

LABORATORY AND FIELD EVALUATION OF CANDIDATE  
TICK REPELLENT MATERIALS TO BE USED  
IN THE FORMULATION OF AEROSOL BOMBS

By

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LABORATORY AND FIELD EVALUATION OF CANDIDATE

TICK REPELLENT MATERIALS TO BE USED

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## PREFACE

The need for laboratory and field evaluations of candidate tick repellent materials to be used in the formulations of aerosol bombs was indicated by Dr. D. E. Howell, Head of the Department of Entomology. The author selected as a thesis problem, laboratory experimentation in which repellent materials would be tested in series against several species of ticks followed by a field test using aerosol bomb formulations of the repellents which proved most efficacious in the laboratory phase of experimentation.

The author wishes to express his sincere appreciation to the officers and enlisted men of Detachment 2, Company A, 9th Special Forces Group (Airborne) 1st Special Forces, who volunteered for and gave their complete cooperation on the field test. Special thanks are expressed to Major Byrd C. Curtis, Detachment Commander, for providing the author with facilities and time during summer camp to run the field test and to Dr. L. D. Goodhue of Phillips Petroleum Company for providing the aerosol bomb formulations used on the field test study.

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## CHAPTER I

### INTRODUCTION

Since time began man has tried to keep insects away from his body. Through the ages he has used smoke from fires, plants hung in his dwellings, and rubbed on his skin oils, pitches, tars and various earths. (Shambough et al., 1957).

Chemical insect repellents were not highly effective until about the beginning of World War II when the needs of service personnel made effective repellents necessary. Many chemicals were tested against various insects to determine their repellency to mosquitoes and other arthropods.

Much of the development of chemicals to be used as repellents involved screening tests run in the laboratory and the field.

Insect repellents serve as warning stimuli which in most cases must compete with an attractant. This is why a chemical may be repellent to an insect when used alone, but not repellent when used with or on an attractant in addition to being repellent per se. (Dethier, 1947).

In the final analysis many so-called repellents are not repellents at all. They are deodorizers that counteract the effect of attractive odors by (1) absorption, (2) adsorption, (3) chemical alteration of potentially odorous compounds, (4) masking of attractive odors, or initial inhibition of attractive odors during formation. (Dethier, 1947).



Standard procedures for testing repellents consist of determining the ability of a test material to counteract an attractant, either an artificial one or one to which the animal is normally subjected in nature. (Dethier, 1947).

The success of evaluation depends upon standardization of variables. The outstanding causes of variation are the differences in different batches of a given chemical due to impurities, oxidation and other changes in test chemicals, the effect of different diluents, possible knockdown effects on insects, physiological factors in insects, the species used, environmental conditions such as temperature and humidity and the different attractive value of each subject. (Dethier, 1947).

Those variables which are inherent in the chemicals are probably the most easily controlled. More emphasis should be placed on controlling environmental conditions, standardization of test animals used, and evaluation of the attractive values of each subject.

## CHAPTER II

### REVIEW OF THE LITERATURE

Early control with repellents - To date, much research has been carried on with repellents used to protect man, but most of this work was done with clothing impregnated with emulsions or solutions of the repellents. Very little work has been done to date to show the effectiveness of the aerosol bomb method of repellent application for human subjects in the field. Improvement of chemical insect repellents began in the late 1930's and received major emphasis at the beginning of the Second World War. Many thousands of chemicals were screened as mosquito repellents, after their repellency to mosquitoes had been demonstrated some were tested against other insects. Later, when the Federal Food and Drug Administration listed only a small number of toxicants as safe for spraying dairy cattle for fly control, an added stimulus was given to the field of insect repellency. This made the development of repellents necessary from both a medical and veterinary standpoint. (Taylor, 1960).

#### Laboratory Screening Techniques

Laboratory animals - Dethier (1947) reported a method for testing repellents using sheep and adjustable headgear, which were attached to the sheep empty one week after the ears were treated. After a two-day delay to allow the sheep to become accustomed to the apparatus, vials containing ticks of both sexes were inserted in a vial holder on each

cage. The ticks had free access to the ears from these vials. Repellency was calculated from the number of ticks found dead, attached, and engorged.

A similar method was reported by Shambough (1957) using cows as the test animals, except the wire cages were attached to the treated ears of a cow. Repellency was determined by the number of ticks dead, attached, or engorged.

Howell (1962) described a method of testing repellency using mice. A large box was used in which a small stand of vegetation was growing. The ticks were released in the box and given time to spread over the entire area, after which a treated mouse was released in the box and after an adequate time interval the mouse was removed and the number of ticks found dead, crawling, and attached were counted and recorded.

Human subjects - King (1954) used a "pen test" in which human subjects wore treated socks in the pens for 15 minutes. The socks were treated from the ankle to the top, a distance of about 10 inches, with 1.11 grams of repellent in 30 ml. of acetone to give a dosage of approximately 2 grams per square foot. The stocking feet were left untreated to avoid contamination of the pen, and short socks were worn under the treated socks to prevent the ticks from biting the feet. Usually two men were in the pen at the same time testing one untreated and three treated stockings. The ticks that crawled to the top of the socks were removed and counted. The percent repellency was then calculated by the formula  $\frac{100(a-b)}{a}$ , a being the number of ticks on the check and b the number of ticks on the treated area.

In determining the protection time of repellents on the arms of three human subjects, Gouck (1957) applied dimethyl phthalate and

diethyl toluamide to measured areas of the forearm. The treated surfaces were confined in glass vessels that were connected to a system of traps containing ethanol. An air stream was passed through the system at a constant rate and the amount of repellent collected in the traps in two hours was determined by ultraviolet spectrophotometry.

A glove test was suggested by Howell (1962) in which a human subject would wear a treated glove that would be placed in a container infested with ticks. The number of ticks crossing the glove and reaching the arm of the subject would be removed and counted.

Treated surfaces - King (1954) determined the number of ticks crossing the midline of a treated 4-inch-square cloth patch which was secured to a small paddle. The 4-inch piece of cloth was impregnated with the test chemical at the rate of 2 grams per square foot. A black line was drawn across the middle of the patch, and the patch was then attached to a small wooden paddle. A folded strip of clean paper toweling 3/4-inch wide was fastened to the lower edge of the patch. The paddle was held in a vertical position and the paper strip brought in contact with the infested container. The ticks crawled onto the paper strip and those that crossed the treated cloth to the marked line were counted. A similar count was made with an untreated cloth. Tests were run on the day after application of the repellent, then at weekly intervals for four weeks, and at two-week intervals thereafter until the repellency dropped below 80 percent on two consecutive days.

Grannett and Sactor (1947) used a "filter-paper test" to determine the repellency of chemicals on treated surfaces. Standard filter paper was treated by spraying the chemical around the outer border. A smaller piece of untreated filter paper was placed in the center of the

treated piece. The ticks were placed on the untreated center and those crossing from the untreated center over the repellent band were counted. It was found while testing repellents with the filter paper method that both nymphal and adult ticks showed a marked attractiveness to differences in the degree of lighting. In cases where no repellent was used the ticks moved toward the side of the filter paper receiving the greater amount of light. Even when repellents were used the attraction to the brighter light caused the ticks to ignore the repellent properties.

#### FIELD TESTS

Smith and Gouck (1946) found that the most consistent results on field tests were obtained when the repellents were applied to the skin or clothing of persons who walked, stood, or sat with untreated persons in tick-infested areas. The ticks that attached to the treated and untreated persons were counted. They also found that repellents were more effective when applied to the clothing than when applied to the skin. Fewer ticks were found when the entire garments were sprayed than when the margins were treated from a bottle.

