EVALUATION OF THE COTTON FABRIC MODEL FOR SCREENING TOPICAL MOSQUITO REPELLENTS[1]

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ABSTRACT. The data of King (1954) were analyzed by rank correlation methods to determine if the results of tests of candidate mosquito repellents on cotton fabric were correlated with the results of tests of the same compounds on the skin. The coefficient of rank correlation was statistically significant ($P < 0.01$) for tests against salt marsh mosquitoes, *Aedes sollicitans* and *Ae. taeniorhynchus*, and yellow fever mosquitoes, *Ae. aegypti*, but not for tests against malaria mosquitoes, *Anopheles quadrimaculatus*. The coefficient of rank correlation was small ($r_s \leq 0.40$), and it was concluded that cotton fabric is not an efficient model for the skin in repellent screening programs.

INTRODUCTION

In 1946 the U.S. Department of Agriculture adopted a method of screening potential mosquito repellents by which candidate materials are tested on cotton fabric in lieu of the skin. A subsequent report stated that "...usually only the compounds rated in class 3 or 4 by these [fabric] tests were evaluated by skin applications ... Compounds that were effective on cloth were submitted to [the Army Environmental Health Laboratory] for skin-irritation tests on rabbits, and if no adverse effects were observed they were subjected to skin tests." (King 1954). Since 1946 more than 15,000 materials have been evaluated in tests on cotton (King 1954, U.S. Department of Agriculture 1967, Schreck et al. 1977), and this method is still the primary method used by the Department of Agriculture to screen potential mosquito repellents for use on the skin (Schreck 1977).

In the original description of the fabric test, King (1954) stated, "Women's mercerized cotton stockings were employed. A measured section a short distance above the ankle was impregnated with the compound at a rate equivalent to 3.3 grams per square foot [3.6 mg/cm^2]. The stocking was spread on a rack to dry and then hung indoors on a line. The usual procedure was to treat one day and make the first test the following day. The stocking was drawn over the arm, with the treated portion about midway of the forearm. The hand was protected with a glove, and the arm was exposed for 1 minute in a test cage.

"If five bites were received, the treatment was considered noneffective. When applied to cloth, repellent materials remain effective for several days or weeks, as compared with hours in skin applications. If less than five bites occurred, the exposures were continued daily until the 14th day and at weekly or biweekly intervals thereafter. Beginning in January 1948, the tests were run weekly until the fourth week and at 2-week intervals thereafter."

Although the fabric model has not been critically evaluated, most reviewers (e.g., Dethier 1956, Busvine 1971, Schreck 1977) have not questioned its validity as a screening method. However, the issue of the validity of the fabric model was recently raised by a subcommittee of the National Research Council (1987). Obviously there are many differences between cotton fabrics and human skin in terms of composition (carbohydrate, protein), structure (fibers, cells), organization (woven, layered) and function (the skin has a number of protective, regulatory, excretory and sensory functions). Accordingly, the present study was conducted to evaluate the validity of the cotton fabric model for screening topical repellents.

MATERIALS AND METHODS

The data of King (1954) were used in the study. These data are still the most extensive data available for the purpose, and, given modern restrictions on the use of human test subjects in research, it is unlikely that they will ever be equalled.

King determined the protection times on both cotton fabric and the skin of 145 compounds against the common malaria mosquito, *Anopheles quadrimaculatus* Say, 49 compounds against salt marsh mosquitoes, *Aedes sollicitans* (Walker) and *Aedes taeniorhynchus* (Wied.) and 1,439 compounds against the yellow fever mosquito, *Aedes aegypti* (Linn.). For reporting purposes, he placed the compounds tested into five classes, defined by the period of protection provided (Table 1).

In the present study, class frequency data for compounds tested on fabric and the skin were compiled from King (1954) with a Zenith Data

[1] The opinions and assertions contained herein are the private views of the authors and should not be construed as reflecting the views of the Department of the Army or the Department of Defense.

Systems model 248-GE microcomputer using WordStar® Professional Release 4 software (MicroPro® International) and analyzed (in part) with a Data General model MV-8000 minicomputer using the BMDP statistical software (University of California). We used rank correlation methods to analyze the class frequency data obtained in the study. Compounds assigned by King (1954) to the same class were treated as tied observations and given the average rank value. For example, Table 2 shows that 107 compounds were classified as class 1 in tests on fabric against *An. quadrimaculatus*. The average rank value for these 107 compounds with respect to tests on fabric was therefore (1 + 107)/2 = 54. Similarly, the average rank value for the 23 compounds classified as class 2 in tests on fabric against *An. quadrimaculatus* (Table 2) was [(107 + 1) + (107 + 23)]/2 = 119. Average rank values with respect to tests on the skin were determined in the same way. For example, the average rank value for the 64 compounds classified as class 1 in tests on the skin against *An. quadrimaculatus* (Table 2) was (1 + 64)/2 = 32.5, and the average rank value for the 58 compounds classified as class 2 in tests on the skin against *An. quadrimaculatus* (Table 2) was [(64 + 1) + (64 + 58)] /2 = 93.5.

