are exposed to the insect repellent in the same way as would be consumers using a similar repellent product. Before testing new repellent compounds with human subjects, companies typically perform a battery of acute toxicity tests to assess the acute hazard of the repellent. The standard acute toxicity tests include oral toxicity, dermal toxicity, inhalation toxicity, eye irritation, dermal irritation, and skin sensitization. (As a matter of EPA policy, a compound with a high acute hazard based on any of these six standard tests is not eligible for testing with human subjects or for registration as a skin-applied repellent.)

In some cases, results of dermal penetration studies in the rat are also available, but these tests are not usually required by EPA to support registration of repellents. The test sponsors and laboratory would also typically submit the protocol for proposed testing to an Institutional Review Board for review and approval. Once the repellent activity and low toxicity of a compound is established and it becomes a candidate for product development, further toxicological testing is conducted as described in 40CFR Part 158 to fully characterize the toxicity, exposure and risk to users of these products in a manner that satisfies EPA's pesticide registration requirements.

Exposure to biting arthropods during laboratory and field-testing.

Subjects participating in repellent efficacy research are likely to be bitten by arthropods. Some people tolerate such bites well, while others find them very unpleasant. A few people have allergic reactions to arthropod bites. Arthropods used in laboratory testing are captive-raised and disease-free. The possibility that wild arthropods encountered in field testing may transmit diseases is discussed in the next section.

The two methods of measuring repellency described in the EPA guideline are discussed below. Note that two limbs of the same subject often serve as an untreated control replicate and as a treated replicate. In such cases the repellent is applied to one arm while the other arm remains untreated. In this way, the number of bites can be compared between treated and untreated areas on the same human subject. This approach reduces variability in test results due to inter-subject differences in attractiveness to pests.

The first method for measuring repellency is the time from treatment until the First Confirmed Bite (FCB). This method has been used for over 50 years to measure the duration of efficacy of insect repellents. In this test, human subjects are exposed to biting mosquitoes at regular intervals—typically for five minutes of every thirty minutes. Failure of a repellent occurs when a human subject receives bites in two consecutive evaluation periods. The second bite confirms that the first bite was a true breakdown of repellency and not just a rare mosquito that may be genetically predisposed to be unaffected by the repellent. The measure of repellency is time to the first bite, as confirmed by the second. Untreated control subjects (or limbs) are exposed at the same intervals as the repellent-treated subjects (or limbs.) Untreated controls are exposed to establish that mosquitoes are active and biting. Once biting pressure is confirmed by mosquitoes landing on untreated subjects and probing the skin for a biting site, the control subjects may remove their arm from the test cage or cover it in a field study.

The SAP recommended a second method, the "Relative Protection" method, which compares the number of bites received by treated control subjects to the number received by untreated controls during the same exposure period. The SAP recommended a standard of repellent efficacy which would require that treated subjects receive 95% fewer bites than the untreated control subjects. To illustrate both the method and the 95% standard, assume a field test is conducted with six treated and six untreated human subjects. For five minutes of every hour the study director exposes the subjects—both treated and untreated—to field populations of mosquitoes. The total number of untreated subject bites is counted; assume this is five bites each for a total of 30 bites.

The repellent would fail this test of 95% protection if the treated subject group as a whole received more than one bite. If, on the other hand, the untreated control subjects received a total of 100 bites, the repellent would pass the test of relative protection if the treated subjects received 5 bites or fewer. At field test sites with more mosquitoes, human subjects can be expected to receive more bites; the same is true in laboratory testing. Therefore, although the 95% protection time method may be the most reliable way to determine repellency, it also exposes test subjects to the greatest number of bites.

Possible exposure to vectors of arthropod-borne diseases in field testing.

EPA guidelines specify that captive-raised arthropods used in laboratory testing be reared under conditions that ensure they are free of disease. Thus only field research is likely to carry any appreciable risk for human subjects of contracting an arthropod-borne disease.

In this draft guideline, EPA describes methods for conducting field-testing in order to test the efficacy of repellent products against mosquitoes and biting flies under "real" use conditions. Some of the biting arthropods to which human test subjects might be exposed are known to transmit diseases. For instance, in many areas of the United States, some of the common mosquito species may transmit West Nile virus. Investigators, however, have no way in the field to determine if a biting arthropod is infected.

**Strategies for Minimization of Potential Risks to Human Subjects in Repellent Efficacy Research**

When the first laboratory efficacy screening studies are conducted, the risks are of allergic or irritation response to bites or of a sensitization, irritation, or toxic response to the repellent material or its matrix. Allergic responses can be reduced with good subject screening and appropriate exclusion factors for volunteers with a history of allergic response to insect bites. Not much can be done to reduce the likelihood of irritation from bites other than to design studies to minimize the duration of exposure, and to exclude subjects with a history of sensitivity to bites. Investigators should have a good idea of the acute toxicity of the test material, and assuming it is low, the risks of acute effects to subjects from this screening exposure would also be low. It would be important, however, to incorporate into the design of the lab studies observations which would note any irritation or other adverse responses among the subjects. Little or nothing would be known about the nature or likelihood of potential subchronic or chronic effects at this stage, and this should be made clear in the information provided to potential subjects. If only acute toxicity data are available it would be prudent to avoid repeat exposures of the same subject to the test material.

When the first field efficacy trials are conducted, to the previously identified risks must be added the risk of contracting a vector-borne disease. This risk can be reduced by choosing sites where vectors are not known to be present, and in some cases may also be reducible through vaccination. CDC/USGS maps are available to indicate where West Nile virus, Lyme disease and Rocky Mountain Spotted-Fever are present; see, e.g.,

http://www.cdc.gov/travel/namerica.htm#risks

http://www.cdc.gov/ncidod/dvbid/lyme/ld_Incidence.htm

http://www.cdc.gov/ncidod/dvbid/lyme/ld_UpClimbLymeDis.htm

http://www.cdc.gov/ncidod/dvbid/lyme/ld_rptmthofill.htm

http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount05_detailed.htm

http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm

http://www.cdc.gov/ncidod/dvbid/arbor/arbocase.htm

According to the scenario above, animal subchronic studies should be in hand by the time of field testing, so something more would be known about the potential for direct adverse effects of the test material. Nonetheless, the duration of field testing is brief, and so hazards associated with chronic exposures would be unlikely. Even so, subjects should be informed about the limits of the investigators' understanding of the potential effects of the material.

**Attachments:**

**OPPTS Draft Product Performance Guideline 810.3700 Insect Repellent Testing**
**Report of the Scientific Advisory Panel October 2000.**
**Copies of references cited in the OPPTS draft Guideline 810.3700.**