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LABORATORY EVALUATION OF THE IRRITANCY OF BENDIOCARB, LAMBDA-CYHALOTHRIN AND DDT TO *ANOPHELES GAMBIAE*

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ABSTRACT. In a laboratory study, the irritancy of bendiocarb, lambda-cyhalothrin and DDT to *Anopheles gambiae* was evaluated at field, ⅓ field and $^{1}/_{10}$ field rates using WHO conical exposure chambers and excito-repellency test boxes. Bendiocarb was the least irritant insecticide at all rates, inducing levels of takeoff, flight and exiting behavior similar to those of a distilled water control treatment. Of those mosquitoes introduced to the bendiocarb-treated boxes, not more than 1% exited and survived at any dose rate. Lambda-cyhalothrin and DDT were highly irritant to *An. gambiae,* inducing a strong stimulation to take off and fly and also a high level of exiting. Exiting–survival rates associated with lambda-cyhalothrin and DDT were between 15 and 51%. The relevance of these findings to the control of mosquito populations and the prevention of malaria transmission is discussed.

INTRODUCTION

Irritancy of insecticides to adults of mosquito vector species has long been recognized (Metcalf et al. 1945, Kennedy 1947). Insecticide irritancy is important because it is a factor governing the number and duration of contacts between the mosquito and the insecticide deposit, and hence is a critical determinant of the probability of mosquitoes acquiring lethal doses (Trapido 1952). Most data so far have been generated for irritancy to DDT, often to the neglect of studies on other insecticides. No method of studying irritancy is yet widely accepted, thus preventing the generation of strictly comparable data (Roberts et al. 1984).

In 1970, the World Health Organization (WHO) proposed their latest methodology for assessing insecticide irritancy to mosquitoes in an attempt to standardize techniques. This consists of observations of mosquitoes placed inside transparent conical exposure chambers held over impregnated papers. With modifications, this method has been used to show a high irritancy of DDT to several *Anopheles* spp. (Bondareva et al. 1986, Quinones and Suarez 1989), a high irritancy of permethrin to 2 anophelines and one culicine (Ree and Loong 1989) and a high irritancy of cyfluthrin, deltamethrin and lambda-cyhalothrin to *Anopheles gambiae* Giles (Pell et al. 1989). More realistic and less time-consuming data on the behavioral effects of insecticides on mosquitoes may be obtained from the use of the excito-repellency (ER) test box (Rachou et al. 1973). In this apparatus, mosquitoes are able to escape completely from perceived unfavorable conditions by movement from a treated box into an untreated location, usually a smaller exit box. Marked escape responses of anophelines to DDT have been reported using this technique (Charlwood and Paraluppi 1978, Rozendaal et al. 1989).

This study reports a laboratory investigation of the irritancy of 3 insecticides currently specified by the WHO for mosquito control: bendiocarb (a carbamate), lambda-cyhalothrin (a pyrethroid) and DDT. Irritancy was assessed using both conical exposure chambers and ER test boxes.

MATERIALS AND METHODS

Mosquitoes: The mosquitoes used in the study were 4–5-day-old, freshly bloodfed, *An. gambiae sensu stricto* (G_3 strain). This strain was colonized from The Gambia and had been held in laboratory culture, with no insecticidal pressure, since 1975.

WHO conical exposure chambers: Sheets of glass (10 cm^2 × 2 mm thick) were covered with glass microfiber paper (Whatman GF/A) and treated with bendiocarb, lambda-cyhalothrin or DDT. A distilled water control treatment was also included. The insecticide formulations and doses applied are shown in Table 1. Glass microfiber paper was chosen because of its lack of interaction with insecticides (Barlow and Hadaway 1968). The treatment procedure involved use of an automated spraying device fitted with a Tee-jet 8004-E nozzle, which moved at a speed of 1 m sec^{-1} and a height of 29.5 cm above the targets. This produced an application rate of 40 ml m^{-2}.

Approximately 18 h later, mosquitoes were placed individually in adaptation tubes (transparent plastic cylinders 2 cm diam × 7.5 cm height), and a stopper fitted to each tube. The base of each tube contained a 2-cm-wide disc of glass microfiber paper that had been soaked in distilled water 24 h earlier. Tubes were placed in an aluminium rack and held horizontally. The bases of all tubes were illuminated by a uniform

Table 1. Insecticide formulations and doses evaluated.

