TESTS OF ULTRASONIC EMISSIONS ON MOSQUITO ATTRACTION TO HOSTS IN A FLIGHT CHAMBER

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ABSTRACT. Five ultrasonic devices generating fundamental frequencies of 20–70 kHz were tested for their efficacy in repelling mosquitoes. Four species (Anopheles quadrimaculatus, Aedes aegypti, Ae. triseriatus and Haemagogus equinus), were used in a flight chamber in which females must fly upwind against the direction of the sound waves and around the ultrasonic devices to reach a trap downwind of a source of human breath and skin emanations. Repellency was rated by the number of mosquitoes entering the trap during a series of 5 min tests. For all species there was no significant difference between the numbers trapped when the devices were switched on or off, when all devices were tested simultaneously. Tests of individual devices against Ae. aegypti also failed to show a repellent effect.

INTRODUCTION

The most common and effective means of personal protection from mosquitoes is the topical application of chemical repellents. These have drawbacks, such as unpleasant odor, greasy feeling, solvent effect on some synthetic materials and limited protection time. The search for alternatives has led to several unconventional approaches, including systemic chemicals, electromagnetic energy and high frequency sound. Manufacturers and distributors of sound generators have put forward at least two rationales to explain the alleged repellent action of sound against mosquitoes. One is that females, once inseminated, are repelled by the flight sound of males. The other is that mosquitoes avoid the ultrasonic cries of bats. Although both explanations are conceivable, we know of no published scientific information to support either idea.

Audible sound generators claimed as mosquito repellers have been sold for over a decade. However, independent studies have failed to show the efficacy of the devices tested so far (Belton 1981, Garcia et al. 1976, Gorham 1974, Helson and Wright 1977, Kutz 1974, Lewis et al. 1982, Rasnitsyn et al. 1974, Schreck et al. 1977, Singleton 1977, Snow 1977). When frequency was measured in these studies, the devices had fundamental frequencies of only 2–5 kHz, but with harmonics extending into the ultrasonic range (Belton 1981). Sound pressure at 1 cm from the source varied from 68 to 84 dB.

Now, ultrasonic repellers are being marketed widely as a means of controlling the behavior of a variety of pests, including mosquitoes. Recent tests of two devices producing ultrasonic frequencies with peaks between 30 and 53 kHz and up to 96 dB at 0.5m (Schreck et al. 1984) nevertheless were unable to show a repellent effect on mosquitoes. Without knowledge of that study we performed similar tests of prototypes of five ultrasonic devices against four mosquito species, measuring repellency during attraction upwind toward a host. Their frequencies covered a wide ultrasonic range (peaks from 22.5 to 68 kHz), with sound pressures up to at least 92 dB at 0.5m. These devices purportedly had been tested in Taiwan, found to be effective mosquito repellers, and were strongly supported by testimonials after field use in the USA.

MATERIALS AND METHODS

MOSQUITOES. Four species were used to test for repellency: Anopheles quadrimaculatus Say (Savannah strain), Aedes aegypti (L.) (Rockefeller strain), Ae. triseriatus (Say) (Columbus strain) and Haemagogus equinus Theobald (Majé strain). The first two were from colonies maintained for >10 years in our laboratory, the last two from colonies 7 and 3 years old, respectively. Specimens for testing were reared at low density and maintained at 27°C and 70%RH under conditions described by Vargo and Foster (1982). The females emerged within a 48 hr period and were provided with 10% sucrose solution at all times except during a test. They were held in $51 \times 36 \times 57$ cm plastic cages in groups of several hundred with an equivalent number of males until the beginning of tests. Females were tested at the following adult ages: An. quadrimaculatus, 4-7 days; Ae. aegypti, 7-12 days; Ae. triseriatus, 11-14 days; Hg. equinus, 7-9 days. At these ages, >90% would already be inseminated, as had been determined by dissection of females at daily intervals in another study using the same conditions (W. A. Foster, personal communication). Between tests, females were held in groups of about 50 in 7-liter plastic cages.