Smith et al. (1949) conducted tests on new materials at Camp Bullis, Texas, and also in South Carolina in areas naturally infested with the lone star tick Amblyomma americanum (L.). Twenty-five enlisted men were assigned to wear treated fatigue uniforms. The materials had been originally screened as tick repellents by Smith and Cole (1949), and had shown a high degree of repellency or toxicity to mosquitoes, mites, and lice. The materials that appeared to be of promise were submitted to the U. S. Food and Drug Administration for

pharmacological tests on laboratory mammals. All of the materials showing harmful effects to the mammals were eliminated. Subjects wearing the treated uniforms were exposed in tick-infested territory for 4 hours, beginning about 9 a. m. The trousers were tucked into combat boots, and the jackets were not tucked into the trousers. After the 4-hour exposure the uniforms were left on for an additional 30 minutes to allow time for the ticks to attach to the subjects or to drop off or die, according to the effectiveness of the chemical. During exposure the subjects usually sat or reclined on the ground, reading, sleeping, or playing cards. Every 30-60 minutes the men were moved to a different focus of infestation. After the uniforms were removed all ticks were picked from the body and clothing by the subjects. The ticks were collected on cellulose tape and counted later. Separate records of attached and unattached ticks were kept, but as the number of attachments proved to be very small, both counts were combined in calculating the effectiveness of the repellent materials. Untreated uniforms were worn by two individuals as checks each day, but the subjects were not informed as to which were treated. Replicate tests were usually not made on the same day, or in the same location. When the average number of ticks on the two checks dropped below 25, the tests for that day were not included in the evaluation of effectiveness.

Brennan (1948) evaluated repellents by using 20 men wearing treated and untreated regulation fatigue uniforms, sixteen of the uniforms were impregnated with 2 ounces of repellent per uniform. The men were exposed for 4 hours per day in areas infested with lone star nymphs and adults at Fort Sam Houston, Texas. The ticks were removed and counted hourly.

Cole and Smith (1948) using a similar field test at Bull's Island, South Carolina, found N-butylacetanilide the most outstanding compound tested when used to impregnate clothing at the rate of 2 grams per square foot. The substance was toxic when applied to the skin directly.

Smith, et al. (1954) conducted field tests with selected tick repellents in South Carolina and Georgia in areas heavily infested with the lone star tick. The uniforms were usually impregnated with emulsions or solutions of the repellents, but a few treatments were made with sprays and aerosols. The ineffective repellents were removed from the test after two weeks. Subjects wearing the treated uniforms were exposed in tick-infested territory for one or two hours, depending on the abundance and activity of the ticks. During this time the men walked about, sat and lay on the ground. In each test one subject wore an untreated uniform as a check. After the exposure they stayed in an uninfested location for  $\frac{1}{2}$  hour, to give the ticks time to attach or to drop off or die according to the effectiveness of the chemical. At the end of the waiting period the uniforms were removed and all the ticks on the clothing and body were counted.

Grannett and French (1950) used treated trousers on field tests to repel American dog ticks, Dermacentor variabilis Say. In these tests the lower portions of trouser legs (approximately 30 inches) were cut from cotton trousers and impregnated with 2 grams of chemical per square foot. In the test an individual wearing untreated trousers rolled one pants leg up to the knee and covered the bare area with a treated pants leg. The bottom was fastened around the ankle with a rubber band so that the untreated sock was covered and the top rolled to attach just above the knee. The other trouser leg was left in place to act as an

untreated check. The tester then walked through the infested area. The ticks were allowed to crawl up to the knee, at which point they were removed and counted. The tests were replicated on successive days.

Grannett and French (1951) used subjects wearing treated fatigue uniforms in further tests of di-n-butyl adipate as a tick repellent against the American dog tick at Long Island, New York. Persons wearing untreated clothing as a check followed 50 yards behind the tester wearing a treated uniform. Because of the heavy infestation, exposure time was standardized to a slow walk of 30 minutes or 1 mile along the network of pathways in the test area. Ticks were allowed to crawl up to the knee or above and then were removed and counted. All of the tests were replicated three times. Although the uniforms were washed twice over the seven-week testing period the di-butyl adipate was 98 percent repellent at the end of the test. The uniforms were treated at a rate of 2 grams per square foot.

Using aerosol treatments applied to trousers, Grannett and French (1951) tested di-butyl adipate at a rate of 1 to 2 grams of active ingredients per square foot of clothing. The protection obtained from such treatments was found to be as good as from emulsion treatments. It was estimated that three or four complete treatments of trousers could be obtained from one low-pressure (1 lb.) aerosol bomb of the 15 percent concentration.

Gouck and Gilbert (1955) following the test procedures of Cole and Smith (1948) found repellent mixture M-1960 the most repellent.

In tests with repellents against the American dog tick, Cole and Lloyd (1955) used human subjects wearing fatigue uniforms impregnated



with emulsions of the repellents. The subjects walked slowly along roadsides well infested with adult ticks, brushing against the vegetation and allowing the ticks to crawl onto the trousers.

#### Chemical Structure and Repellency

Dethier (1947) published information on the comparative effectiveness of organic compounds of homologous series in producing certain given physiological phenomena with different arthropods. In the majority of cases there is a logarithmic increase in effectiveness as the carbon chain increases in length. It is of interest, therefore, that studies of the relative effectiveness of homologous compounds in stimulating certain chemoreceptors should reveal a modification of this premise. Dethier noted that the curves for different series occurred at increasing chain lengths in passing from the less to the more water-soluble compounds. In addition, the break in each series occurred consistently near the point which marked the division between those members which are miscible in water in all proportions and those with infinite solubilities in water. This stimulating effectiveness of the latter members was shown to be inversely proportional to their molar solubility in water. Of all chemical properties examined, solubility alone agrees consistently with the accumulated data. The fact that the threshold values for individual compounds are frequently different from those which would be expected solely on the basis of the correlation between threshold and solubility in water, suggests that other factors which have not been identified are also concerned in stimulation.

## CHAPTER III

### MATERIALS AND METHODS

Chemical Materials for Surface and Field Testing - Seven chemicals were used in the laboratory and field investigations of this study. These chemicals are listed below with their chemical formulae. The formulations depended upon the test for which they were used. The specific formulations used are given with each test description.

1. MGK 264 N-(2-ethylhexyl)-bicyclo(2.2.1)-hept-5-ene-2,3-dicarboximide
2. MGK 11 2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydrofurfural
3. MGK 326 di-n-propyl isocinchomeronate
4. MGK 1207 3-chloropropyl n-octyl sulfoxide
5. Delphene N,N-diethyl-meta-toluamide
6. N-butylacetanilide
7. di-n-butyl adipate

Test Animals - Larvae, nymphs, and adults of three species of ticks were used in the laboratory investigations.

Rhipicephalus sanguineus (Latreille) adults were collected from an infested apartment house in Stillwater, Oklahoma. The adults were used in testing as needed.

Amblyomma americanum (Linnaeus) replete females were collected in Southwestern Arkansas from cattle and unfed nymphs and adults were collected with the aid of a drag. Larvae were reared in the laboratory.

Dermacentor variabilis (Say) adults were collected in Southwestern Arkansas. Replete females were collected from a heavily infested dog. Larvae, nymphs and adults were used in the laboratory testing.

Amblyomma americanum and Dermacentor variabilis were present during the field test. The two species of ticks were collected for classification purposes only since facilities for keeping the collected animals alive during the testing period in the field were unavailable.

Methods of Collecting - A 4-foot-square cloth drag was used for collecting unfed adult lone star and American dog ticks in heavily infested pastures.

Replete females were collected from cattle and dogs. Engorged females were collected from cattle which were being held in working pens in readiness for application of livestock spray. The author removed the replete females and placed them in pill boxes.

