(4) R. Venkataramanan and J. E. Axelson, *J. Pharmacol. Exp. Ther.*, **215**, 231 (1980).
(5) R. Venkataramanan and J. E. Axelson, in "Abstracts," vol. 9 (1), A.Ph.A. Academy of Pharmaceutical Sciences, Anaheim, Calif., 1979, p 92.
(6) R. Venkataramanan, F. S. Abbott, and J. E. Axelson, *J. Pharm. Sci.*, 71, 491 (1982).
(7) R. A. Ronfeld, E. M. Wolshin, and A. J. Block, *Clin. Pharmacol. Ther.*, 31, 384 (1982).
(8) A. T. Elvin, J. B. Keenaghan, E. W. Byrnes, P. A. Tenthorey, P. D. McMaster, B. H. Takman, D. Lalka, C. V. Manion, D. T. Baer, E. M. Wolshin, M. B. Meyer, and R. A. Ronfeld, *J. Pharm. Sci.*, **69**, 47 (1980).
(9) R. L. Smith and R. T. Williams, *J. Med. Pharm. Chem.*, **4**, 147 (1961).
(10) J. V. Kilmartin and L. Rossi-Bernardi, *Physiol. Rev.*, **53**, 836 (1973).
(11) B. N. LaDu, H. G. Mandel, and E. L. Way, in "Fundamentals of Drug Metabolism and Drug Disposition," Williams and Wilkins, Baltimore, Md., 1971, pp. 187–205.
(12) P. L. Grover and P. Sims, *Biochem. J.*, **90**, 603 (1964).
(13) D. Lalka, M. B. Meyer, B. R. Duce, and A. T. Elvin, *Clin. Pharmacol. Ther.*, **19**, 757 (1976).
(14) R. Venkataramanan, Ph.D. Thesis, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada, 1978.
(15) R. Venkataramanan and J. E. Axelson, *Xenobiotica*, **11**, 259 (1981).
(16) D. M. Roden, S. B. Reele, S. B. Higgins, R. K. Carr, R. F. Smith, J. A. Oates, and R. L. Woosley, *Am Heart J.*, **100**, 15 (1980).
(17) G. J. Mulder, J. A. Hinson, and J. R. Gillette, *Biochem. Pharmacol.*, **27**, 1641 (1978).
(18) R. A. Cardona and C. M. King, *ibid.*, **25**, 1051 (1976).

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In Vitro Skin Evaporation and Penetration Characteristics of Mosquito Repellents

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Abstract □ An *in vitro* apparatus was used to study mosquito repellent evaporation and penetration characteristics with skin. The mosquito repellents 2-ethyl-1,3-hexanediol, *N,N*-diethyl-*m*-toluamide, *N,N*-diethyl-*p*-toluamide, 1-(butylsulfonyl)hexahydro-1*H*-azepine, and *N,N'*-dicyclohexamethyleneurea were studied. *In vitro* repellent duration, calculated from repellent evaporation rates, was compared to *in vivo* duration at the same dose (0.3 mg/cm²) to assess the validity of the model. *In vitro* durations for 2-ethyl-1,3-hexanediol, *N,N*-diethyl-*m*-toluamide, *N,N*-diethyl-*p*-toluamide, and *N,N'*-dicyclohexamethyleneurea correlated with *in vivo* durations ($r^2 = 0.94$), although *in vitro* duration was longer than *in vivo* duration. 1-(Butylsulfonyl)hexahydro-1*H*-azepine, which had the longest *in vivo* duration, had an *in vitro* duration that exceeded the test period (12 hr). The 0–12-hr *in vitro* percutaneous penetration correlated with corresponding data available from *in vivo* studies.

Keyphrases □ Mosquito repellents—*in vitro* skin evaporation, penetration □ Evaporation—*in vitro* skin penetration, mosquito repellents □ Penetration—*in vitro* skin evaporation, mosquito repellents

Evaporation of mosquito repellents from the skin surface and percutaneous penetration represent important modes of loss of mosquito repellents from the skin surface. Various estimates of the percutaneous penetration of mosquito repellents have been made (1–4). However, only one repellent (*N,N*-diethyl-*m*-toluamide) whose loss from the skin surface by evaporation and skin penetration has been quantified (5). The percentages of *in vitro* skin evaporation and percutaneous penetration of the following five mosquito repellents are reported in this paper: 2-ethyl-1,3-hexanediol (I), *N,N*-diethyl-*m*-toluamide (II), *N,N*-diethyl-*p*-toluamide (III), 1-(butylsulfonyl)hexahydro-1*H*-azepine (IV), and *N,N'*-dicyclohexamethyleneurea (IV). Two dose levels were used: a dose corresponding to a repellent's minimum effective dose against *Aedes aegypti* mosquitoes (6) and a dose of 0.3 mg/cm², which has been used to determine the effective duration of the repellents on the skin of humans (6).