Insecticide	Concentration of active ingredient (AI) in formulation[1] (% w/w)	Dose (mg AI m^{-2})
Bendiocarb	80	400.0[a]
		133.3[b]
		40.0[c]
Lambda-cyhalothrin	10	30.0[a]
		10.0[b]
		3.0[c]
DDT	75	2,000.0[a]
		666.7[b]
		200.0[c]

[1] All formulations were water-dispersible powders. All spray solutions were made up in distilled water. [a] = standard field rate, [b] = ⅓ field rate, [c] = $1/_{10}$ field rate. Doses less than the standard field rates were evaluated to estimate the irritancy of aged deposits, originally treated at field rate.

light source (40-W clear light bulb) resulting in a light intensity of 86 lx inside each tube. The adaptation period lasted 30–40 min for each mosquito and served to mimic the main experimental conditions under which observations were made (i.e., a contact surface of glass microfiber paper and a transmitted light intensity of 86 lx).

Adaptation tubes were individually taken from the rack as required. With minimal disturbance, the stopper was removed while holding the end of the tube over the exit hole of a WHO conical exposure chamber held in a ceramic box. With gentle tapping of the tube, the mosquito was induced to fly into the chamber and the exit hole then sealed with a cotton wool plug. The box contained 3 grooves into which fitted a sheet of translucent glass (10 cm^2 × 2 mm thick), a sheet of glass covered with treated glass microfiber paper and the exposure chamber. The exposure chamber fit tightly onto the paper surface. By means of a circular hole (9 cm diam) in the back of the box, transmitted light entered the exposure chamber via the translucent tile. The box was positioned at a distance (*ca.* 25 cm) from the light source sufficient to produce a light intensity in the chambers equal to that in the adaptation tubes.

After an adaptation period of 1 min, the behavior of each mosquito was monitored for 7 min following placement in the exposure chamber. During observations, the following parameters were recorded: 1) length of time to first take off from the paper (once having landed for the first time on the paper during the observation period), 2) number of takeoffs from the paper, and 3) total time in flight. If a mosquito landed on the paper prior to the 7-min observation period, but did not take off from the paper during the course of this period, a score of 420 sec was given for time to first take off. Similarly, if a mosquito landed on the paper during the observation period and had not taken off by the end of this period, a score equal to the length of time spent on the paper was given. Mosquitoes maintaining contact with either the exposure chamber or the cotton wool plug for a period >30 sec were induced to move by gentle tapping of the apparatus. Any immediate flight induced was not recorded in (3). Each treatment was replicated 20 times and the order in which treatments were observed was randomized to control for effects of circadian rhythm on mosquito behavior, experimenter fatigue, etc. All observations took place between 0830 and 1900 h at 23–25°C and 55–65% RH.

ER test boxes: Light-proof test boxes, each consisting of 6 plates of aluminium (30 cm^2) that could easily be assembled and dismantled, were constructed (Fig. 1). Adjacent plates could be attached by means of 2 screws positioned through flanges running along the edges of the plates. One plate of each box had a circular exit hole (10 cm diam) cut into it. The plates, on the sides without the flanges, were covered by wrapping glass microfiber paper around each plate and by taping it down on the other side. The plates containing the exit holes were covered although the holes were not. The plates were treated as shown in Table 1, using the same procedure as outlined previously. Prior to their assembly into boxes, the plates were dried for 4 h. Each treatment was replicated 3 times.

After assembly, a truncated paper cone (height 10 cm) with a 2-cm-diam hole at the apex was fitted exactly over the hole in each box and attached with tape. The inner surface of each cone had previously been coated with 'Fluon' (polytetrafluoroethylene) to provide a surface unattractive for mosquito settling. All boxes were orientated such that the exit holes and cones were on the front vertical faces. A glass cylinder (4.5 cm diam × 12.5 cm length) was then tightly fitted over the apex of each cone. Nylon netting was used to seal the cylinders at the ends farthest from the cones. The cylinders were held in place by means of clamps attached to retort stands.