The brown dog ticks were collected from the rug of an infested apartment in Stillwater, Oklahoma, with the use of an aspirator.

Holding Cages - The adult ticks were kept in small pill boxes, 25 adult ticks per box. The species of ticks were kept separate to facilitate use in testing. The ticks were kept at 80°F. and a RH of 84 percent maintained by a supersaturated solution of potassium bromide in a wide-mouthed 1-gallon jar which was kept securely closed except when ticks were needed (Figure 1).

#### Laboratory Procedures

Filter paper test - Round pans 18 centimeters in diameter were used as test chambers. The sides of the pan had an angle of approximately 95° with the bottom. To reduce loss of the test animals a ¼-



Figure 1. Holding cage for ticks.

inch ring of petrolatum was applied to the top of the pan.

Whatman number 4 filter paper 18.5 centimeters in diameter was used to cover the bottom of the pan. Standard Whatman 15 centimeter filter paper was used for the testing surface. The filter paper was cut into quarters allowing the evaluation of three chemicals and one check for each test replication. The quarters were impregnated by dipping in the test repellents. The filter papers were air dried for 24 hours. When testing the aerosol formulations the quarters of filter paper were sprayed until the entire quarter was thoroughly covered and allowed to air dry for 24 hours. All repellents were applied at the rate of 2 grams per square foot of surface.

After air drying for 24 hours the individual quarters were assigned random positions relevant to the other three quarters. The top right quarter was designated as the number one position, lower right quarter number two, lower left quarter number three, and the upper left quarter as the number four position in the clockwise numerical order. The positions assignments for each replication were made by drawing four numbered cards from a box. In each test, then, there was a random distribution of the treated pieces of filter paper and the untreated check.

After the quarters were placed in their random positions they were put together with strips of masking tape so that the pieces of filter paper fit in their natural round structure.

The test structure was placed in the bottom of the test chamber on top of the bottom covering piece of filter paper, with the masking tape holding it together on the bottom side.

A piece of untreated  $7\frac{1}{2}$  centimeter filter paper was placed in the center of the test structure to provide an untreated center on which

the test animals were placed at the start of the experiment (Figure 2).

Five nymphal or adult ticks or 25 larval ticks were used per replication. When adult ticks were used there was no time limit required per replication because they moved off the untreated center and crossed the test structure very rapidly. A 10-minute time limit was placed on each replication when seed ticks were used, however, because of the small size of the test animals and the slower speed of their movements. The number of ticks crossing the individual test quarters was counted and recorded. In the case of larval ticks the number of seed ticks killed while trying to cross the test structure was also recorded.

The repellent materials were tested in series. Each series consisted of individual filter paper tests, in which the different species of test animals were used in order to show the percent repellency of the repellent chemicals to the different species of ticks. Larval ticks were used to determine the effect of the repellents on the immature stages of the test animals.

Series number one consisted of three separate filter paper tests. The comparative repellency of N,N-diethyl-m-toluamide, N-butyl adipate, and N-butylacetanilide was determined for D. variabilis, R. sanguineus, and A. americanum.

Series number two consisted of four separate filter paper tests used to evaluate the repellency of the three aerosol formulations used in the field test, OSS# 41877, OSS# 41878, and OFF. A. americanum, D. variabilis, and R. sanguineus adults and A. americanum larvae were used as test animals.

Series number three was designed to test the repellency of the repellent materials used with N,N-diethyl-m-toluamide in the formulation

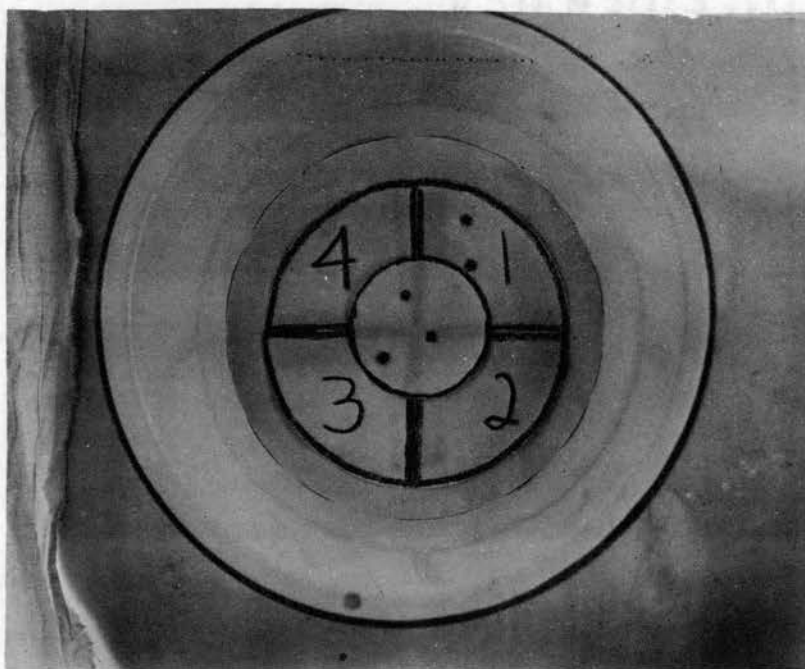


Figure 2. Filter paper test; test chamber, test arrangement, and test animals.

of the aerosol bombs used on the field test. MGK 11, MGK 326, and MGK 1207 were the repellent materials tested in this series using D. variabilis adults and A. americanum larvae.

Series number four consisted of testing the highest performing repellent used in series number three with N,N-diethyl-m-toluamide and NGK 264. Adult A. americanum and D. variabilis larvae were used as test animals for this series of tests.

Following are the repellents used on the filter paper test and their formulations:

1. Army issue prior to 1962 - M-1960

N-butylacetanilide -----	30.00%
2-butyl-2-ethyl-1,3-propanediol -----	30.00%
benzyl benzoate -----	30.00%
emulsifier -----	10.00%

2. New Army issue - M-6

N,N-diethyl-m-toluamide -----	75.00%
Ethanol -----	25.00%

Series number 1:

1. N,N-diethyl-m-toluamide -----	100%
2. N-butylacetanilide -----	100%
3. N-butyl adipate -----	100%

Series number 2:

1. OSS# 41877	
N,N-diethyl-m-toluamide -----	7.00%
N-(2ethylhexyl)-bicyclo(2.2.1.)-hept-5-ene-	
2,3-dicarboximide -----	2.00%
3-chloropropyl n-octyl sulfoxide -----	2.00%



Soltrol 170 -----	10.00%
Isopropanol -----	29.00%
Freon 12 -----	50.00%
2. OSS# 41878	
N,N-diethyl-m-toluamide -----	6.30%
Other isomers -----	.70%
N-(2ethylhexyl)-bicyclo(2.2.1)-hept-5- ene-2,3-dicarbonimide -----	2.00%
2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydro- furfural -----	.50%
Di-N-propyl isocinchomeronate -----	.50%
Isopropanol, anhydrous -----	30.00%
Di-chloro-di-fluoromethane -----	30.00%
Trichloro mone-fluoromethane -----	30.00%
3. OFF	
N,N-diethyl-m-toluamide -----	12.75%
Other isomers -----	2.25%
Inert ingredients -----	85.00%

## Series number 3:

1. 2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydro- furfural -----	90.00%
Ethanol -----	10.00%
2. di-n-propyl isocinchomeronate -----	90.00%
Ethanol -----	10.00%
3. 3-chloropropyl n-octyl sulfoxide -----	90.00%
Ethanol -----	10.00%

## Series number 4:

- |    |  |        |
|----|--|--------|
| 1. | di-n-propyl isocinchomeronate -----          | 90.00% |
|    | Ethanol -----                                | 10.00% |
| 2. | N-(2-ethylhexyl)-bicyclo(2.2.1.)-hept-5-ene- |        |
|    | 2,3-dicarboximide -----                      | 90.00% |
|    | Ethanol -----                                | 10.00% |
| 3. | N,N-diethyl-m-toluamide -----                | 90.00% |
|    | Ethanol -----                                | 10.00% |

Paddle test - Metal pans 12 by 8 inches were used as the test chambers. The sides of the pan had an angle of approximately 95 with the bottom. White petrolatum was applied to the top of the pan to reduce loss of the test animals from the test chamber. The ring of petrolatum was approximately  $\frac{1}{4}$  inch deep.