The duration of steady-state evaporation rate of repellents from aluminum planchets has been compared with the duration of effectiveness of several mosquito repellents on the skin of humans (7). The findings suggest a possible relationship between evaporation rate from skin and repellent duration. In this report, this possible relationship was examined by computing the *in vitro* durations for each repellent from *in vitro* evaporation rates and comparing them to previously reported values for *in vivo* duration (6).

EXPERIMENTAL

Labeled Compounds—The following radiolabeled mosquito repellents were used: [1,3-^{14}C]2-ethyl-1,3-hexanediol(Ia)[1], specific activity, 6.06×10^4 dpm/μg; [carbonyl-^{14}C]*N,N*-diethyl-*m*-toluamide(III*a*) (8), specific activity, 1.15×10^4 dpm/μg; [carbonyl-^{14}C]*N,N*-diethyl-*m*-toluamide(II*a*) (8), specific activity, 2.47×10^4 dpm/μg; 1-(butylsulfonyl)-[2,2'-^{14}C] hexahydro-1*H*-azepine(IV*a*), specific activity, 332 dpm/μg; and *N,N'*-[2,2'-^{14}C]dicyclohexamethyleneurea(V*a*), specific activity, 174 dpm/μg. For skin applications of I at the minimum effective dose and the 0.3 mg/cm² dose, cold I[3] was used to dilute the radiolabeled samples to give total radioactive doses of 0.02 and 0.14 μCi, respectively. For one replicate (skin No. A8478) of skin application of III at 0.3 mg/cm², cold III[4] was used to dilute the radiolabeled sample to give a final ra-

[1] New England Nuclear Corp., Boston, Mass.
[2] SRI International, Menlo Park, Calif.
[3] Niagara Chemical Division, FMC, Middleport, N.Y.
[4] Hercules, Inc., Wilmington, Del.

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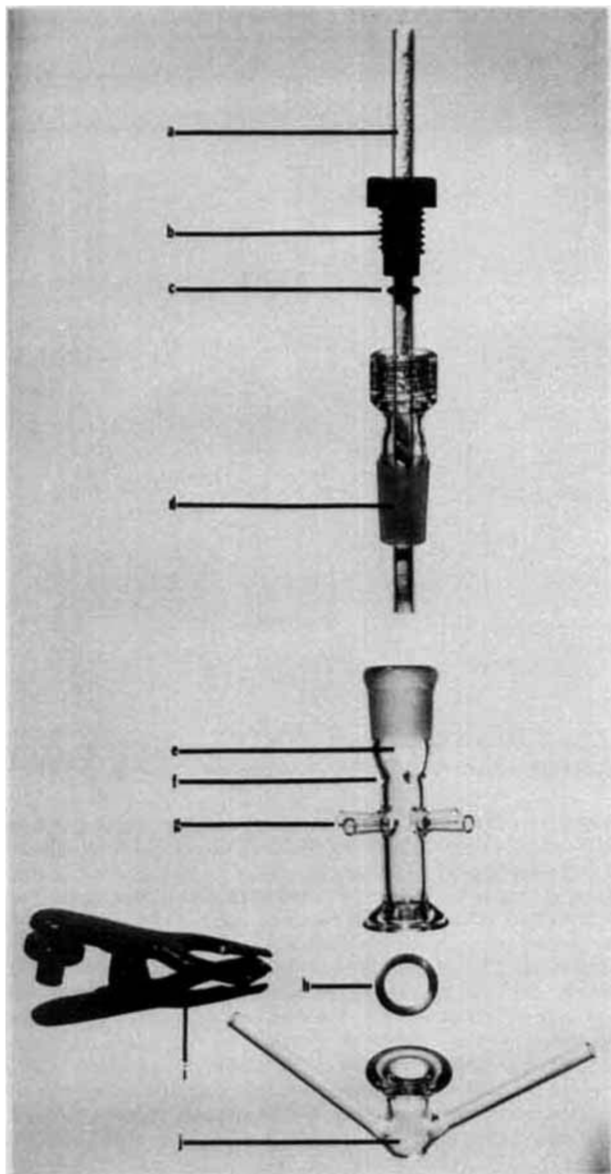