The coefficient of rank correlation (Steel and Torrie 1980) was calculated from the sum of squares of differences of the paired average rank values corrected for ties by the method of Langley (1970). This correction is necessary because a tie involving x observations reduces the sum of squares by an amount equal to $(x^3 - x)/12$ (Langley 1970). Example: The correction for the 107-way tie in tests on fabric against malaria mosquitoes (Table 2) was $(107^3 - 107)/12$ = 102,078. Similarly, the correction for the 64-way tie in tests on the skin against malaria mosquitoes (Table 2) was $(64^3 - 64)/12$ = 21,840. The corrected coefficient of rank correlation was tested for significance by the standardized difference method (Langley 1970).

The rank data were also graphed as an aid in visualizing the results obtained (Fig. 1). Table 3 shows how the data of Fig. 1 were obtained, using the data for compounds classified as class 1 in tests on fabric against *An. quadrimaculatus* (Table 2) as an example.

Table. 1. System of classification used by King (1954) in reporting the protection times of chemicals tested at Orlando, Florida, 1942–52.

	Tests on cotton fabric		Tests on the skin	
Class[a]	Class limits (days)[a]	Class value (days)[b]	Class limits (min)[a]	Class value (min)[b]
Tests against *Anopheles quadrimaculatus*				
1	<1	0.5	0–30	15
2	1–5	3	31–60	45.5
3	6–10	8	61–90	75.5
4	11–21[c]	16	91–150[c]	120.5
4A	>21	-	>150[c]	-
Tests against *Aedes sollicitans*, *Ae. taeniorhynchus* and *Ae. aegypti*				
1			0–60	30
2			61–120	90.5
3	(Same as above)		121–180	150.5
4			181–300[c]	240.5
4A			>300[c]	-

[a] King (1954).
[b] Midpoint of class interval.
[c] These limits have been modified from those given on pages 14 and 15 of King (1954) to agree with his stated intent (page 2) and summary of results obtained (pages 19 and 20).

Table 2. Class frequency data for chemicals tested by King (1954) on cotton fabric and the skin at Orlando, Florida, 1942–52.

Classification in tests on fabric	Classification in tests on the skin: 1	2	3	4	4A	Total
Compounds tested against *Anopheles quadrimaculatus*						
1	51	41	11	4	0	107
2	8	12	2	0	1	23
3	3	2	0	2	0	7
4	1	3	1	0	0	5
4A	1	0	0	2	0	3
Total	64	58	14	8	1	145
Compounds tested against *Aedes sollicitans and Ae. taeniorhynchus*						
1	0	3	1	0	0	4
2	2	5	2	2	2	13
3	0	3	4	2	2	11
4	0	3	0	3	0	6
4A	0	1	3	8	3	15
Total	2	15	10	15	7	49
Compounds tested against *Aedes aegypti*						
1	91	176	17	15	0	299
2	33	170	27	20	1	251
3	13	118	28	27	2	188
4	10	167	24	82	15	298
4A	4	196	41	129	33	403
Total	151	827	137	273	51	1439

RESULTS AND DISCUSSION

Table 2 gives the class frequency data obtained from Tables 1 and 7 of King (1954) in this study. Nearly all of the 25 possible combinations of fabric and skin classification were observed. One compound (dibutyl tartrate) was classified as class 4A on fabric and class 1 on