The paddles used in testing the repellents were made by attaching two pieces of light weight wood together with glue forming the base and handle of the paddle. The material used to make the cloth patches was white cotton oxford cloth which had been thoroughly cleaned. The cloth was cut into 4-inch square pieces. A median line was drawn in black across the cloth at the 2-inch mid-line. The cloth patch was treated with the repellents by dipping them into 50 millimeter beakers containing the repellents. The cloth patches were impregnated with the test repellent at a rate of 2 grams per square foot. After treating, the 4-inch patches were attached to the paddles by thumb tacks. The paddles were then suspended from a string and allowed to air dry for 24 hours. Prior to running the test a  $\frac{3}{4}$ -inch piece of clean paper toweling was attached to the bottom of the treated cloth patch. The paper toweling was attached to the cloth by straight pins (Figure 3).

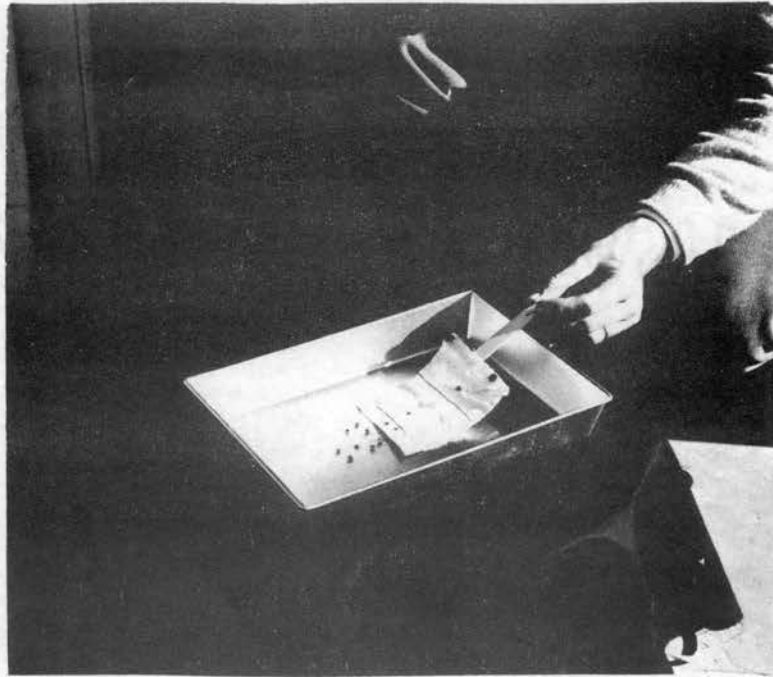


Figure 3. Paddle test; test chamber, paddle and test animals.

Twenty-five adult ticks were used per test. The ticks were placed in the test chamber and the test paddle brought in contact with the infested area. The paddles were numbered and randomized for position or order in the testing. Three repellents and one check were used in each replication. The ticks crossing the 2-inch mid-line at the end of a 5-minute time period were counted and recorded.

The repellent materials tested on the paddle test method were tested in series. Two separate paddle tests were used to test N,N-diethyl-m-toluamide, N-butyl adipate, and N-butylacetanilide using adult D. variabilis and A. americanum ticks as test animals.

Following are the repellents and their formulations used on the paddle test:

1. N,N-diethyl-m-toluamide ----- 100%
2. N-butylacetanilide ----- 100%
3. N-butyl adipate ----- 100%

Glove test - Metal pans 12 by 8 inches were used as test chambers for the glove tests. The test chamber was placed in a larger pan containing water to produce a water moat to prevent the test animals from escaping.

Two pair of medium sized cotton gloves were used as the treated surfaces. A black line was drawn on the gloves at one-half the distance from the index finger tip to the point where the wrist piece was attached.

Three gloves were used in testing the three repellents used on this test, the fourth glove being used as an untreated check. The gloves were treated with aerosol formulations of the repellents used on the field test. Each glove was treated at a rate of 2 grams per

square foot and each was suspended in the air by string for 30 minutes prior to testing (Figure 4).

Twenty-five adult ticks were used per replication. Each replication consisted of testing three treated gloves and one untreated check. The gloves were numbered and the numbers were randomly selected as to the numerical order each glove would have in each replication. The ticks which had crossed the mid-line at the end of the 10-minute time limit were counted and recorded.

Following are the repellents used on the glove test and their formulations:

1. OSS# 41877

N,N-diethyl-m-toluamide -----	7.00%
N-(2ethylhexyl)-bibyclo(2.2.1.)-hept-t-ene-2, 3-dicarboximide -----	2.00%
3-chloropropyl n-octyl sulfoxide -----	2.00%
Soltrol 170 -----	10.00%
Isopropanol -----	29.00%
Freon 12 -----	50.00%

2. OSS# 41878

N,N-diethyl-m-toluamide -----	6.30%
Other isomers -----	.70%
N-octyl bicyclo heptene dicarboximide -----	2.00%
2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydrofurfural -----	.50%
Di-N-propyl isocinchomeronate -----	.50%
Isopropanol, anhydrous -----	30.00%
Di-chloro-di-fluoromethane -----	30.00%
Trichloromono-fluoromethane -----	30.00%

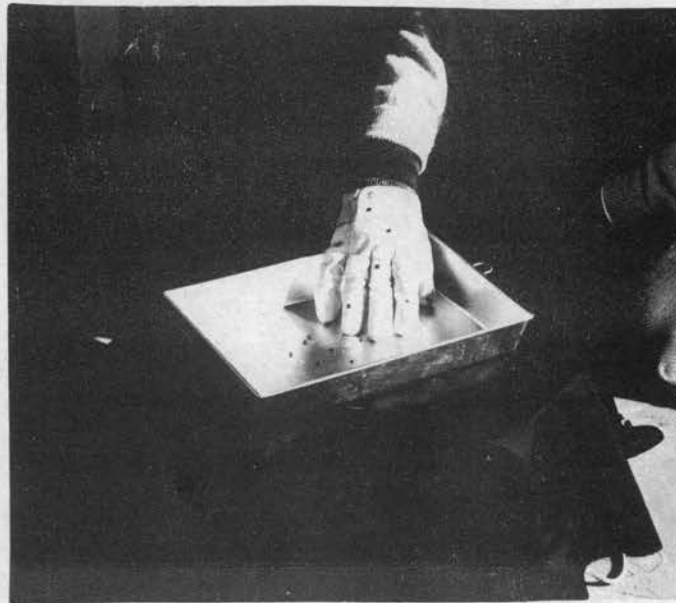


Figure 4. Glove test; test chamber, treated glove, and test animals.

## 3. OFF

N,N-diethyl-m-toluamide -----	12.75%
Other isomers -----	2.25%
Inert ingredients -----	85.00%

FIELD TEST

The field test started 1 July 1962 and ended 15 July 1962, the actual test covering 9 days in the field. The subjects were Army Reserve personnel of Detachment 2, Company A, 9th Special Forces Group (Airborne), 1st Special Forces. Two men of the 5th Special Forces Group were also used on the test. The men were randomly assigned to three groups of 5 men each.