Approximately 18 h later, 40–50 mosquitoes were introduced into each test box. Mosquitoes that found conditions in the test boxes unfavorable were able to escape by exiting into the cones and then passing into the glass cylinders. Once inside the cylinders, mosquitoes could be seen and removed using an aspirator pushed through a hole in the netting. Between collections, these

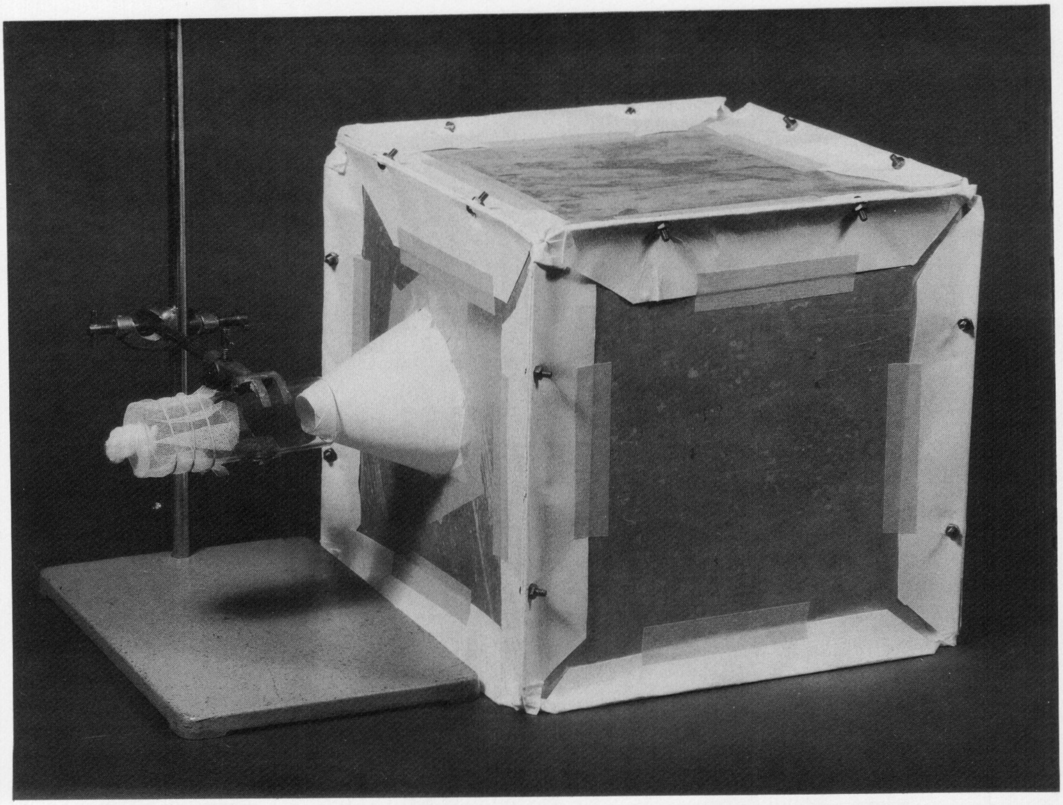

Fig. 1. An excito-repellency test box as used to study the exiting behavior of mosquitoes exposed to insecticides.

holes were sealed with cotton wool. Mosquitoes accumulating in the cylinders were removed at intervals over an exiting period lasting 2 h and the individual batches of mosquitoes placed into plastic holding containers (6 cm base diam. × 7.5 cm height), with cotton wool soaked in 10% glucose solution available. The order in which treatments were observed was randomized. Experiments took place between 0830 and 1700 h at 23–25°C, 55–65% RH and at a light intensity of 0.8 lx (comparable with the 0.3 lx used by Rowland [1991] to simulate a moonlit night). At the end of the 2-h exiting period, the cone of each box was sealed to prevent further escape. On the following day, mortality counts of the mosquitoes present in the holding containers were made and 6 days later, all test boxes were opened and mosquitoes inside were counted.