Figure 1—*Evaporation-penetration cell, Key: (a) vapor trap i.d. = 0.38 cm, o.d. = 0.63 cm, length = 15.2 cm; (b) threaded portion of adapter; (c) rubber o-ring; (d) 14/20 standard taper adapter; (e) evaporation manifold, joint size 18/9, i.d. = 0.9 cm, length = 7.25 cm; (f) indents for centering vapor trap located 1 cm above air inlets; (g) air inlets i.d. = 0.3 cm, o.d. = 0.5 cm, length = 1.5 cm, located 2.75 cm above lower joint; (h) polytetrafluoroethylene o-ring; (i) clamp No. 18A; (j) lower chamber, joint size 18/9, i.d. = 0.9 cm, length = 2.5 cm, inlet and outlet i.d. = 0.3 cm, o.d. = 0.5 cm, length = 4 cm.*

dioactive dose of 0.03 μCi. All compounds were homogeneous as determined by TLC (silica gel[5] and chloroform).

Procedure—Whole skin (abdominal), obtained at autopsy, was stored at $-65°$ in sealed plastic bags before use. Storage time did not exceed 3 months. Subcutaneous fat was removed from the thawed sample ($\sim$7 cm^2) before use.

The apparatus shown in Fig. 1[6] was used. A magnetic stirrer was placed in the lower chamber filled with Ringer's lactate solution[7], a skin sample was placed over the lower chamber visceral side down, a polytef o-ring which served as a seal between the lower and upper chamber was placed on top of the skin, and the evaporation manifold was clamped into place. Air bubbles underneath the skin were removed by tipping the assembly, allowing the skin to come in contact with the Ringer's lactate. After 20 min. the evaporation manifold was removed and the desired dose of labeled compound dissolved in ethanol ($\sim$10 μl) was applied to the skin area circumscribed by the o-ring (1.27 cm^2) by use of a syringe[8]. For controls, an equal volume was placed into counting vials. The evaporation manifold was then clamped to the lower chamber and the lower chamber was immersed in a water bath at 37°. One arm of the lower chamber was connected to a precision pump[9] which delivered 1.6 ml of Ringer's lactate/hr. The other arm of the lower chamber was connected to a short length of flexible tubing which led into a counting vial.

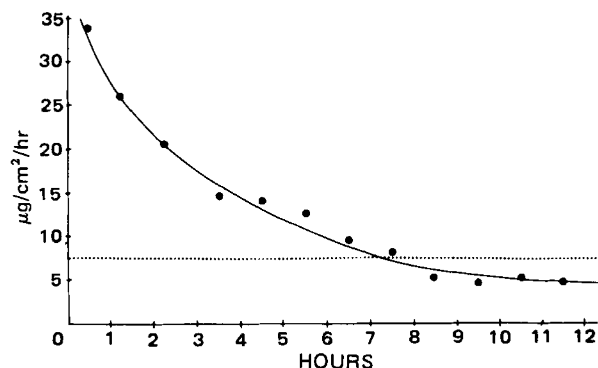

Figure 2—*Mean evaporation rate of I versus time at 0.3 mg/cm^2 (—); the minimum effective evaporation rate (·····).* In vitro *duration is 7 hr.*

The vapor trap, a glass tube packed with 200 mg of absorbant[10] and plugged with cotton at its ends, was inserted into the threaded 14/20 standard taper adapter[11], and the assembly slipped into the ground-glass joint of the evaporation manifold so that the lower end of the vapor trap tube was 0.65 cm above the surface of the skin. The vapor trap was connected by flexible tubing to a bubbler trap which contained aqueous counting solution[12] and served as a safety trap. The bubbler trap was connected to a peristaltic pump[13], which pulled in air at 30 ml/min. The outlet from the peristaltic pump was connected to a flow gauge[14]. Air entering the evaporation manifold was entrained in the following manner: The four intake tubes of the evaporation manifold were connected by adapters to a common tube which was connected to a calcium sulfate drying tower. The inlet port of the drying tower was connected by tubing to an air flow gauge, whose reading corresponded to the other flow gauge reading if there were no leaks or plugged tubes in the system. Dry air at