The area used for the field test was the northeast section of the Camp Gruber military reservation near Muskogee, Oklahoma. The area used by Detachment 2 for its summer activity duty training was in the northeast corner of the Camp Gruber Military Reservation. The area used was bounded on the east and north sides by the boundaries of the Military Reservation itself and bounded on the south by Greenleaf Creek. The western boundary was decided by the officers planning the field problems for the entire company. Detachment 2 utilized Greenleaf Creek for carrying out its field problems and it was in this area that the field test was conducted (Figure 5).

The vegetation in this area was very dense, with very few open areas. The vegetation varied from large oak trees to smaller scrub oak trees along the area used for the field test. In most areas the grass and brush was very dense with numerous game trails or rodent trails through it. This area was very similar to the area used by another

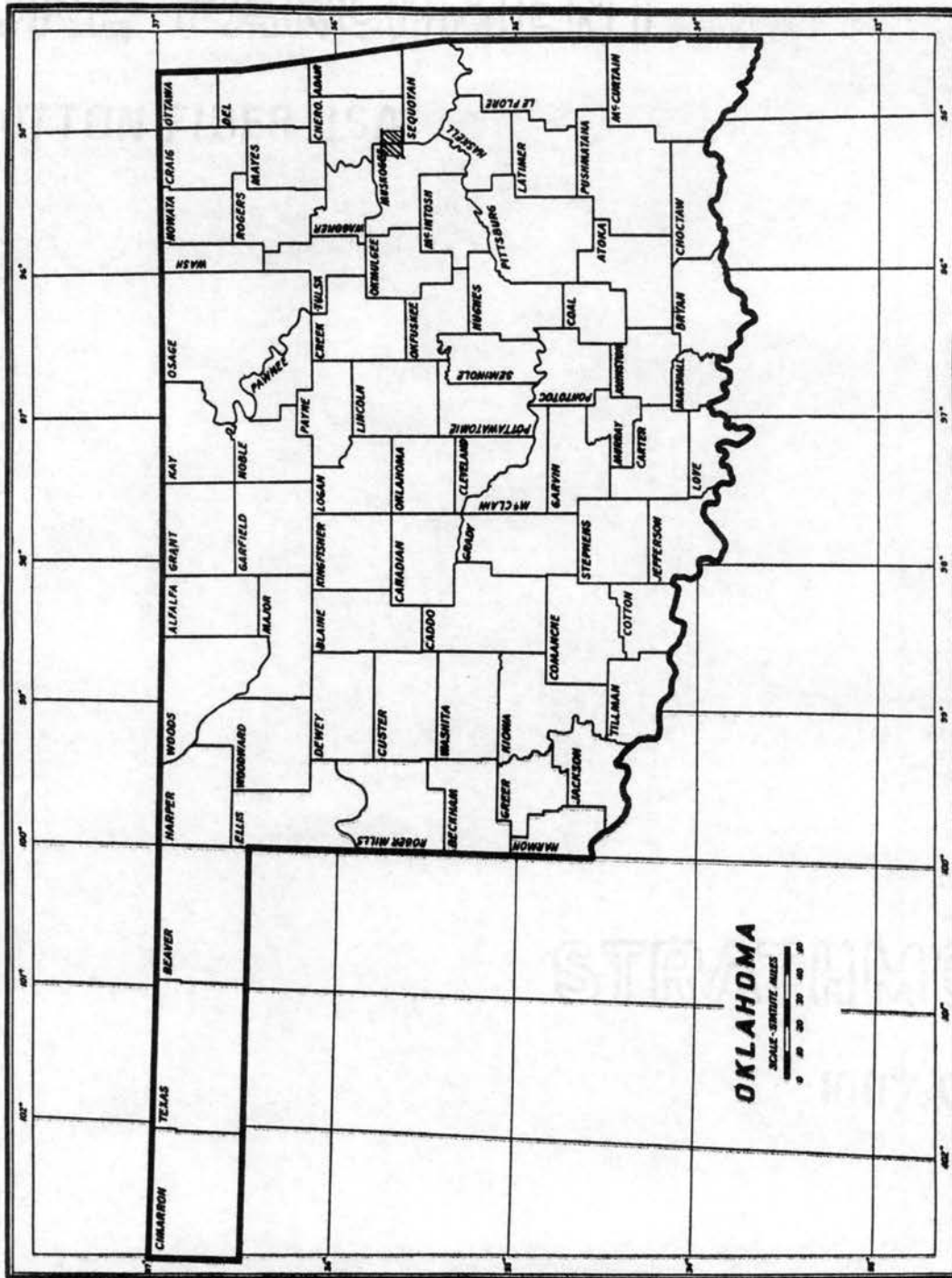


Figure 5. Geographic location of the field test area



detachment, using regular Army issue repellents, which the author was able to observe from time to time on the field problems. The main difference in the two areas was that in the area to the west open meadows were more predominant than in the area used for the field test.

Upon arrival in the field, demonstrations were given as to the parts of the outer clothing to be sprayed for good protection and also the effective method for applying the repellents. After the demonstration each man was given his individual aerosol bomb. The author then observed while the men applied the repellents to their bodies and clothing. Regulation Army two-piece fatigues (HBT) uniforms were worn during the entire field test.

Areas treated with the repellents - The tongue of the combat boots was sprayed so as to prevent chiggers and larval ticks from entering around this area. The tops of the boots were treated along with the area above them where the trouser legs were bloused, so as to protect the subject against ticks gaining entrance to the body in this area. The fatigue trousers were treated around the waist and also the fatigue jacket was treated in this area because it was worn tucked into the trousers. The fly opening on the trousers was also treated. The entire button front and collar of the fatigue jacket was treated as well as the area around the sleeve openings. The face and neck were also treated. This was done by placing a hand over the eyes, holding the mouth shut and spraying the entire face area. Less accessible areas were sprayed by an associate.

The repellents were applied in the mornings soon after rising as the men were dressing and again in the late evening. They were observed as much as possible during this time and corrections were made on their

methods of application if needed. The reason for the retreatment was that at the end of the training day, the men would bathe in the creek and the repellent was washed from the skin. After bathing and prior to leaving on night problems the men treated themselves on the exposed areas of their body and lightly over the areas already treated on the fatigue uniforms. It was at this late afternoon time that the author checked the subjects for crawling and attached ticks. As they undressed and prepared to bathe, they examined themselves and each other for attached and crawling ticks. The author examined as many individuals as possible during this time. Ticks found crawling or attached on the body were counted and recorded by the author.

To keep a daily account of the number of ticks found crawling or attached on each subject, the author prepared a data form on which to record this information. This form was employed to record the data for the entire group (Figure 6).

Data recorded throughout the day included the number of ticks crawling or attached on the areas of the body where the ticks were found. The group total for the day was not recorded until the end of day when the subjects were completely examined.

Following are the formulations of the aerosol repellent bombs used on the field test along with the code numbers assigned to them.

1. OSS# 41877

N,N-diethyl-m-toluamide -----	7.00%
N-(2ethylhexyl)-bicyclo(2.2.1.)-hept-5-ene-2, 3-dicarboximide -----	2.00%
3-chloropropyl n-octyl sulfoxide -----	2.00%
Soltrol 170 -----	10.00%

## DATA FORM

Name _____	Repellent No. _____	Group No. _____	Days								
			1	2	3	4	5	6	7	8	9
<u>Ticks crawling</u>											
<u>Ticks attached</u>											
<u>Total for day</u>											
<u>Parts of the Body</u>											
<u>Where Ticks Found</u>											
<u>Arms</u>											
<u>Underarms</u>											
<u>Chest</u>											
<u>Neck</u>											
<u>Head</u>											
<u>Waist</u>											
<u>Crotch</u>											
<u>Legs</u>											
<u>Ankles</u>											
<u>Feet</u>											

Figure 6. Data form for recording data on field test.

