northwest. The results indicate that near Laramie adult *Aedes* move approximately 5 mi. in a period of 14 to 26 days. Apparently a second brood of plains *Aedes* is seldom of importance near Laramie. This confirms the work of Owen (1951) as to the relative importance of second brood *Aedes* in the high plains of Wyoming.

Adult control may be practical in mosquito producing fields near populated areas if 2 or 3 weeks of control can be achieved by adulticiding these nearby mosquito producing fields. These efforts can give relief from mosquito infestations in centers of population.

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EVAPORATION RATES AND PROTECTION TIMES OF MOSQUITO REPELLENTS

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ABSTRACT. Evaporation rates and protection times of 5 mosquito repellents were determined, and an inverse relationship of the evaporation rate to the protection time was shown. Three new repellents were found to have a low volatility compared to deet. It is proposed that repellent evaporation rate can be used to determine a reasonable dose for screening new repellents against *Aedes aegypti* prior to appliction to man or animal.

The duration of repellent protection against mosquitoes is partially affected by the rate of loss of repellent due to abrasion, penetration, and evaporation. Consequently, the repellent evaporation rates can be regarded as one physical property of repellents which might affect repellent efficacy. In early work on repellent evaporation, Kasman et al. (1953) measured the evaporation rate of dimethylphthalate from filter paper and determined that the rate of evaporation depended on the surface area of the filter paper. Later Smith et al. (1963) reported repellent evaporation rates for dimethylphthalate, 6–12, and deet from the skin of guinea pigs and humans. They attributed the differences in repellent protection time to differences in the minimum effective dose of repellents necessary to repel mosquitoes and also to differences in the rate of loss of repellent from the skin.

In studies to relate repellent evaporation rate to repellent protection time, the most widely used repellent, deet, was shown to have a volatility approximately one-half that of 6–12 (Smith et al 1963). However, for most new repellents volatility at *ambient* temperature has been evaluated only indirectly by boiling point consideration (Johnson et al. 1967 and 1975). The current report relates evaporation rates to protection time against mosquitoes for 5 repellents. In addition, the relative volatilities are compared to deet which previously has been considered as a low volatility repellent.

MATERIALS AND METHODS. Repellents used were Eastman's 2-ethyl-1,3-hexanediol (6–12)®, Practical; N,N-diethyl-m-toluamide (deet), Practical; Stanford Research Institute's 3,6,9-trioxapentadecan-1-ol (SRI-6), (Johnson et al. 1975) 96 percent; n-butane-hexamethyleneimine-sulfonamide (sulfonamide), (Pervomaiskii et al. 1963) 99 percent; and cyclohexamethylene-carbamide (carbamide), (Stephanor et al. 1969) 99 percent. Instrumentation consisted of a Cahn RTL millibalance coupled to a Honeywell recorder inside a 46 x 75 x 91 cm incubator. Drierite® was used to control the humidity inside the incubator. Temperature was regulated by means of a heating strip and a refrigerated water bath. A 1.13 cm^2 planchet containing 100 mg of repellent was placed on the weighing pan of the millibalance. The planchet and repellent were then tared to 9.0 mg, and the repellent was allowed to evaporate over a 100-hour period. Four replicates were run for each repellent at selected temperatures between 25° C and 60° C. Tare runs were made using a planchet without repellent to correct for electronic drift or other variations in signal which were not caused by evaporation of repellent.

REPELLENT PROTECTION TIMES. Repellents in ethanol solution were applied to 7 x 10 cm sites on the ventral forearms of test subjects such that the applied dose would be 0.32 mg/cm^2. Subjects were tested in groups of 4 with 4 repellent-treated sites (2 on each forearm) per individual (Shimmin et al. 1974). Application of formulations was rotated among the 4 sites so that each repellent appeared on a different site among the 4 subjects and every repellent was paired with every other repellent on the same forearm at least twice in a group of 8 subjects. Data were analyzed by paired comparison with the deet control using Tukey's w-procedure. Subsequent to application, the subject inserted his arm into a plastic sleeve with holes corresponding to the repellent-treated sites and introduced his forearm into a cage containing 250 active female *Aedes aegypti* mosquitoes for a 3-minute exposure. The test was repeated at hourly intervals until 2 bites were received on a treated site. Then that site was covered; the protection time was recorded; and testing continued until all sites had failed.

MINIMUM EFFECTIVE DOSE (MED). The repellent dosage necessary to repel mosquitoes was determined by application of a repellent at 0.05 mg/cm^2 to one of four 7 x 10 cm test sites on the ventral forearms. After a 15-minute drying period, the site was exposed to mosquitoes as described above. If the repellent failed, a higher dose was applied to a second site, and after 15 minutes that site was exposed to mosquitoes. If the first site did not fail in the 3-minute exposure, a lower concentration was applied to the second site. The third and fourth application for each individual was used to bracket the MED precisely with repellent concentrations ranging from 0.0025 to 0.20 mg/cm^2.