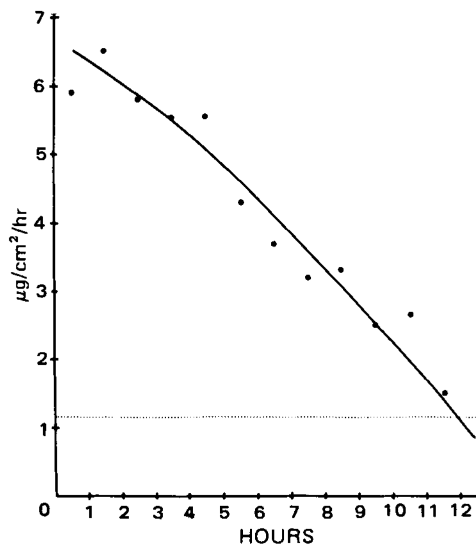

Figure 3—*Mean evaporation rate of II versus time at 0.3 mg/cm^2 (—); the minimum effective evaporation rate (·····).* In vitro *duration is 12 hr.*

[5] Silica gel G Applied Science Division, Milton Roy Co. Laboratory Group, State College, Pa.
[6] Laboratory Glass Apparatus, Berkeley, Calif.
[7] Cutter Laboratories, Inc., Berkeley, Calif.
[8] Hamilton Co., Reno, Nev.
[9] Bodine Electric Co., Chicago, Ill.
[10] Tenax GC, Alltech Associates, Arlington Heights, Ill.
[11] Ace Glass Inc., Vineland, N.J.
[12] Aqueous Counting Scintillant, Amersham Corp., Arlington Heights, Ill.
[13] Masterflex pump, Cole-Parmer Instrument Co., Chicago, Ill.
[14] Flowmeter No. 10, Gilmont Instruments, Inc., Great Neck, N.Y.

Table I—Disposition of Radioactivity 12 Hr after Application of Radiolabeled Repellents to Excised Skin at a Dose of 0.3 mg/cm²

Skin Code	Percent of Applied Radioactive Dose: Evaporation[a]	Percutaneous Penetration	Skin Surface	Skin Oxidation	Total Recovery[b]
			I		
A6178	45.9	12.9	13.2	10.9	83.0
A6178	42.7	15.0	10.3	19.0	87.7
A6178	50.6	10.7	16.9	9.4	90.5
A6178	44.0	13.6	7.3	20.1	86.6
—[c]	52.7	7.7	18.9	10.1	91.9
Mean ± *SD*	47.2 ± 4.3	12.0 ± 2.9	13.3 ± 4.7	13.9 ± 5.2	87.9 ± 3.5
			II		
A5578	12.6	8.6	17.9	35.7	80.5
A5578	17.5	4.2	32.6	19.1	75.3
A8378	19.9	6.9	16.2	34.4	77.7
Mean + *SD*	16.7 ± 3.7	6.6 ± 2.2	22.2 ± 9.0	29.7 ± 9.2	77.8 ± 2.6
			III		
A13479	15.6	—[d]	14.2	43.8	74.7
A13579	15.5	4.7	39.8	13.8	75.3
A13679	14.6	3.6	34.4	30.1	83.5
Mean ± *SD*	15.2 ± 0.6	4.2 ± 0.8	29.5 ± 13.5	29.2 ± 15.0	77.8 ± 4.9
			IV		
A1279	4.2	2.6	37.0	31.7	75.7
A2779	7.5	2.7	25.7	33.8	70.3
A2779	7.5	3.1	35.3	29.3	75.7
—[c]	5.4	1.8	61.6	15.0	86.8
Mean ± *SD*	6.2 ± 1.6	2.6 ± 0.5	39.9 ± 15.3	27.5 ± 8.5	77.1 ± 7.0
			V		
A5778	2.5	3.0	28.1	36.8	71.1
A8378	0.8	4.1	27.6	46.1	83.6
A8678	5.9	4.5	50.1	18.7	86.1
Mean ± *SD*	3.0 ± 2.6	3.9 ± 0.8	35.3 ± 12.9	33.9 ± 13.9	80.3 ± 8.0

[a] Radioactivity recovered from the vapor trap. [b] Total recovery includes small percentages of radioactivity recovered from the evaporation manifold. [c] Skin code not recorded. [d] Sample lost.