To check the validity of adopting a 2-h exiting period, the knockdown speeds of each insecticide at each dose evaluated were observed. Between 10 and 15 freshly bloodfed mosquitoes were introduced into WHO exposure chambers attached to 10-cm² glass plates covered with treated glass microfiber paper. Exposure chambers were placed in a vertical orientation and knockdown was monitored. A distilled water control treatment was used and all treatments were replicated 3 times.

RESULTS

WHO conical exposure chambers: Data were analyzed using one-way analysis of variance (Fig. 2). A very low degree of irritant behavior was observed in the control treatment as indicated by long times to first takeoff (mean = 323.5 sec), low numbers of takeoffs (mean = 0.93) and short total flight durations (mean = 7.9 sec). Only for time to first takeoff at the lower 2 dose rates was bendiocarb significantly more irritant than the control ($P < 0.05$). Of the 3 insecticides, results for bendiocarb were closest to those of the control, indicating bendiocarb to be the least irritant insecticide.

Lambda-cyhalothrin was significantly more irritant than the control at both field and ⅓ field rate with the greater difference apparent at ⅓ field rate. Higher irritancy was indicated by significantly shorter times to first takeoff ($P < 0.001$),

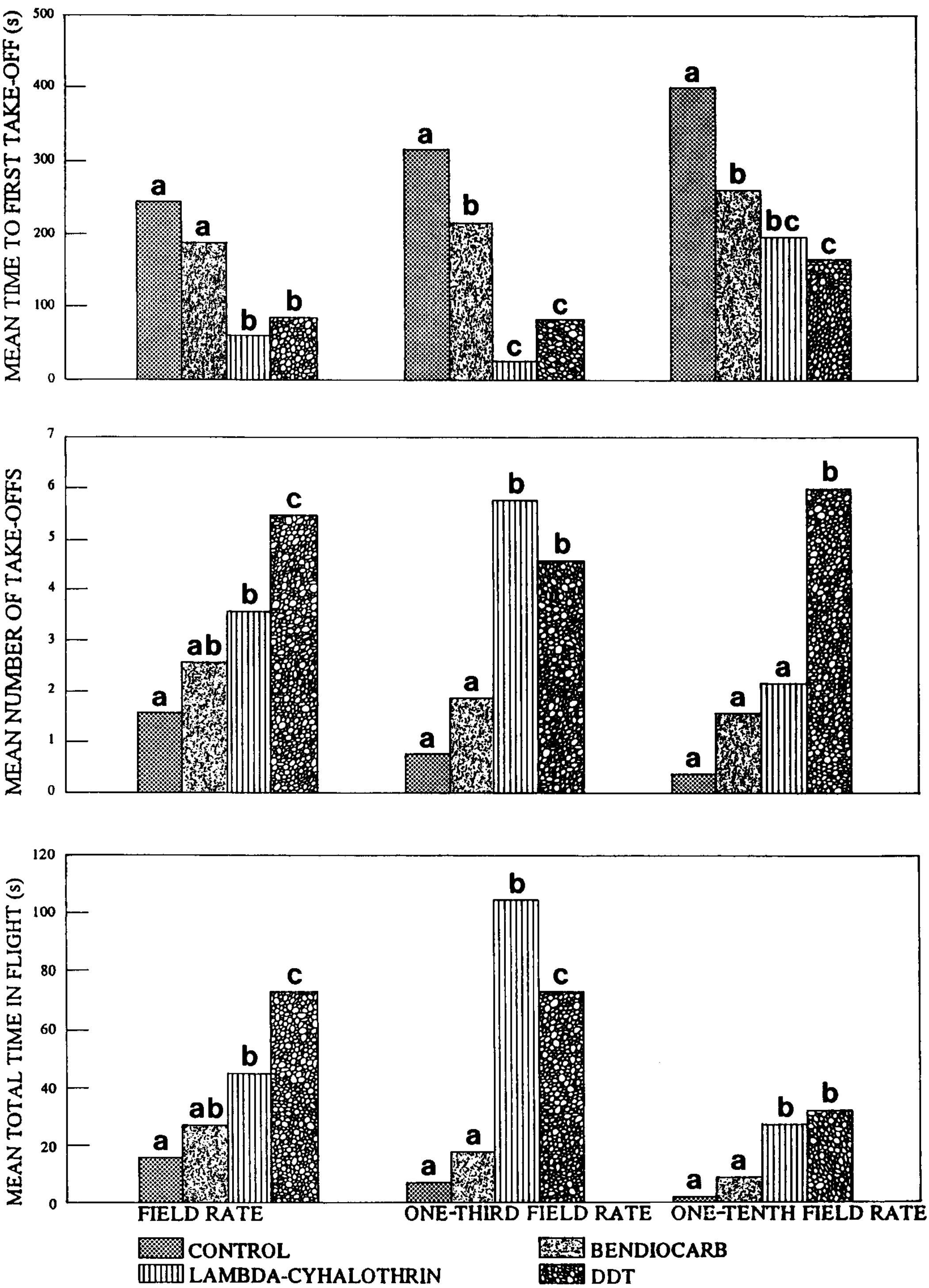

Fig. 2. Observations of the behavior of mosquitoes placed in WHO conical exposure chambers. Bars sharing the same letter at each dose rate in each histogram are not significantly different ($P > 0.05$).

greater numbers of takeoffs ($P < 0.05$) and longer total flight durations ($P < 0.05$). Irritancy was least pronounced at $1/10$ field rate with only time to first takeoff and total flight duration significantly different from the control ($P < 0.001$). Lambda-cyhalothrin was more irritant than bendiocarb at all rates evaluated. The greatest difference occurred at $1/3$ field rate, where for all behavioral measures, lambda-cyhalothrin had significantly greater irritancy ($P < 0.001$). At field and $1/10$ field rate, lambda-cyhalothrin was significantly more irritant than bendiocarb for one measure of behavior only ($P < 0.05$).

For all measures of behavior, DDT was significantly more irritant than the control and bendiocarb at all 3 dose rates ($P < 0.05$). In comparison with lambda-cyhalothrin, DDT resulted in significantly more takeoffs and a longer total flight duration at field rate, and significantly more takeoffs at $1/10$ field rate ($P < 0.05$). Lambda-cyhalothrin resulted in a significantly longer total flight duration than DDT at $1/3$ field rate ($P < 0.05$).

