RELATIVE EFFECTIVENESS OF DIETHYL TOLUAMIDE AND M–2020 AGAINST AEDES SCAPULARIS (RONDANI)

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FIELD TESTS of the effectiveness of the new insect repellent, diethyl toluamide, have been reported in recent years by Altman and Smith against *Aedes* spp. in Alaska (*1*) and by Gilbert and associates against *Mansonia* spp. in the Canal Zone (*2*), *Aedes aegypti*, *Aedes taeniorhynchus*, *Aedes* spp., and *Psorophora confinnis*, in Florida (*3,4*), and *Aedes dorsalis* and *Aedes* spp. in Oregon (*5*). Such tests have usually been based on the average protection time (the interval between application and first "confirmed" bite, that is, a bite followed by another bite within 30 minutes), and the ratio between the average protection time obtained with diethyl toluamide and that obtained with some "standard" formulation, either M–250 or M–2020. Results of these tests are summarized in table 1. The average protection time afforded by formulations containing 50 percent or more diethyl toluamide was more than 4 hours.

In each of the tests shown in table 1, the treated area was confined to the forearm of volunteer test subjects in an attempt to limit the high degree of variability inherent in this type of test, Travis (*6*) having proved that there were no significant differences in the results obtained on any of the limbs. Unfortunately, we assumed as possibly other workers not thoroughly familiar with this test have, that the protection time based upon application of repellent to the forearm could be interpreted as the protection time to be expected for all exposed skin surfaces to which repellent was applied.

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Background and Biological Observations

During a routine collecting trip in the vicinity of Brownsville, Tex., in May 1959, we encountered a relatively large population of *Aedes scapularis* (Rondani) in a wooded area approximately one-half mile west of Port Brownsville. In the United States this species is known to occur only in the lower Rio Grande Valley and possibly in extreme southern Florida. It occurs southward from Mexico and Central America to Argentina and in the Antilles (*7*). *A. scapularis* has successfully transmitted yellow fever virus from monkey to monkey under laboratory conditions (*8*).

Since routine application of a commercial preparation of 50 percent diethyl toluamide to all exposed skin areas gave protection for less than 1 hour, we decided to conduct additional tests that would produce results directly comparable to results reported by other authors and at the same time provide the following information:

1. Relative effectiveness of 100 percent M–2020 and 75 percent diethyl toluamide. (The Department of the Army is considering substi-

tution of diethyl toluamide for M–2020 as the standard insect repellent for issue to the Armed Forces.)

2. Relative ability of 75 percent diethyl toluamide and 100 percent M–2020 to withstand removal by rubbing.

3. Relation between protection time on a single forearm and protection time on all exposed skin areas.

Tests were conducted in the Brownsville area on two occasions, June 17–19 and 29–30, using volunteers of Mexican extraction who were obtained through the labor office of the Texas Employment Commission, Brownsville. Because Travis (*6*) had proved that perspiration can influence the effectiveness of repellents, we required our subjects to remain relatively inactive during the test periods. We discovered that the *A. scapularis* in this area landed and bit readily during the daylight hours, that activity began at sunrise, was prolonged into the twilight hours, and ceased during the hours of darkness.

Description and Results of Tests

For one series of tests of the relative effectiveness of diethyl toluamide and M–2020, equal dilutions of the repellents were used. One milliliter was applied to the forearms of each of 10 individuals, 50 percent diethyl toluamide to the right forearm and 50 percent M–2020 (diluted from 100 percent with ethanol) to the left forearm. Under the conditions of the tests, 50

Table 1. Published results of tests of relative effectiveness of diethyl toluamide and other repellents [1] in protecting against bites of various species of mosquitoes