22° (ambient laboratory temperature) was thus drawn into the evaporation manifold, over the skin surface, and through the vapor trap, which absorbed any repellent evaporating from the skin surface. Air flowed above the skin and Ringer's lactate flowed below the skin for 1 hr after application of the minimum effective dose and for 12 hr after the 0.3 mg/cm² dose. During the 12-hr runs, the vapor trap and counting vial were changed at hourly intervals.

Radioactivity Measurements—After minimum effective dose applications, the Ringer's lactate in the lower chamber and the rinses of the lower chamber were combined with the Ringer's lactate in the counting vial used to collect lower chamber outflow. After the 0.3-mg/cm² dose, each of the 12 counting vials used to collect lower chamber outflow and the residual fluid in the lower chamber were counted separately[15]. The contents and counting solution rises of each vapor trap were placed in separate counting vials. The resultant disintegrations per minute were corrected for loss in counting efficiency introduced by the absorbant powder, determined by spiking vapor traps with a known amount of radiolabeled repellents. Efficiency varied from 89 to 92% of control for the five repellents. The stratum corneum surface of the skin sample was washed with aqueous counting solution and rinses were collected in a counting vial. The skin sample was then cut into pieces (each <250 mg in weight) and the separate pieces were oxidized[16]. The disintegration per minute was determined by the standard spike method. The evaporation manifold was rinsed with aqueous counting solution and the radioactivity recovered was added to the total percent recovery. The percentage of the applied radioactive dose, recovered from the polytef o-ring by counting solution rinse, was added to the percentage accounted for by the skin surface rinse.

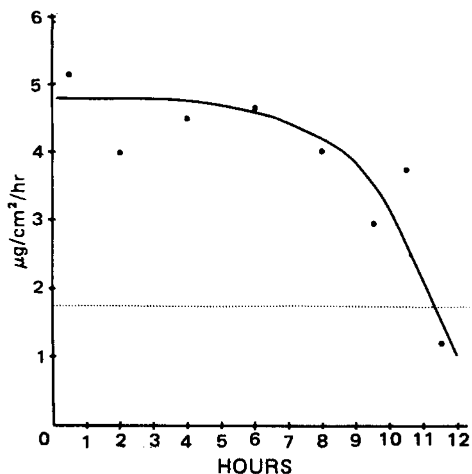

Figure 4—*Mean evaporation rate of III* versus *time at 0.3 mg/cm² (—); the minimum effective evaporation rate (·····).* In vitro *duration is 12 hr. Because of irregularly timed sample collection during the first 9 hr, hourly Sample 2 was combined with 3, 4 with 5, 6 with 7, and 8 with 9.*

RESULTS AND DISCUSSION

The mean evaporation rate *versus* time after the 0.3-mg/cm² dose for each repellent is plotted in Figs. 2–6 relative to the minimum effective evaporation rate of the repellent, the evaporation rate obtained from the

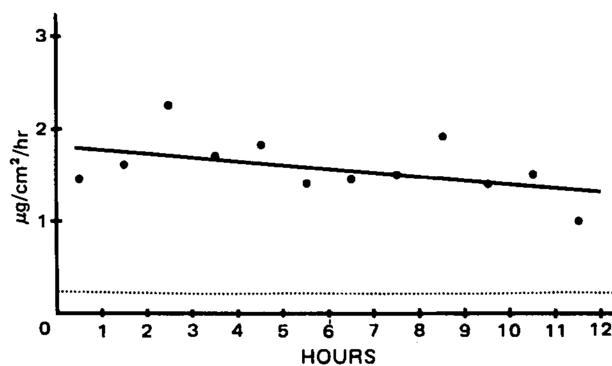

Figure 5—*Mean evaporation rate of IV* versus *time at 0.3 mg/cm² (—); the minimum effective evaporation rate (·····).* In vitro *duration is >12 hr.*

[15] Disintegration per minute determined by automatic external standard on a Packard model 3390-AAA scintillation spectrometer, Packard Instrument Co., Downers Grove, Ill.