ER test boxes: Exiting data exhibited a very clear response to treatment (Fig. 3). Mosquitoes placed into the control boxes consistently showed a very low level of exiting with <8% leaving the boxes during the 2-h observation period. The level of exiting induced by bendiocarb was similarly very low (<12%) at all 3 dose rates. Lambda-cyhalothrin and DDT induced far greater levels of exiting, with both insecticides causing >50% exit rates at all dose rates. Mosquitoes generally exited the boxes during the first 15 min of the observation period, but, particularly at $1/10$ field rate, exiting was spread more evenly with several mosquitoes exiting after 30 min.

The proportion of mosquitoes that had exited the boxes at the end of the observation period was calculated for each treatment and a one-way analysis of variance performed (Table 2). Prior to analysis, data were arcsine transformed to stabilize the error variability and validate the assumption of normality. At no dose rate was the level of exiting associated with bendiocarb significantly different from the control treatment ($P > 0.05$). Both lambda-cyhalothrin and DDT caused significantly greater exiting than either the control or bendiocarb at all dose rates ($P < 0.01$). DDT consistently resulted in greater exiting than lambda-cyhalothrin, although significantly greater exiting occurred only at field rate ($P < 0.01$).

The proportion of mosquitoes that had exited the boxes after 2 h and were alive in 24-h mortality counts (i.e., exiting-survivors) was calculated for each treatment, and after arcsine transformation, one-way analysis of variance was performed (Table 3). Treatment effects were closely correlated with those apparent for the proportion exiting data. Bendiocarb did not cause significantly greater numbers of mosquitoes to exit and survive than the control at any dose rate. The number of exiting-survivors associated with lambda-cyhalothrin and DDT was significantly greater than for either the control or bendiocarb at all dose rates ($P < 0.01$). In comparison with lambda-cyhalothrin, DDT resulted in significantly greater exiting-survival at field rate ($P < 0.01$), with no significant difference between the 2 at the lower rates ($P > 0.05$).