MOSQUITOES. *Aedes aegypti* mosquitoes, 7–10 days old, were maintained at 27° C and 80 percent relative humidity under constant light. Sugar solution from cotton was available *ad libitum*.

VOLUNTEERS. Healthy, active duty military personnel were selected at random from a volunteer population of 30 males, averaging 22 years in age (range 20–28).

All volunteers gave written, informed consent prior to participation in the tests.

RESULTS. Data from the strip chart recorder were analyzed by determining the evaporation at a given temperature over a measured time period (Table 1). Over the temperature ranges included in our testing, the plots of weight loss vs. time were consistently linear with r-correlations of 0.973 or greater for 20 data points in each run. This indicates that the air in the incubator did not become saturated with repellent vapor. Saturation would cause a decrease in the measured evaporation rate with time.

Table 1. Physical properties of five repellents

Repellent	Evaporation Rate at 30° C ($\mu g/cm^2/hr$)	Energy of Activation of Vaporization (kJ/mole)	Boiling Point (°C) at 0.5mm Hg
6-12[R]	56.5±5.01	70.7	95
Deet	22.6±2.83	97.0	105
SRI-6	9.31±2.97	71.5	122
Sulfonamide	8.25±1.45	91.6	115
Carbamide	2.23±1.91	96.2	120

Repellent evaporation rates at 30° C are shown in Table 1. The evaporation rates determined at different temperatures were then plotted using the Arrhenius relation of ln K vs. 1/T, where K is the rate of repellent loss and T is temperature in degrees Kelvin (Figure 1). The slope (Figure 1) is $-E_a/R$, where R is the gas constant and E_a is the activation energy of vaporization, or the relative energy necessary to vaporize molecules of repellent from the planchet. E_a for the repellents studied is shown in Table 1. By extrapolation, the $-E_a/R$ provides an estimate of evaporation rates at specified temperatures other than those tested. Assuming pressure effects are constant, extrapolation over a reasonable temperature range is accurate (Moore 1972). However, when dealing with Arrhenius plots over a large temperature range, 100° C or more, one might expect deviation from linearity due to the temperature dependence of E_a (Moore 1972). Repellent protection times and MED's are shown in Table 2.

DISCUSSION. Duration of repellent protection on human skin is influenced by the mosquito population, loss of repellent from the skin, human variables and the intrinsic repellency[1] of the repellent. Changing any of these variables can alter the duration of protection against mosquitoes. Two of these variables, the intrinsic repellency and repellent-skin interaction, are difficult to measure and will be considered to be approximately the same for the repellents studied. With these factors in mind, the following discussion relates the effects that repellent evaporation rates have on the evaluation of topical vapor-phase mosquito repellents.

The most obvious effect of evaporation is repellent loss from the skin which affects the duration of repellent protection from mosquitoes. Carbamide and sulfonamide with lower evaporation rates than deet provide longer protection times against mosquitoes, while 6-12 with a higher evaporation rate has a shorter protection time than deet (Tables 1 and 2). A plot of protection time versus the logarithm of the evaporation rate produces a statistically significant correlation ($r = -0.943$, $n = 5$, Table 2). There is an

[1] Intrinsic repellency is a term which is used to compare the repellent effect of a given number of molecules of one repellent with an equal number of molecules of a second repellent. In recognition that different repellents might act by different mechanisms, the intrinsic repellency will be defined as the minimum concentration of repellent present in the air adjacent to the receptor of an arthropod which will prevent the arthropod from biting the host. This definition has a precise meaning only when associated with specific species and test conditions.

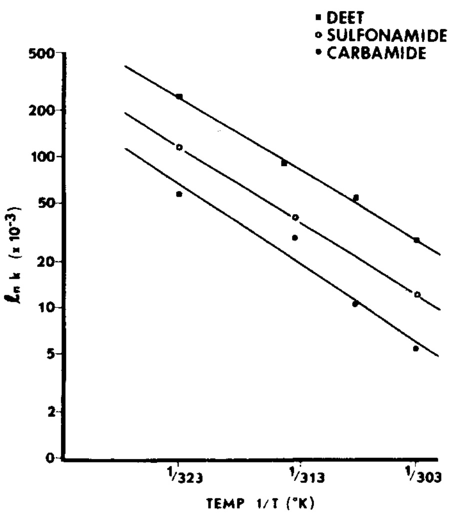

Fig. 1. Arrhenius plot of evaporation rate vs. 1/T, the slope ($-E_a/R$) is used to determine the activation energy of vaporization of the repellent. Points indicate an average of four runs.