[16] Samples were oxidized in a Packard model 306 Sample Oxidizer, liberated radioactive CO_2 trapped in Packard Carbo-sorb, and disintegration per minute determined on a Packard model 3375 scintillation spectrometer.

Table II—Disposition of Radioactivity 1 Hr after Application of Radiolabeled Repellents to Excised Skin at a Minimum Effective Dose Against *A. aegypti* Mosquitoes

Skin Code	Percent of Applied Radioactive Dose				
	Evaporation[a]	Percutaneous Penetration	Skin Surface	Skin Oxidation	Total Recovery[b]
			I, 0.046 mg/cm²		
A5778	16.5	0.3	40.1	30.8	89.5
A6178	12.3	2.0	23.5	41.6	80.1
A6378	19.8	0.8	30.7	33.5	87.6
Mean ± *SD*	16.2 ± 3.8	1.0 ± 0.9	31.4 ± 8.3	35.3 ± 5.6	85.7 ± 5.0
			II, 0.027 mg/cm²		
A5778	3.1	0.3	41.3	44.5	90.0
A8378	5.5	0.0	64.1	22.1	92.7
A5678	4.2	0.2	53.0	22.1	81.0
Mean ± *SD*	4.3 ± 1.2	0.2 ± 0.1	52.8 ± 11.4	29.6 ± 12.9	87.9 ± 6.1
			III, 0.032 mg/cm²		
A8678	8.4	0.4	47.6	25.0	83.1
A8378	4.6	0.2	37.0	38.4	80.7
A6378	3.6	0.1	44.1	26.0	78.8
Mean ± *SD*	5.5 ± 2.5	0.2 ± 0.1	42.9 ± 5.4	29.8 ± 7.5	80.9 ± 2.1
			IV, 0.020 mg/cm²		
A13479	1.4	0.2	81.2	15.5	98.6
A13579	0.6	0.0	65.7	10.8	84.6
A13679	0.9	0.1	82.5	17.4	101.3
Mean ± *SD*	1.0 ± 0.4	0.1 ± 0.1	76.5 ± 9.4	14.6 ± 3.4	94.8 ± 9.0
			V, 0.196 mg/cm²		
A6378	0.5	1.0	47.3	30.9	80.3
—[c]	0.6	0.3	52.6	24.4	78.5
A2779	0.5	0.6	67.8	18.8	88.4
Mean ± *SD*	0.5 ± 0.1	0.6 ± 0.3	55.9± 10.6	24.7 ± 6.1	82.4 ± 5.3

[a] Radioactivity recovered from the primary vapor trap. [b] Total recovery includes small percentage of radioactivity recovered from the evaporation manifold. [c] Skin code was not recorded.

minimum effective dose. (In Figs. 2–4, curves were fitted by eye. In Figs. 5 and 6, least-squares regression lines were drawn.)

The radioactivity recovered from each hour outflow from the lower chamber during the 0.3-mg/cm² dose–12-hr runs represents a time average of the amount of radioactivity in the lower chamber during a given hour. The amount of radioactivity recovered from the residual Ringer's lactate in the lower chamber at the end of the 12-hr runs was always higher than could be accounted for by the 12th-hr outflow. This was probably the result of adsorption of repellent to the glass walls of the lower chamber. Therefore, the amounts of radioactivity in each of the 12 counting vials from the lower chamber outflow, and residual radioactivity in the lower chamber were summed, expressed as the percent of applied dose, and termed as the percutaneous penetration (Table I).

The disposition of radioactivity, expressed as the percent of applied radioactive dose, following the minimum effective dose, is given in Table II, and following the 0.3-mg/cm² dose is given in Table I. The relative volatilities for the repellents are: V < IV < II < I[17]. The percent of radioactivity lost from the skin by evaporation was greater as volatility increased (Tables I and II). Except for I (the most volatile compound) in the 12-hr runs, the majority of the applied radioactive dose for each repellent was recovered from the skin surface and from the skin tissue.

In vitro duration for each repellent at the 0.3-mg/cm² dose was computed by determining the time it took for the evaporation rate from the 0.3-mg/cm² dose to reach the evaporation rate arising from the minimum effective dose or minimum effective evaporation rate for that repellent. The *in vitro* duration so obtained was plotted against the duration on humans (6) of each repellent at 0.3 mg/cm² (Fig. 7). Although *in vitro* duration was always longer than *in vivo* duration, a good correlation existed between them ($r^2 = 0.94$).