Isopropanol -----	29.00%
Freon 12 -----	50.00%
2. OSS# 41878	
N,N-diethyl-m-toluamide-----	6.30%
Other isomers -----	.70%
N-(2ethylhexyl)-bicyclo(2.2.1.)-hept-5-ene-2, 3-dicarboximide -----	2.00%
2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydrofurfural -----	.50%
Di-N-propyl isocinchomeronate -----	.50%
Isopropanol, anhydrous -----	30.00%
Di-chloro-di-fluoromethane -----	30.00%
Trichloro mono-fluoromethane -----	30.00%
3. OFF	
N,N-diethyl-m-toluamide -----	12.75%
Other isomers -----	2.25%
Inert ingredients -----	85.00%

## CHAPTER IV

### RESULTS

#### LABORATORY EXPERIMENTATION

Filter paper tests - In preliminary tests using the filter paper method A. americanum nymphs and adults were used to compare U. S. Army repellent M-1960 issued before 1962 with the newly issued repellent M-6.

The results tabulated in Table 1 indicate, on a percent repellency basis, the value of each of the repellents against the nymph and adult ticks.

TABLE I

Comparative repellency of M-1960 and M-6 against nymph and adult A. americanum.

Repellents	% Repellency against adults	% Repellency against nymphs	% Repellency both stages
M-1960	91	100	96
M-6	83	66	74

Series number one - This series of tests consisted of three individual filter paper tests evaluating the effectiveness of three repellents against three species of ticks. Table II details the percent repellency provided by each of the repellents used in this series of tests and the species they were tested against.

TABLE II

Percent repellency of N,N-diethyl-m-toluamide, N-butyl adipate, and N-butylacetanilide against A. americanum, R. sanguineus, and D. variabilis adults.

Repellent	% Repellency <u>A. americanum</u>	% Repellency <u>D. variabilis</u>	% Repellency <u>R. sanguineus</u>
N,N-diethyl-m-toluamide	76	88	92
N-butyl adipate	92	94	78
N-butylacetanilide	42	77	50

Series number two - The second series consisted of four individual filter paper tests. The repellent materials used for this series of tests were those repellent formulations used in aerosol bombs for the field test. The three materials were tested against R. sanguineus, D. variabilis, and A. americanum adult ticks and A. americanum larval ticks.

Table III compares the repellency of three repellent materials against the three species of adult ticks and against one larval species. Also included in this table is the number of larvae killed while trying to cross the treated surfaces.

TABLE III

Percent repellency of aerosol bomb formulations to several species of ticks.

Repellent	Percent Repellency			Larval <u>A. americanum</u>	No. larvae killed
	<u>A.</u> <u>americanum</u>	<u>D.</u> <u>variabilis</u>	<u>R.</u> <u>sanguineus</u>		
OSS# 41877	85	93	88	81	32
OSS# 41878	100	97	95	99	21
OFF	95	97	97	100	20

Series number three - Series number three consisted of two individual filter paper tests using the repellent materials that were used in combination with N,N-diethyl-m-toluamide in the aerosol bomb formulations. Adult D. variabilis and A. americanum larvae were tested against MGK 11, MGK 326, and MGK 1207. The results of this series of tests are tabulated in Table IV. The number of larvae killed trying to cross the treated surfaces is also recorded in this table.

TABLE IV

Repellency of MGK 11, MGK 326, and MGK 1207 against D. variabilis adults and A. americanum larval ticks.

Repellent	Percent Repellency		No. larvae killed
	<u>D. variabilis</u>	Larvae <u>A. americanum</u>	
MGK 11	91	99	27
MGK 326	88	99	12
MGK 1207	73	94	37

Series number four - This series compared the effectiveness of MGK 326, MGK 264, and N,N-diethyl-m-toluamide in repelling D. variabilis larvae and A. americanum adults. These results are tabulated in Table V, showing the effectiveness of the repellent materials to the species of ticks used and the number of seed ticks killed trying to cross the treated surfaces.

TABLE V

Percent repellency of MGK 326, MGK 264, and N,N-diethyl-m-toluamide to D. variabilis larvae and A. americanum adult ticks.

Repellent	Percent Repellency		Number of larvae killed
	<u>A. americanum</u> adults	<u>D. variabilis</u> larvae	
N,N-diethyl-m-toluamide	77	100	3
MGK 264	9	99	5
MGK 326	86	100	3

Paddle test - This test consisted of two individual paddle tests. The comparative effectiveness of three repellents against two species of adult ticks is shown in Table VI.

TABLE VI

Percent repellency of N,N-diethyl-m-toluamide, N-butylacetanilide, N-butyl adipate to A. americanum and D. variabilis adult ticks.

Repellent	Percent Repellency	
	<u>A. americanum</u> adult	<u>D. variabilis</u> adult
N,N-diethyl-m-toluamide	-17	-26
N-butyl adipate	-17	-80
N-butylacetanilide	75	66

Glove test - The three repellents used on the field test in the form of aerosol bombs were tested using adult D. variabilis ticks. The results of this test are shown in Table VII.



TABLE VII

Percent repellency of OSS#41877, OSS# 41878 and OFF to D. variabilis adult ticks.

Repellent	Percent Repellency	
	<u>D. variabilis</u> adults	
OSS# 41877	80	
OSS# 41878	90	
OFF	95	

## FIELD TESTS

Two species of ticks were found in the field test area, the lone star tick, A. americanum, and the American dog tick, D. variabilis.

The total number of ticks found crawling, attached, and the total number of ticks on the body are recorded for each of the three groups in Table VIII.

TABLE VIII

Number of ticks found crawling and attached on experimental field test groups.

Tick numbers	OSS# 41877 Group 1	OSS# 41878 Group 2	OFF Group 3
Number ticks found crawling	233	235	174
Number ticks found attached	144	149	93
Total number ticks found on body	377	384	267

The areas of the body where the ticks were observed crawling and attached are shown in Table IX. Ticks were found most often in the waist, crotch, and leg areas. The exact number of ticks found in the various areas of the body was not recorded because of the lack of time.

TABLE IX

Parts of the body where ticks were found crawling and attached.

Part of Body	Group 1		Group 2		Group 3	
	Crawling	Attached	Crawling	Attached	Crawling	Attached
Arms	*		*	*	*	*
Underarms						
Neck	*					
Head				*		
Chest	*					
Waist	*	*	*	*	*	*
Crotch	*	*	*	*	*	*
Legs	*	*	*	*	*	*
Ankles	*					
Feet	*		*	*	*	*

\*Indicates one or more ticks.

All ticks crawling and attached per day for all groups are recorded in Table X. Included in Table X are the totals for all three test groups on a per day basis.

The data for the three test groups using OSS# 41877, OSS# 41878, and OFF are recorded in Table XI.

TABLE X

Numbers of ticks found on experimental groups in the field test.

Groups	Days																	
	1		2		3		4		5		6		7		8		9	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C
1	20	45	7	27	14	20	7	11	23	29	21	31	26	30	17	32	9	8
2	6	40	8	15	14	18	8	9	30	34	25	43	23	24	21	36	14	16
3	15	33	1	3	13	21	3	10	12	27	10	22	18	19	13	25	8	14
Total	41	118	16	45	41	59	18	30	65	90	56	96	67	73	51	93	31	38

A - The number of ticks attached

C - The number of ticks crawling

TABLE XI

Total number of ticks found on test subjects.