Species	Location	Type test	Percent diethyl toluamide	Average protection time (hours)	Standard [2]	Ratio diethyl toluamide to standard
Mansonia spp.: *titillans* (Wlkr.), *indubitans* D. & S., *fasciolata* (L.-Arr.) *nigricans* (Coq.) (*2*)	Canal Zone	Field	[3] 100	5. 81	375	1. 12
Aedes aegypti L. (*4*)	Florida	Lab	[4] 100	11. 1	M–2020	2. 13
Aedes aegypti L. (*4*)	Florida	Lab	[4] 50	6. 80	M–2020	2. 43
Aedes aegypti L. (*3*)	Florida	Lab	[3] 100	4. 15	M–250	2. 71
Aedes taeniorhynchus (Wied.) (*4*)	Florida	Field	[4] 100	9. 62	M–2020	1. 89
Aedes taeniorhynchus (Wied.) (*4*)	Florida	Field	[4] 50	6. 05	M–2020	2. 80
Aedes spp.: *communis* (DeG.) *punctor* (Kirby), *excrucians* (Wlkr.), *fitchii* (F.&Y.), *stimulans* (Wlkr.) (*1*)	Alaska	Field	[3] 10	4. 68+	----------	----------
Aedes spp.: *communis* (DeG.) *punctor* (Kirby), *excrucians* (Wlkr.), *fitchii* (F.&Y.), *stimulans* (Wlkr.) (*1*)	Alaska	Field	[3] 25	6. 62	375	1. 69
Aedes dorsalis (Meig.) (*5*)	Oregon	Field	[4] 50	6. 73	M–2020	1. 80
Aedes dorsalis (Meig.) (*5*)	Oregon	Field	[3] 50	5. 60	M–2020	1. 45
Aedes spp.: *fitchii* (F. & Y.), spp. (dark legged) (*5*)	Windigo Pass, Oreg.	Field	[4] 25	7. 45	M–2020	1. 92
Aedes spp.: *fitchii* (F.&Y.), *cinereus* (Meig.), spp. (dark legged) probably mostly *communis* (DeG.) (*5*)	Diamond Lake, Oreg.	Field	[4] 25	5. 42	M–2020	2. 07
Aedes spp.: *taeniorhynchus* (Wied.), *sollicitans* (Wlkr.) (*3*)	Florida	Field	[3] 50	4. 42	M–2020	1. 73
Psorophora confinnis (L.-Arr.) (*3*)	Florida	Field	[3] 25	1. 88	375	1. 20

[1] Applied at the rate of 1 ml. to the forearm except tests in Canal Zone where 0.5 ml. was used.

[2] Standards used at same dilution as test material in all instances. Composition of the standards as follows: 375: 1, 3-hexanediol, 2-ethyl. M–250: dimethyl phthalate, 60 percent; 1, 3-hexanediol, 2-ethyl, 20 percent; indalone, 20 percent. M–2020: dimethyl phthalate, 40 percent; 1, 3-hexanediol, 2-ethyl, 30 percent; dimethyl carbate, 30 percent.

[3] Meta isomer.

[4] Technical grade diethyl toluamide, ca. 70 percent meta isomer.

NOTE: Figures in parentheses are reference numbers.

percent diethyl toluamide gave appreciably more protection than 50 percent M–2020. The results are summarized in table 2.

In a second series of tests of relative effectiveness, 75 percent diethyl toluamide was compared with 100 percent M–2020. Other conditions of these tests were the same as described above. At these concentrations equal protection was afforded the test subjects by the two repellents, as shown below:

Repellent:	*Mean protection time (hours)*	*Standard deviation (hours)*
75 percent diethyl toluamide	5. 22	0. 50
100 percent M–2020	5. 26	. 62

The test of the relative ability of 75 percent diethyl toluamide and 100 percent M–2020 to withstand removal by rubbing was suggested by Dr. Carrol N. Smith and members of his staff at the laboratories of the Bureau of Entomology and Plant Quarantine, U.S. Department of Agriculture, Orlando, Fla. The procedure used was the same as that of the first series except that the forearms of the subjects were rubbed with a paper towel approximately 10 minutes following application of the repellent. The towel was wrapped around the forearm and slid under light pressure, 10 times on each arm, from the elbow to the wrist and back to the elbow as the subject gently rotated his arm. With duration of protection as the criterion for determining resistance to rubbing, we found that 75 percent diethyl toluamide was greatly superior to 100 percent M–2020, as shown in table 2.