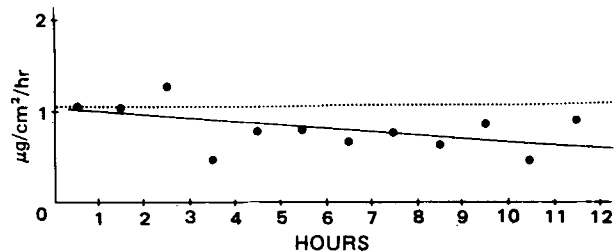

Figure 6—*Mean evaporation rate of V* versus *time at 0.3 mg/cm² (—); the minimum effective evaporation rate (·····).* In vitro *duration is 1 hr.*

[17] June R. Jaeger, Research Chemist, Letterman Army Institute of Research, personal communication.

The minimum effective evaporation rate for each repellent represents the minimum amount of repellent vapor necessary to repel *A. aegypti* mosquitoes under given test conditions and, therefore, is a measure of the intrinsic repellency or potency of a compound. Minimum effective evaporation rate is calculated by dividing the amount (micrograms) of repellent evaporating from the skin surface by the skin surface area (1.27 cm²) and by the time (1 hr). The minimum effective evaporation rates (µg/cm²/hr ± *SD*) for the repellents studied are as follows: IV, 0.20 ± 0.09; V, 1.1 ± 0.1; II 1.2 ± 0.3; III, 1.8 ± 0.8; I, 7.5 ± 1.7. Compound IV is the most potent repellent, V, II, and III are equipotent, and I is the least potent repellent among the five. Repellent minimum effective evaporation rate will not necessarily be the same for different types of mosquitoes and would be expected to increase when test conditions are more severe (*e.g.*, increased avidity of mosquitoes). For V, the least volatile repellent, the evaporation rate following the 0.3-mg/cm² dose (Fig. 6) is essentially

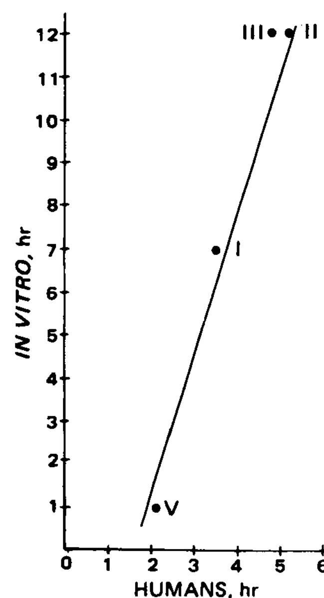

Figure 7—*Calculated* in vitro *duration* versus in vivo *duration of protection of five repellents at a dose of 0.3 mg/cm² against* A. aegypti *mosquitos.*

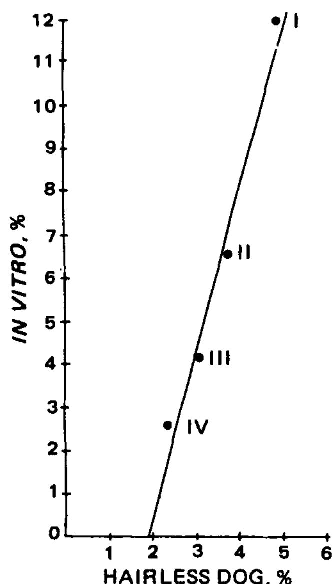

Figure 8—*Mean 12-hr percutaneous penetration (percent of applied radioactive dose)* in vitro versus *hairless dog.*

a horizontal line near its minimum effective evaporation rate. In this instance, the repellent failed almost immediately when challenged with mosquitoes (6). However, if the minimum effective evaporation rate were lower, the repellent could last a long time. The low volatility of V probably accounts for its sporadic performance[18].

In Fig. 8, 12-hr *in vitro* percutaneous penetration at the 0.3-mg/cm^2 dose is compared to 12-hr percutaneous penetration in the hairless dog at the same dose (2, 9) for four of the repellents studied (data for *in vivo-in vitro* comparison of IV were not available). Although *in vitro* percutaneous penetration was always greater than *in vivo* penetration, a good correlation exists between them ($r^2 = 0.96$).