	Days									
	1	2	3	4	5	6	7	8	9	
Group I										
Crawling	45	27	20	11	29	31	30	32	8	
Attached	20	7	14	7	23	21	26	17	9	
Total	65	34	34	18	32	52	56	49	17	
Group 2										
Crawling	6	8	14	8	30	25	23	21	14	
Attached	40	15	18	9	34	43	43	36	16	
Total	46	23	32	17	64	68	47	57	30	
Group 3										
Crawling	15	1	13	3	12	10	18	13	8	
Attached	33	3	21	10	27	22	19	25	14	
Total	48	4	34	13	39	32	37	38	22	

The data presented in Table XII gives the percent of the total number of ticks in each test group each day.

TABLE XII

Daily tick infestations on experimental groups.

Groups	Days								
	1	2	3	4	5	6	7	8	9
1	41	56	34	38	34	34	40	34	25
2	29	38	32	35	41	45	34	40	43
3	30	6	34	27	25	21	26	26	32

Table XIII indicates the actual number of ticks found each day on each group.

TABLE XIII

Total number of ticks found on body for all groups per day.

Groups	Days								
	1	2	3	4	5	6	7	8	9
1	65	34	34	18	52	52	56	49	17
2	46	23	32	17	64	68	47	57	30
3	48	4	34	13	39	32	37	38	22
Total	159	61	100	48	155	152	140	144	69

The group percentages of all the ticks found on the 9-day field test are shown in Table XIV. Group number three in which individuals were treated with OFF had the lowest percentage of ticks, slightly over 25 percent of the total number of ticks. The groups treated with

OSS# 41877 and OSS# 41878 had the same number of ticks during the entire test.

TABLE XIV

Percentage of total number of ticks found on each experimental group.

Groups	Total number of ticks	Percentage
1	377	37
2	384	37
3	267	26
<u>Total</u>	<u>1028</u>	<u>100</u>

The analysis of variance for the field test data is given in Table XV.

TABLE XV

Analysis of variance of field test data.

Source	df	ss	ms	F
Total	134	3,359.97		
Days	8	1,074.18	134.27	34.70
Replications	44	1,784.64	40.56	10.48
Treatments	2	191.39	95.70	24.72
Error	80	309.76	3.87	

The mean tick numbers for each repellent formulation are presented in Table XVI. The vertical lines connecting these values indicate that OFF was significantly different from the other two repellents at the 99 percent level of probability as determined from the analysis of variance. There was no significant difference between OSS# 41877 and OSS# 41878 at either the 99 percent or 95 percent level of probability.

TABLE XVI

Mean tick numbers for each repellent formulation used on the field test.

Repellent number	Mean
OSS# 41878	8.53
OSS# 41877	8.37
OFF	5.93

\*Mean numbers of ticks not connected by the same vertical line are significantly different from each other at the 99 percent level of probability.

## CHAPTER V

### DISCUSSION

#### LABORATORY TECHNIQUES

During the course of these experiments three types of tests were conducted in screening candidate chemicals as possible repellent materials to be used in aerosol bomb formulations to protect military personnel against ticks and other biting arthropods.

In the laboratory, filter-paper, paddle, and glove tests were used as methods of determining the repellency of various compounds. The filter-paper tests, however, are somewhat more effective because three candidate materials and a check can be tested during one replication against a species of tick.

Filter-paper tests - The results of the preliminary test showed M-1960 to have greater repellent value against A. americanum than N,N-diethyl-m-toluamide. Cole and Lloyd (1955), in field tests with D. variabilis found M-1960 to be slightly more effective.

The filter-paper test in the laboratory indicated that N-butyl adipate provided a higher percent repellency to A. americanum, D. variabilis, and R. sanguineus adults than N,N-diethyl-m-toluamide and N-butylacetanilide. Similar results were observed by Granett and French (1950 and 1951) in their studies with D. variabilis. In contrast Smith, et al. (1949), Brennan (1948), Cole and Smith (1948),

and Smith et al. (1954) found N-butylacetanilide to be the most effective repellent on field tests.

Aerosol formulations of OSS# 41878 were slightly more repellent than OFF to A. americanum adult ticks. OFF was slightly more repellent to R. sanguineus adults and A. americanum larvae than OSS# 41877. Both OSS#41878 and OFF were higher in percent repellency than OSS# 41877 to all species and stages of test animals used.

Many of the test animals used in testing the repellency of OSS# 41877, OSS# 41878, and OFF, were observed by the author to be killed by the repellent materials. After moving out onto the treated area the test animals apparently could not find their way back to the untreated center, and while searching for a way off the treated surface were killed by the repellents. The larval ticks were observed to circle in a agitated manner and usually continued to do so until incoordination was noted as the legs were contracted against the body and the ticks flipped over on their backs shortly before death. These data suggest that the repellents are effective toxicants as well as repellent and may protect in both ways.

When the components of OSS# 41878 were tested individually MGK 11, gave the highest percent repellency to both A. americanum larvae and D. variabilis adults. While MGK 1207 was slightly less repellent than MGK 11 and MGK 326 it caused the highest death rate to the A. americanum larvae. MGK 326, gave a higher percent repellency to A. americanum adults and D. variabilis larvae than either N,N-diethyl-m-toluamide or MGK 264. The high degree of specificity of MGK 264 is shown by its relatively low percent repellency (9 percent) against A. americanum adults while a repellency value of 99 percent was observed against D.



variabilis larvae. These data indicate the need for testing against species and stages of ticks.

Paddle test - Data obtained by the use of the paddle test and filter-paper test were apparently contradictory. N-butylacetanilide gave 75 and 66 percent repellency to A. americanum and D. variabilis, respectively, using the paddle test method while the number of ticks on the paddles treated with N,N-diethyl-m-toluamide and N-butyl adipate was slightly greater than that observed on the check paddles.

These data seem to show opposite results with respect to the percent repellency found using the same repellents on the filter-paper test. The repellents showing the highest repellency on the filter-paper test, show a minus percent repellency on the paddle test. These repellents were N,N-diethyl-m-toluamide and N-butylacetanilide. However, N-butyl adipate which showed the lowest repellency on the filter-paper test gave the highest repellency on the paddle test. The comparison of these data are shown by observing Tables II and VI.

The author observed that the test animals moved much faster and covered a larger area on the repellents that had a high repellency on the filter-paper test, while they moved in a more normal manner on the repellent showing a lower percent repellency on the filter-paper test and also on the check. The ticks seemed to move very quickly on the high performing repellents seemingly searching for a way back to the untreated area of the paddle. The ticks moved quickly over the treated area and when reaching the untreated part of the paddle (the handle area) they would again assume a normal, hunting, movement.

It is suggested that the above data explain the seemingly contradictory results observed using the paddle test method of screening repellents.

Glove test - The results of the glove test indicate that with regard to the effectiveness of this method of screening repellents, the glove test would be intermediate between the filter-paper and paddle test methods. The data obtained from this method of testing in the laboratory gives an indication of the repellent value of candidate materials when they are used with a human attractant. Of the three repellents used on the glove test, OFF gave the highest percent repellency (95 percent) to D. variabilis adults. OSS# 41878 was second high giving 90 percent repellency to this species of adult test animals. OSS #41877 gave 80 percent repellency to D. variabilis adult ticks using this method of screening candidate materials. The results of the field test using these same repellent formulations (Table XVI) coupled with the data recorded in the laboratory experimentation indicate the usefulness of the glove test in screening candidate repellent materials.

#### FIELD TEST

The data obtained from the three types of laboratory tests were evaluated and three promising repellents were formulated for use in evaluating their effectiveness under field conditions. These repellents were packaged in pressure containers using Freon 12 as a propellant. With the cooperation of Detachment 2, Co. A, 9th Special Forces Group (Airborne) 1st Special Forces these repellents were applied by the Army personnel in a standard manner. Test groups were randomly selected and exposed to heavy populations of ticks while taking part in field problems similar to those expected under combat conditions.