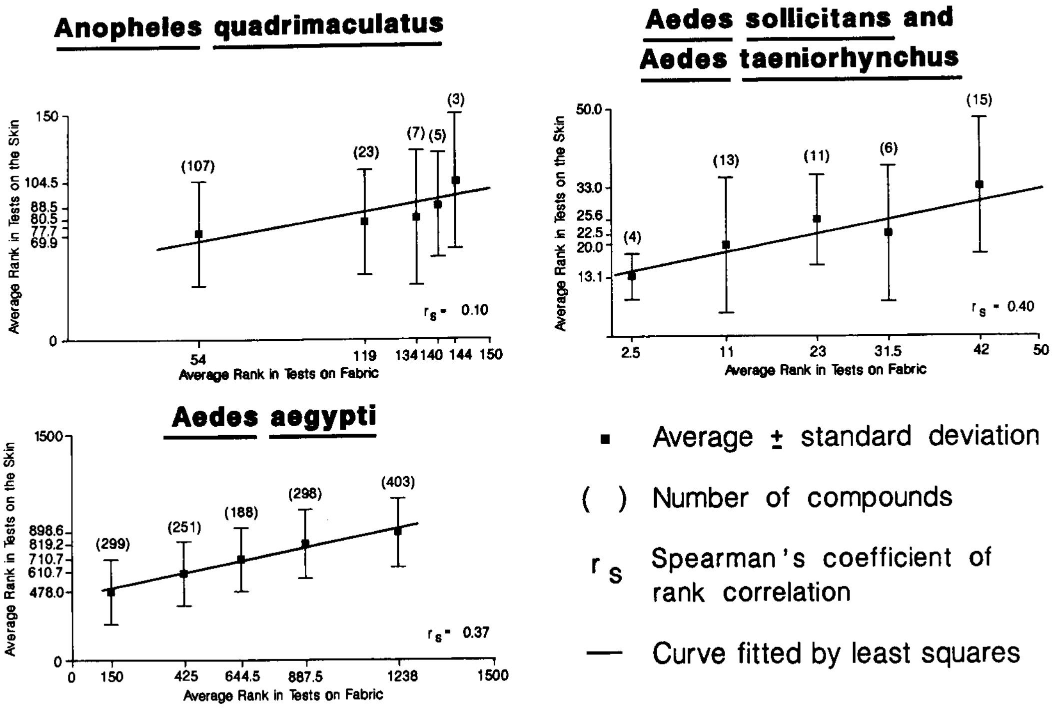

Fig. 1. Rank correlation of protection times of repellents tested on fabric and skin against *Anopheles quadrimaculatus*, *Aedes sollicitans*, *Ae. taeniorhynchus* and *Ae. aegypti.*

Table 3. Calculation of average rank and standard deviation for compounds classified as class 1 in tests on fabric against *Anopheles quadrimaculatus* (example).

Classification			Average rank			
Fabric[a]	Skin[a]	Frequency[a] (f)	Fabric[b] (X)	Skin[b] (Y)	(fY)	(fY^2)
1	1	51	54	32.5	1,657.5	53,868.75
1	2	41	54	93.5	3,833.5	358,432.25
1	3	11	54	129.5	1,424.5	184,472.75
1	4	4	54	140.5	562.0	78,961.00
1	4A	0	–	–	–	–
Total		107[c]			7,477.5[c]	675,734.75[c]

[a] For source see Tables 1 and 2.
[b] For method of calculation see text. Value shown for the independent variable (X) and the first two values shown for the dependent variable (Y) are calculated in the text as examples.
[c] Mean and standard deviation given in Figure 1 for the dependent variable (Y) were obtained from these totals by standard formulae.

the skin in tests against *An. quadrimaculatus*, and four compounds (pentaerythrityl tetrapelargonate, propyl-2-one benzoate, isobutyl N,N-diethylsuccinamate and vanillin) were so classified in tests against *Ae. aegypti*. However, no compounds were classified as class 1 on fabric and class 4A on the skin.

Figure 1 shows the correlation of the rank in tests on the skin with the corresponding rank in tests on fabric. The coefficient of rank correlation was not statistically significant in tests against *An. quadrimaculatus* (r_s = 0.10), but it was significant ($P < 0.01$) in tests against *Ae. sollicitans* and *Ae. taeniorhynchus* (r_s = 0.40) and *Ae. aegypti* (r_s = 0.37).

The standard deviations of the dependent variable (rank in tests on the skin) for each class of the independent variable (rank in tests on fabric) are also shown in Fig. 1. The high values of the standard deviation reflect the low correlations between the dependent variable and the independent variable in the data analyzed.

CONCLUSIONS

The results obtained in tests on the skin against *Ae. sollicitans*, *Ae. taeniorhynchus* and *Ae. aegypti* were significantly correlated with those given by the cotton fabric model. However, the coefficients of correlation were small ($\leq$ 0.40), indicating that only about $0.4^2 \times 100 =$ 16% of the variation in the dependent variable (rank in tests on the skin) is explained by the variation in the independent variable (rank in tests on fabric).

In view of the fact that extensive toxicity testing is required before tests of repellents on the skin can be approved (National Research Council 1987), we conclude that cotton fabric is not an adequate model for the skin in repellent tests. This conclusion substantiates the recent recommendation of the National Research Council (1987) for use of an animal model in lieu of the fabric model in screening topical mosquito repellents. However, the fabric model will still be needed in screening repellents for use on clothing.

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