EQUIPMENT. The ultrasonic devices were supplied by International Trade Development Corporation. They were battery-operated, from 7–19.5 cm tall, variously shaped, and fabricated of medium green plastic with black speaker covers. Since only one device emitted

any audible sound (a clicking noise), a lightemitting diode next to the on/off switch of all devices indicated when the sound emitter was on and was functioning. Each device either provided a choice of two different frequencies ("selectable") or produced a range of frequencies ("scanning"). The manufacturer had assigned an emitting frequency to each device. These were tested using an uncalibrated condenser microphone, a 3L5 Tektronix spectrum analyzer, and a 564 Tektronix storage oscilloscope. Sound pressure level was measured in an open room with a Brüel and Kiaer meter type 2203 (set in the linear position) with a Carthridge type 4133 condenser microphone. Sensitivity of this system drops by 10 dB at 30 kHz and by more than 20 dB above 45 kHz. Thus, devices emitting frequencies in the 60-75 kHz range probably produced considerably greater pressure levels than we measured. Measurements were made both at 0.5 - 1.0 cm and at 50 cm from the speaker covers. The emitted frequencies and sound pressure levels we recorded are presented in Table 1.

The repellent effect of the ultrasonic devices was tested under the environmental conditions for mosquito maintenance (above) in a flight chamber similar in design to the olfactometer described by Klowden and Lea (1978). A host compartment had separate openings to introduce exhaled human breath (by tube) and to insert a hand, with sufficient space around the wrist to allow entry of air into the compartment and over the hand. This air then passed into a trap, consisting of a cardboard cylinder 20 cm $long \times 18$ cm diameter. The trap was screened across the upwind end and contained an inwardly-directed screen funnel on the downwind end. The trap was connected to a 120 cm \times 30 cm² flight section of clear acrylic plastic. At its downwind end was a 30 cm³ clear

acrylic holding chamber for the mosquitoes, separated by a screen from a fan box that generated an air flow of about 3 cm/sec (0.1 km/h) in the flight section. The trap, flight section and holding chamber were separated by sliding screen gates before and after a test. The ultrasonic devices were placed together or individually directly in front of, and 7-20 cm downwind of, the entrance to the trap. The speakers on devices were always aimed downwind. To be trapped, a mosquito had to fly from the holding chamber, through the length of the flight section, around (within 15 cm) of one or more devices, and into the trap. When all five devices were tested together, two of the smaller (selectable) models (A and C) were placed on top of the large (scanning) models (B and D). When tested individually, the smaller models were mounted on clear plastic cups. The 40 kHz and 22.5 kHz positions, respectively, were used when the selectable devices (A and C) were switched on.

TEST PROCEDURE. Simultaneous tests of the five devices were performed in pairs, once with the devices switched on and once switched off. The order was random. Five pairs (replicates) of tests were conducted with Ae. triseriatus and four pairs with the other three species. For each species, a different human host was used in each pair of tests, and three of these hosts were used against each of the four species. It was known from previous laboratory and field studies that these hosts comprised a broad range of attractiveness for mosquitoes. For each test, the devices were switched on (or off), then about 50 females (range 43–51, $\overline{x} = 48.6$) were transferred gently by mouth aspirator to the holding chamber and allowed to settle for about 10 min while air moved through the flight chamber. At the start of each test, the host placed his hand into the host compartment and

Model	Type and size (cm)	Current (ma)	Nominal fr e quency (kHz)*	Recorded	Recorded pressure** (dB)	
				frequency (kHz)	0.5–1 cm	50 cm
Α	Selectable	9.2	40	42	115	83
	(6×10)		60	62	<63	<63
В	Scanning	18.0	22.5-60	22-70	109	78
	(7×11)			(42 peak)		
С	Selectable	2.0	22.5	22.5	111	76
	(4 ×7)		60	60	95	63
D	Scanning	18.0	20-60	60-75	95	66
	(9 × 19.5)			(68 peak)		
E	Scanning	19.0	20-60	20-75	115	92
	(17.5×15.5)			(25 peak)		

Table 1. Characteristics of ultrasonic devices tested.

* Frequency assigned by manufacturer.

** Only highest values given; values <63 dB were indistinguishable from background noise.

also began exhaling into it while an experimenter removed the screened gates and began observations on the flight behavior of the mosquitoes. At the end of 5 min the gates were replaced, and the mosquitoes in the trap (including any remaining on the outside of the screen funnel), flight section, and holding chamber were collected and counted. Those in the trap were anesthetized with CO_2 . A fresh group of mosquitoes was used for the second test in a pair, and mosquitoes were not used more than once in a 24 hr period.

Tests were run with individual ultrasonic devices to rule out possible interference caused by the emission of different frequencies simultaneously. These tests were conducted as above except that only *Ae. aegypti* was used (in groups of 48–50), and only one human host was involved.