For a comparison of the relative effectiveness of a test repellent and a standard repellent, the paired forearm technique is undoubtedly the best method developed to date. From the standpoint of field utilization, however, the length of time that all treated exposed skin surfaces can be expected to repel biting insects becomes of primary importance. Undoubtedly, this time will be highly variable and dependent upon a large number of factors, many of which have not yet been identified and defined.

To gain information on this subject, additional observations were taken during the test with equal dilutions described above. All exposed skin areas were treated with undetermined amounts of 50 percent diethyl toluamide at a rate comparable to that recommended for troops in the field. The time at which the first confirmed bite occurred on any exposed skin surface was recorded. Omitting bites on the forearm treated with M–2020, the average protection time for all exposed skin areas was 3.73 hours. Including bites on the forearm treated with M–2020, the average protection time for all exposed skin areas was 2.61 hours. It was

Table 2. Relative effectiveness of diethyl toluamide and M–2020 against Aedes scapularis (Rondani) and ability to withstand removal by rubbing [1]

Repellent	Mean protection time (hours)	Standard deviation	Mean difference in protection times (hours)	Standard error of mean difference in protection times (hours)	"t"	Probability of obtaining a larger value of "t"	Ratio diethyl toluamide to M–2020
	Relative effectiveness of equal dilutions						
50 percent diethyl toluamide	4. 23	0. 44	1. 64	0. 170	9. 62	<0. 001	1. 63
50 percent M–2020	2. 59	. 38					
	Ability to withstand removal by rubbing						
75 percent diethyl toluamide	4. 52	1. 01	1. 77	0. 274	6. 45	<0. 001	1. 64
100 percent M–2020	2. 75	. 97					

[1] 1 ml. of repellent applied to paired forearms of 10 subjects.

established in the test using equal dilutions that bites occurred sooner on the forearm treated with M–2020 than on the forearm treated with an equal dilution of diethyl toluamide. Hence it may be concluded that the average protection time, if all exposed areas of the skin had been treated with 50 percent diethyl toluamide, would have fallen between 2.61 and 3.73 hours. This protection time is appreciably less than the 4.23 hours obtained on a single forearm treated with 50 percent diethyl toluamide. Further investigation of these relationships is planned.

Summary

Field tests of the relative effectiveness against *Aedes scapularis* (Rondani) of the standard U.S. Army insect repellent, M–2020, and diethyl toluamide at various dilutions and under various test conditions were made. Compared with the current standard Army formulation, diethyl toluamide appears to be markedly superior when tested at equal concentrations. On the other hand diethyl toluamide at 75 percent strength gives protection approximately equal to that obtained with the current Army standard at 100 percent strength, although it is markedly superior to the standard from the standpoint of resistance to removal by rubbing.

ADDENDUM: Since this study was made, the Department of the Army has substituted 75 percent diethyl toluamide for 100 percent M–2020 as its standard insect repellent.

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(8) Davis, N. C., and Shannon, R. C.: Studies on yellow fever in South America. V. Transmission experiments with certain species of *Culex* and *Aedes*. J. Exper. Med. 50: 803–808, December 1929.

Causes of Fish-Kills

First reports from 31 States indicate that agricultural pesticides and industrial wastes caused 70 percent of the 135 fish-kills attributed to pollution along more than 600 miles of streams and 5,000 acres of lakes from June to October 1960. The Public Health Service and the States are continuing the national collection of these reports in order to get some indication of nationwide prevalence and causes of fish-kills.

Fish-kills are deaths of varying numbers of fish in a specific area within a short period of time, usually occurring from foreign matter in a river. This may come from a large amount of chemicals or slow accretion of material that makes the water intolerable to fish.

The first nationwide study showed 73 out of 185 stream fish-kills were caused by agricultural poisons; 57 by industrial wastes; 15 by domestic sewage; 17 by other means such as pipeline breaks and shipping pollution; and 28 by unknown agents.