Knockdown speed data as assessed using the exposure chambers showed bendiocarb to have the most rapid action, followed by lambda-cyhalothrin and then DDT. In no insecticide treatment at any dose rate did the time for 100% of the mosquitoes to be knocked down exceed 90 min. These data confirmed the validity of adopting a 2-h cut-off point with the ER boxes.

DISCUSSION

Anopheles gambiae exposed to the water-treated control paper showed a strong adaptation to conditions in both the exposure chambers and ER boxes, although considerable variations in behavioral response occurred over the course of the 3 insecticide dose rate evaluations. These were probably due to different generations of mosquitoes being used in each case. Mosquitoes showed a clear behavioral response to each of the insecticides evaluated. Exposure to bendiocarb resulted in a low stimulation to take off and fly and low exiting behavior, with less than 2% of the mosquitoes introduced into the ER boxes exiting and surviving. Lambda-cyhalothrin and DDT had far higher irritancy inducing a stronger stimulation to take off and fly and also a higher level of exiting behavior. Of those mosquitoes exiting the lambda-cyhalothrin- and DDT-treated boxes, a high proportion had not picked up a lethal dose and were alive 24 h later. Levels of exiting-survival associated with lambda-cyhalothrin and DDT were consistently at least 15%, and reached as high as 51% for lambda-cyhalothrin at $1/10$ field rate. Of these 2 insecticides, the irritancy associated with DDT was slightly the greater.

These findings are in agreement with the widely reported low irritancy of bendiocarb and high irritancy of DDT to mosquitoes. Irritancy of lambda-cyhalothrin has not yet been widely researched, although Pell et al. (1989) did report a laboratory evaluation of the irritancy of this insecticide to *An. gambiae* using conical exposure chambers. Lambda-cyhalothrin could not be distinguished from DDT in terms of time to first flight, although for both flight frequency and the

