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Headquarters U.S. Army Medical Research and Development Command Office of the Surgeon General V ashington 25, D. C.

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Attention: Lt. Col. Donald Howie

Subject: Report No. 3222 /1 (Letter Report) 0. 222

Period Covered: May 1 to July 31, 1962

Title: "Development of an Orally Effective Insect Repellent"

Gentlemen:

The objective of the research program is to develop orally administered insect repellents affording better and longer-lasting protection than conventional surface repellents. Current efforts are concerned with securing selected surface repellents, establishing certain physico-chemical properties of these chemicals and the development of a mosquito colony.

Based on their reported effective repellency a selected number of surface repellents have been designated for initial study on this program. The list of 98 compounds which will be utilized in this phase of the study is enclosed. Contact has been made with Dr. C. N. Smith, USDA, Entomology Research Division, Orlando, Florida, to obtain various compounds. Universities and various commercial organizations have also been contacted. To date the following chemicals have been received:

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Lt. Col. Donald Howie

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San Contraction

Methyl anthranilate N-n-propylacetanilide O,O-dimethyl-o-2,4,5-Trichlorophenyl-phosphorothioate 10-Undecenoic acid N,N-diethyl-meta-Toluamide Dimethyl Phthalate Benzyl benzoate Decanoic acid Alpha, alpha-dimethyl-alpha-carbobutoxydihydro-gamma-pyrone 2-Ethyl-1,3-hexonediol

The physico-chemical properties of these compounds will be determined to establish those agents which might be localized in skin excretions. Subsequently these agents will be evaluated in vivo for their effectiveness as mosquito repellents when given internally.

A mosquito colony has been established. Eggs of the Yellow Fever mosquito (Aedes aegypti) were obtained from the Department of Biology, University of Notre Dame and are now maintained in the Armour Research Foundation laboratory.

The eggs are hatched in deoxygenated water and the larva are raised and fed in an incubator kept at 80 °F and 80% relative humidity. Pups swimming to the top of the water surface are hand picked and separated according to sex. Adult mosquitoes are maintained in the incubator, fed 10% sucrose solution except when they are required to lay eggs. Prior to egg laying the mosquitoes are fed mouse blood meal.

We are currently engaged in developing a reliable and sensitive test for determining repellency in mice treated with a test compound. Protein bound dyes as well as various radioactive compounds are being used for this purpose. Initially we have used urea- C^{14} and iodinated serum albumin (RISA-I¹³¹) as indicators. Preliminary experiments indicated applicability of RISA-I¹³¹ for this procedure. Lt. Col. Donald Howie

- 3 -

July 31, 1962

Female mosquitoes fed on mice previously injected with RISA-I¹³¹ showed high radioactivity. When these mice were sprayed with commercially available mosquite repellent, "OFF" (N,N-diethyl-m-toluamide 12.75%) the exposed mosquitoes did not show any radioactivity. Therefore, this procedure may provide a sensitive method for measuring repellency of a compound applied on the surface or given internally to a laboratory animal such as a mouse.

Respectfully submitted,

ARMOUR RESEARCH FOUNDATION of Illinois Institute of Technology

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HL/cg