Federal Publications

Physicians' Age, Type of Practice, and Location. Health Manpower Source Book. *PHS Publication No. 263, Section 10; 1960; by William H. Stewart and Maryland Y. Pennell; 199 pages; 55 cents.*

Text, tables, and charts present basic data on age and type of practice of physicians according to their location in mid-1959 by region, State, county group, standard metropolitan statistical area, and county. Changes in age distribution and in the character of practice, grouped into six major categories, are indicated during the past three decades.

The number and location of osteopathic physicians, dentists, and veterinarians are also given, with a view to providing background information for persons and organizations concerned with the provision of health services for the civilian population.

Syphilis. Modern diagnosis and management. *PHS Publication No. 743; 1960; 63 pages; $2.00.*

Directed to the general practitioner, the manual gives in concise form and comprehensible format most of what he needs to know in addition to his formal medical training to manage the average case of syphilis.

The importance of a detailed history and complete physical examination is emphasized. Dark-field and spinal fluid examinations, serologic tests, the course of syphilis, and manifestations of the various stages are described. Treatment, epidemiology, and special problems in diagnosis are discussed.

Forty-three color photographs of lesions, an annotated bibliography, and a note on source material are included in the appendix.

An Industrial Waste Guide to the Potato Chip Industry. *PHS Publication No. 756; 1960; 12 pages; 20 cents.*

Procedures for economic reduction or elimination of wastes which may reach fresh water streams are given.

Use of wastes as animal fodder, disposal of untreated wastes on agricultural lands, and stabilization ponds are discussed. Source and volume of wastes, factors which cause deviations from normal waste loads, and suggestions for meeting these deviations are also described.

The guide, prepared by the National Technical Task Committee on Industrial Wastes, is intended primarily for management and operators of the industry. It should be helpful also to consultants and regulatory personnel.

Sanitation in the Control of Insects and Rodents of Public Health Importance. Insect control series. *PHS Publication No. 772, part 4; revised 1960; by Wilfred H. Johnson; 46 pages; 35 cents.*

Proper storage, collection, and disposal of refuse are discussed with special emphasis on the sanitary landfill as an economical method of disposal for communities of 50,000 population or less.

The manual, directed to supervisors in health and sanitation departments at the local, county, and State levels, also lists references and audiovisual aids.

Public Health Service Grants and Awards by the National Institutes of Health, Fiscal Year 1960.

HEALTH RESEARCH FACILITIES CONSTRUCTION AND RESEARCH PROJECTS. *PHS Publication No. 777, part 1; 1960; 445 pages; $1.25.*

TRAINING GRANTS, RESEARCH FELLOWSHIPS AND TRAINEESHIPS. *PHS Publication No. 777, part 2; 1960; 175 pages; 50 cents.*

National Goals in Air Pollution Research. Report of the Surgeon General's Ad Hoc Task Group on Air Pollution Research Goals. *PHS Publication No. 804; 1960; 39 pages.*

America's needs in air pollution research in the next decade are delineated in terms of 10 specific national goals and the requisite financial effort.

Areas covered include effects on man and agriculture, economic losses, measurement and identification, surveys and monitoring, meteorology and atmospheric reactions, control, administrative and legal aspects, and information, education, and training.

Besides briefly summarizing air pollution problems and trends, this report charts the cost of research needed to achieve each goal and the recommended allocations of financial responsibility among the Federal, State, and local governments and industry.

Fluorescent Antibody Techniques in the Diagnosis of Communicable Diseases. *PHS Publication No. 729; 1960; by W. B. Cherry, M. Goldman, and T. R. Carski; 73 pages; 45 cents.*

Designed for public health laboratory workers and others interested in applying fluorescent antibody techniques to the diagnosis of communicable diseases, this manual emphasizes the practical details of preparation, testing, and use of fluorescent antibody reagents.

Status of these techniques in relation to a variety of diagnostic problems in microbiology is discussed.

Pertinent procedures and information previously not easily accessible to the worker are given in the appendixes.

This section carries announcements of new publications prepared by the Public Health Service and of selected publications prepared with Federal support.

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