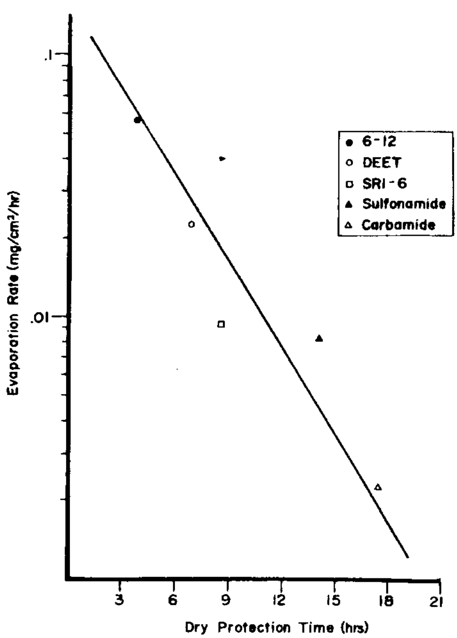

Fig. 2. Evaporation rate of repellents is plotted vs. the protection time afforded human subjects against *Aedes aegypti* mosquitoes under laboratory test conditions.

upper limit to the increase in protection time. When the evaporation rate of a repellent is too low, there will not be enough repellent above the skin to repel mosquitoes. The evaporation rate indicates only the relative rate of loss by evaporation exclusive of intrinsic repellency or penetration through the skin; the last two factors are more difficult to measure and in some cases may have a greater effect on protection time than the evaporation rate.

In addition to protection time, the repellent evaporation rate has a less obvious influence on the minimum effective dose of repellent (MED) necessary to repel mosquitoes. The MED has been proposed as the principal cause for differences in repellent efficacy (Smith et al. 1963); however, repellent evaporation rates can have a direct effect on the MED. Comparison of MED and evaporation rates indicates that MED is closely aligned with evaporation rates of repellents (Tables 1 and 2). When a repellent has a low evaporation rate, more repellent (a higher MED) might be required on the skin to maintain the vapor concentration necessary to repel mosquitoes. Hence, repellent volatility should be considered when comparing compounds which have been shown to exhibit repellency.

In order to use evaporation rates to help evaluate new repellents, one must have an accurate determination of the repellent evaporation rate. Repellent volatility has been determined sparingly by gravimetric analysis in the past (Kasman et al. 1953), and has been related to the repellent boiling point by Roadhouse (1953) and Johnson et al. (1967, 1975). However, boiling point analogies at high temperatures or low pressures should not be extrapolated to volatilities at ambient conditions without caution. Extrapolations like those in Figure 1 are valid only over limited temperature ranges (Moore 1972). Since repellents are generally polar compounds of various chemical entities, boiling point extrapolations to ambient conditions are adequate only when chemicals from ho-

Table 2. Repellent efficacy against *Aedes aegypti* mosquitoes

Repellent	Protection time		Minimum effective dose	
	Mean±sd (hr)	Replicates	Mean±sd (mg/cm²)	Replicates
6-12	3.9±1.6[a]	40	..	..
Deet	6.8±2.0	40	0.025±0.019	16
SRI-6	8.5±4.6[b]	12	0.039±0.015	8
Sulfonamide	14.9±5.6[a, b]	8	0.042±0.026	7
Carbamide	17.4±5.1[a, b]	12	0.078±0.048	24

[a] Significantly different from deet at 95% level ($p<0.05$).
[b] Shimmin et al. 1974.

mologous series of compounds are being compared. For example, comparison of boiling points with evaporation rates at 30° C shows a 20-fold variation in evaporation rate within a narrow range of boiling points.

Once the volatility is determined, a standard describing a low volatility, long-lasting repellent would be helpful for comparison. Definition of low volatility is difficult for repellents. Deet, the most widely used repellent, is considered to have a low volatility with one-half the volatility of 6–12 as reported by Smith et al. (1963) and as seen in Table 1. However, the evaporation rate of deet is 2½ times greater than the evaporation rate of the test compound SRI–6. If deet is considered to be a low volatility repellent then the test compounds in Table 1 must be considered in a new class of repellents with still lower volatility. For a specified applied dose, low volatility implies a lower repellent vapor concentration. If repellent screening programs use low dosages of the standard repellent deet, the new class of low volatility repellents might be unfairly discriminated against. For example, if deet and carbamide were applied at a dose of 0.08 mg/cm² under the test conditions used in this report, deet with a lower MED and a higher volatility would afford 2 to 3 hours protection (Spencer, unpublished results). Conversely, carbamide would afford little or no protection because the carbamide MED is 0.078 mg/cm², slightly less than the applied dose. On the other hand, with application of both repellents at 0.32 mg/cm² carbamide has a significantly longer protection period than deet (Table 2). Hence, in a repellent screening program volatility might be an important factor in determining initial test concentration for a candidate compound.

Since the effective level of repellent vapor will vary with the species, density and biting activity of the mosquito population, the baseline for evaluation of repellent evaporation rates must be adjusted to the test conditions. However, the evaporation rate can be used as a simple physical measurement to aid in preliminary evaluation of a repellent compound along with other factors such as toxicity, cosmetic acceptability and repellency to various species of mosquitoes *in vitro*. In addition, once a compound has been shown to exhibit repellent properties, the evaporation rate will provide a means of estimating approximate application doses and relative duration of persistence prior to application directly to man's skin.

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