RESULTS

The results of the tests using all five ultrasonic devices simultaneously are summarized in Table 2. The differences were generally quite small between the numbers of mosquitoes attracted into the trap when the devices were on or off. Two-way analysis of variance and Duncan's multiple range test of arcsine-transformed proportions indicated that these differences were not significant (P > 0.8). Comparisons within each species and among all species combined, using a t-test for paired arcsinetransformed data, also revealed no significant differences. However, there was a significantly smaller proportion of Hg. equinus and Ae. triseriatus trapped, compared to An. quadrimaculatus and Ae. aegypti (P < 0.0001). The majority of the former two species remained in the holding chamber at the end of the tests, whereas the majority of the latter two species flew into the trap (Table 2). Furthermore, there was a much larger proportion of the former species in the flight section. These general species differences occurred in all replicates, regardless of the host used as the attractant.

Comparisons of individual ultrasonic devices tested against *Ae. aegypti* are summarized in Table 3. The differences in the numbers trapped, when the devices were on or off, were again generally small. One-way analysis of variance and paired *t*-tests of arcsine-transformed proportions indicated that there was neither a difference between modes in tests of any of the individual devices, nor in all data combined (all P > 0.25).

Observations made during the course of tests did not reveal any behavioral differences in the mosquitoes flying toward the host when the ultrasonic devices were on or off. When the mosquitoes reached the vicinity of the devices, they typically veered around them, often circling them or lingering in their vicinity before proceeding upwind into the trap, regardless of device mode. Mosquitoes commonly alighted on the devices, occasionally right on the speaker covers. Those mosquitoes that remained in the holding chamber at the end of a test had generally not flown at all during the test: those collected from the flight section were usually at rest on or near the devices at the upwind end.

DISCUSSION

The results indicate that ultrasound in the 20-70 kHz range has little or no effect on oriented flight of female mosquitoes toward the source of breath and skin stimuli from human hosts. This study does not rule out the possibility that other frequencies or increased sound pressure might have a deterrent effect, nor that the devices tested might have an effect under different environmental conditions. It does, however, add to the accumulating weight of repellents.

 Table 2. Distribution of females in flight chamber after tests of five ultrasonic devices simultaneously. Totals of all replicates.

	Device mode	Replicates	Total tested	Flight chamber section			
Species				Holding	Flight	Trap	Trapped
An. quadrimaculatus	off	4	198	32	8	158	79.8%
4	on	4	197	23	11	163	82.7%
Ae. triseriatus	off	5	235	121	40	74	31.5%
	on	5	232	128	32	72	31.0%
Ae. aegypti	off	4	200	21	17	162	81.0%
	on	4	196	19	14	163	83.2%
Hg. equinus	off	4	198	124	33	41	20.7%
	on	4	197	104	43	50	25.4%
Totals	off	17	831	298	98	435	52.3%
	on	17	822	274	100	448	54.5%

Model	Device mode	Replicates		Flight chamber section			
			Total tested	Holding	Flight	Trap	Trapped
A	off	2	100	2	1	97	97.0%
	on	2	99	4	1	94	94.9%
В	off	2	96	1	5	90	93.8%
	on	2	100	2	2	96	96.0%
С	off	2	100	0	4	96	96.0%
	on	2	100	2	3	95	95.0%
D	off	2	99	5	4	90	90.9%
	on	2	99	1	6	92	92.9%
E	off	7	345	16	13	316	91.6%
	on	7	345	26	21	298	86.4%
Totals	off	13	640	14	26	600	93.8%
	on	13	645	30	29	586	90.9%

 Table 3. Distribution of Aedes aegypti females in flight chamber after tests of each of five ultrasonic devices individually. Totals of all replicates.

All four species usually deviated from their direct flight path when approaching the ultrasonic devices, whether they were on or off, and this was probably caused by the confusing effect of air turbulence downwind of the devices. The visual target they presented also provided a distraction, so that the mosquitoes often circled the devices before again moving upwind into the trap.

The lower trapping efficiency of Hg. equinus and Ae. triseriatus in the flight chamber test system was probably due to one or both of these factors: 1) They had been in culture a relatively short time and were thus more easily disturbed by handling and confined conditions; 2) they are zoophilic sylvan species, unlike the domestic and peridomestic Ae. aegypti and An. quadrimaculatus, and are poorly adapted to seeking human hosts in enclosed situations. The larger numbers of Hg. equinus and Ae. triseriatus remaining in the holding chamber and flight section after each test, compared to the other species, indicate that both the initial host-seeking response and upwind orientation were inhibited.

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