The disposition of radioactivity 1 hr after topical application of radiolabeled II at its minimum effective dose (0.025 mg/cm^2) has been previously reported (5), both *in vitro* and *in vivo*. For the *in vitro* studies, 9.7 ± 5.9% of the applied radioactive dose evaporated, 19.7 ± 3.1% remained on the skin surface, and 50.8 ± 15.0% remained in the skin. For the *in vivo* studies, 9.6 ± 3.6% of the applied radioactive dose evaporated and 27.1 ± 11.6% remained on the skin surface. In this study, a lower percentage (4.3 ± 1.2%) of the radioactive dose of II evaporated 1 hr after *in vitro* application at the minimum effective dose. This difference may result from a closer proximity of the vapor entraining tube to the skin surface (1.5 mm *versus* 6.5 mm) in the previous report (5). A larger portion (52.8 ± 11.4%) of the applied radioactive dose was recovered from the skin surface and a correspondingly smaller portion (29.6 ± 12.9%) of the applied radioactive dose was recovered by skin oxidation, compared to the percentages cited in the previous study (5). This difference may result from the thoroughness of the skin surface rinse procedure, as the sum of the percentages of applied radioactive dose recovered by skin rinse and skin oxidation in the two studies are similar in magnitude.

The *in vitro* apparatus described here can be a useful tool for the screening of mosquito repellent formulations that incorporate a repellent whose evaporation and penetration characteristics and minimum effective evaporation rate are known. Formulations can be selected that reduce excessive evaporation, maintain evaporation rates above the minimum effective evaporation rate for longer periods of time, and reduce percutaneous penetration as compared with the unformulated repellent.

[18]W. Reifenrath and W. Akers, unpublished data.

REFERENCES

(1) R. J. Feldmann and H. I. Maibach, *J. Invest. Dermatol.*, **54,** 399 (1970).

(2) W. G. Reifenrath, J. A. Hill, P. B. Robinson, D. L. McVey, W. A. Akers, D. M. Anjo, and H. I. Maibach, *J. Environ. Pathol. Toxicol.*, **4,** 249 (1980).

(3) H. L. Snodgrass and M. H. Weeks, *Am. Ind. Hyg. Assoc. J.*, **39,** 540 (1978).

(4) L. Blomquist and W. Throsell, *Acta Pharmacol. Toxicol.*, **41,** 235 (1977).

(5) T. S. Spencer, J. A. Hill, R. J. Feldmann, and H. I. Maibach, *J. Invest. Dermatol.*, **72,** 317 (1979).

(6) J. A. Hill, P. B. Robinson, D. L. McVey, W. A. Akers, and W. G. Reifenrath, *Mosquito News*, **39,** 307 (1979).

(7) M. L. Gabel, T. S. Spencer, and W. A. Akers, *ibid.*, **36,** 141 (1976).

(8) A. P. Kurtz, "Annual Progress Report," Letterman Army Institute of Research, San Francisco, Calif., p. 5 (1971).

(9) W. G. Reifenrath, P. B. Robinson, V. Bolton, and R. E. Aliff, *Fd. Cosmet. Toxicol.*, **19,** 195 (1981).

High-Performance Liquid Chromatographic Analysis of Digitoxin Formulations

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Abstract □ A rapid, selective, and simple high-performance liquid chromatographic assay for digitoxin formulations is described. The method utilizes a conventional octadecyl-bonded phase column with detection at 220 nm. The isocratic solvent system resolves digitoxin from its potential degradation products and provides an accurate assay for tablet and injectable formulations with a relative standard deviation of 1.4 and 3.3%, respectively. The method is sufficiently sensitive to monitor content uniformity of tablets and the minimum quantifiable amount of digitoxin was determined to be 20 ng. The total chromatograph time was ~15 min.

Keyphrases □ Digitoxin—high-performance liquid chromatographic analysis of formulations, content uniformity □ Formulations—digitoxin, high-performance liquid chromatographic analysis, content uniformity □ High-performance liquid chromatography—content uniformity, analysis of digitoxin formulations

Digitoxin is a cardiac glycoside obtained from the leaves of *Digitalis purpurea* and is used in the treatment of congestive heart failure. Due to its long biological half-life the unit dose is generally low (0.1 mg). Assurance of potency and content uniformity of tablets is, therefore, a necessity for proper dosage. The determination of such a potent drug in the dosage form requires a method that is accurate, selective, and sensitive.

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