Data obtained from these tests indicated that OFF was the most effective material tested. However, it is necessary to compare the

concentrations of repellents used when evaluating the data. OFF contained 12.75 percent N,N-diethyl-m-toluamide and 2.25 percent related isomers while OSS# 41877 contained 7.0 percent N,N-diethyl-m-toluamide, 2.0 percent MGK 264, and 2.0 percent MGK 1207. OSS# 41878 contained 6.30 percent N,N-diethyl-m-toluamide, .70 percent related isomers, 2.0 percent MGK 264, 0.5 percent MGK 11, and 0.5 percent MGK 326. OSS# 41877 and OSS# 41878 repellent mixtures were formulated to obtain repellency to a larger number of tick species and stages based on data collected in the laboratory. Applications of OFF contained 50 percent more N,N-diethyl-m-toluamide than OSS# 41877 or OSS# 41878. During the time the materials were tested in the field 85 percent of all the ticks collected were A. americanum adults and larvae, and the effectiveness against other stages and species was not adequately evaluated.

The effectiveness of the three repellents applied as aerosols was compared with results obtained by the use of N,N-diethyl-m-toluamide applied as a liquid by troops in contiguous areas.

All test groups had equal exposure to the ticks, as they lived and worked together over the entire test period. It was impossible to set up a check or control group for the field test as all Army units working in the area were issued Army repellents. The author did, however, observe these troops on two occasions. The men of this detachment had an average of 25 to 30 mosquito bites on their backs and necks. The waist and crotch areas were almost completely covered with tick and chigger bites. The lower portions of the legs were covered with numerous bites of both ticks and chiggers. The only men in this unit who seemed to get appreciable protection against ticks and chiggers were the men using a supplemental application of sulfur in their socks, and this gave them protection in the foot and ankle area.

Group number three using OFF had the lowest percentage of ticks on the field test as shown in Table XIV. There was no difference found between groups one and two which were using OSS# 41877 and OSS# 41878 respectively.

OFF was significantly different from OSS# 41877 and OSS# 41878 at the 99 percent level of probability, however, the author believes that this difference in repellency between OFF and the other two repellent mixtures can be explained by the difference in the amount of repellent in the aerosol formulations.

#### EFFECT OF REPELLENTS ON CHIGGERS AND TABANIDS

Chiggers - The chigger population was found to be very high in the area covered by the field test. All three of the repellents used in the field test were very effective against the chiggers. The first evening spent in the field before the repellents were used, gave the author an indication of the population of chiggers. All men in the unit had several bites by the following morning. After the repellents were issued and were being used by the test subjects on the field test very few new chigger bites were noted. In some cases, however, some individuals were not applying the repellents in the right manner, and until this was corrected, these individuals were heavily attacked.

Tabanids - Horseflies and deerflies caused considerable irritation during the first evening in the field. Almost all of the unit personnel were bitten at least once during the course of the evening and some were bitten many times. These flies bit through the undershirts or on the neck, arms and ears of the men as they carried out their duties. After the repellents were issued and had been applied, the biting flies would

not light on the treated areas. Repellents OSS# 41877 and OSS# 41878 did an excellent job of keeping these troublesome pests away from the body.

## CHAPTER VI

### SUMMARY AND CONCLUSIONS

Laboratory and field experiments were conducted to aid in the selection of repellents that would be suitable to protect personnel against the bites of ticks and other biting arthropods.

Laboratory experiments utilizing three screening techniques, filter paper, paddle, and glove test, were conducted to evaluate various repellent materials for possible use in aerosol bomb formulations. Adults, nymphs, and larvae of three species of ticks were used in the laboratory tests to show the differences in repellency of test materials for the different species and stages of ticks.

The three top performing repellents observed on the filter paper test were N-butyl adipate, 2,3,4,5-bis( $\Delta$ -2-butylene) tetrahydro furfural, and di-n-propyl isocinchomeronate. The highest performing repellent using the paddle test was N-butylacetanilide. OFF was observed to give the highest percent repellency using the glove test method of screening repellent materials.

In the field, the repellent chemicals applied as aerosol formulations, were evaluated on human subjects. The repellent formulations used in the aerosol bombs were formulated in the hope of finding a mixture of materials that would provide suitable protection for military personnel against several species and stages of ticks.

The results of these experiments, when interpreted with the supportive information obtained by close observation of each species

of test animal and chemical formula coupled with the evaluation of previous literature, suggest possible explanations for the effectiveness of the materials which were evaluated by the use of three species of ticks.

N,N-diethyl-m-toluamide, N-butyl adipate, N-butylacetanilide, MGK 11, MGK 326, MGK 1207, and MGK 264, demonstrated different degrees of repellency depending on the method of testing and the species of test animals used.

The amount of N,N-diethyl-m-toluamide contained in the aerosol bomb formulations seemed to have a direct influence on the effectiveness of these materials in the field test.

The best repellents formulated as a result of laboratory tests provided effective control of chiggers, tabanids, and mosquitoes in the field test.

#### LITERATURE CITED

- Brennan, J. M. 1948. Field tests with tick repellents. U. S. Public Health Ser. Reports. 63: 339-46.
- Cole, M. M. and C. N. Smith. 1948. Tick repellent investigations at Bull's Island, S. C. Journ. Econ. Ent. 42: 880-3.
- Cole, M. M. and G. W. Lloyd. 1955. Tests with repellents against the American dog tick. Journ. Econ. Ent. 48: 772-3.
- Dethier, V. G. 1947. Chemical insect attractants and repellents. 289 pp. The Blakiston Co. Philadelphia, Pa.
- Gouck, H. K. and I. H. Gilbert. 1955. Field tests with new tick repellents in 1954. Journ. Econ. Ent. 48: 499-500.
- Gouck, H. K. and M. C. Bowmann. 1959. Effect of repellents on the evolution of carbon dioxide and moisture from human arms. Journ. Econ. Ent. 52: 1157-9.
- Grannett, Philip and Bertram Sactor. 1947. Testing tick repellents and observations of phototropic effects. Journ. Econ. Ent. 44: 259-263.
- Grannett, Philip and Carl F. French. 1950. Field tests of clothing treated to repel American dog ticks. Journ. Econ. Ent. 43: 41-44.
- Grannett, Philip and Carl F. French. 1951. Further tests of di-butyl adipate as a tick repellent. Journ. Econ. Ent. 44: 93-7.
- Grannett, Philip. 1960. Factors affecting choice of insect repellents. New Jersey Agriculture. 42: 7-9.
- King, W. V. 1954. Chemicals evolved as insecticides and repellents at Orlando, Florida. USDA Agriculture Handbook No. 69, p. 17.
- Shambough, G. F., R. F. Brown, and J. J. Pratt, Jr. 1957. Repellents for biting arthropods. Advances in pest control research. 1: 277-305. Interscience Publishers, Inc. New York.
- Smith, C. N. and H. K. Gouck. 1946. Observations on tick repellents. Journ. Econ. Ent. 39: 374-8.
- Smith, C. N. and M. M. Cole. 1949. Investigations of tick repellents at Camp Bullis, Texas 1948. Journ. Econ. Ent. 42: 716-20.



Smith, C. N., M. M. Cole, I. H. Gilbert, and H. K. Gouck. 1954. Field tests with tick repellents. 1949, 1950, and 1952. Journ. Econ. Ent. 47: 13-19.

Taylor, Robert Tieche. 1960. The effect of repellent treated surfaces on insect behavior. Unpublished thesis. p. 1. Oklahoma State University.

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