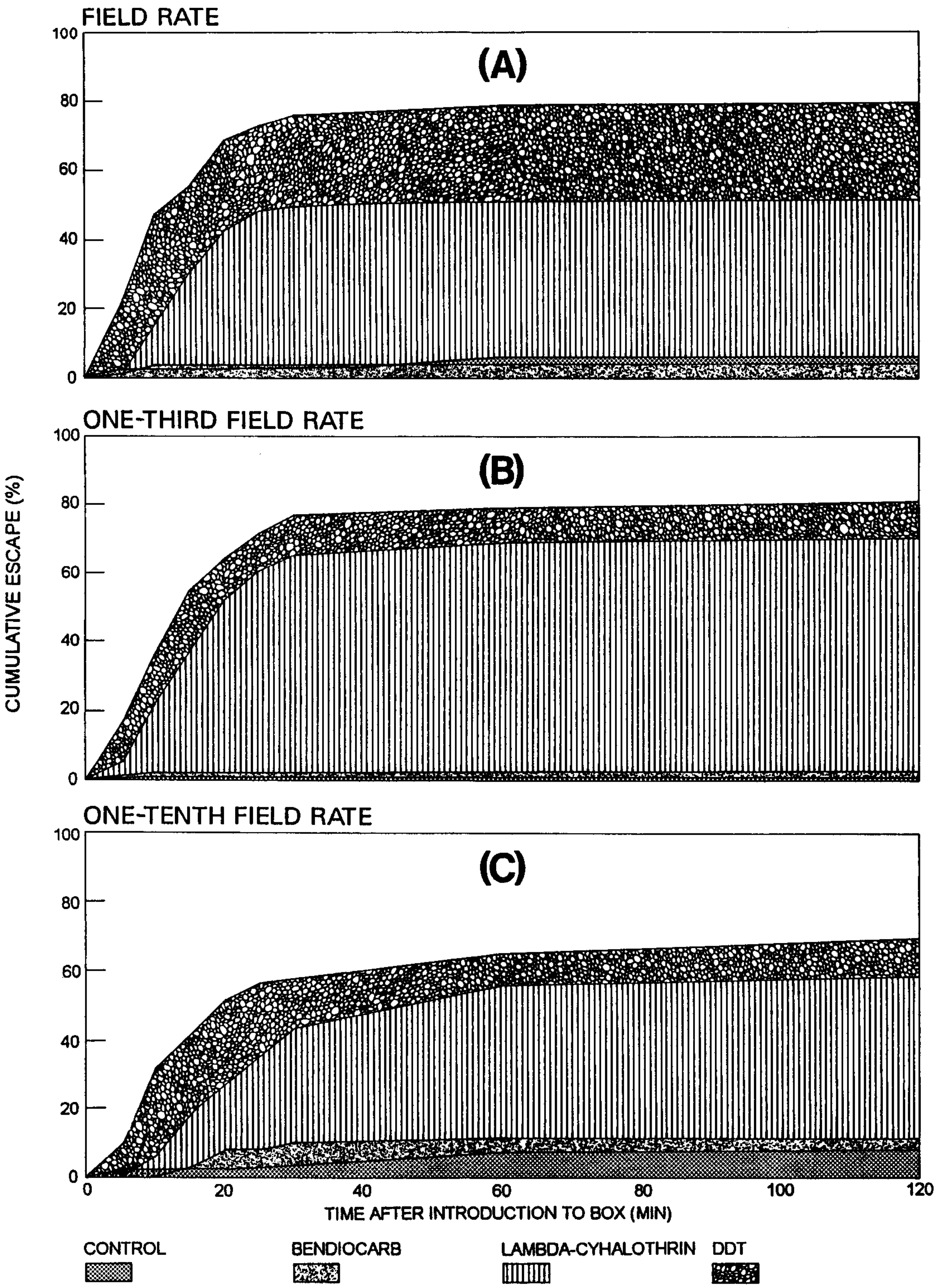

Fig. 3. Numbers of mosquitoes exiting the excito-repellency test boxes over the 2-h exiting period. A. Field rate; B. One-third field rate; C. One-tenth field rate.

Table 2. Mean ± SE arcsine-transformed proportion mosquitoes exiting ER boxes. Values sharing the same letter at each dose rate are not significantly different ($P > 0.01$).

	Dose		
Treatment	Field rate	One-third field rate	One-tenth field rate
Distilled water control	12.27 ± 4.15a	2.34 ± 4.06a	16.48 ± 5.12a
Bendiocarb	9.73 ± 2.77a	6.56 ± 5.93a	13.71 ± 12.16a
Lambda-cyhalothrin	45.16 ± 7.45b	56.35 ± 4.07b	51.38 ± 3.24b
DDT	63.32 ± 3.76c	63.93 ± 7.67b	64.11 ± 12.78b

relative amount of time spent on a treated surface or in flight, lambda-cyhalothrin induced a significantly different response from that of DDT. In comparison with deltamethrin and cyfluthrin, lambda-cyhalothrin was the least irritant pyrethroid for all aspects of behavior examined. The irritancy of bendiocarb was also assessed and was found to be low. Artem'ev et al. (1991) compared the irritancy of lambda-cyhalothrin and bendiocarb and concluded that because neither insecticide led to any redistribution of mosquitoes between sprayed and unsprayed buildings, both insecticides were nonirritant. Artem'ev et al. (1991), however, did not monitor the numbers of mosquitoes exiting the sprayed buildings and hence their data do not strictly represent the irritant effect of either insecticide, as mosquitoes may have migrated to the external environment.

In the present study, the evaluation of dose rates lower than the standard field rates was not completely realistic of the field situation. As deposits age in the field, breakdown products may result that themselves have behavioral effects and a changing ratio of active ingredient to the inert components of the insecticide formulation may occur. The marked peak of irritant behavior seen at ⅓ rate for lambda-cyhalothrin suggests that in the field, irritancy to this insecticide may not reach a maximum until considerably after application. Irritancy to lambda-cyhalothrin and DDT may also remain high for a considerable time thereafter, as both induced marked irritant responses at the $\frac{1}{10}$ field rate dose. Coosemans and Sales (1977) reported a constant irritant effect of permethrin and deltamethrin as the insecticide deposits became older. Ree and Loong (1989) observed *An. maculatus* Theobald to show a graded response of irritable behavior to increasing doses of permethrin, although this did not occur with *An. farauti* Laveran or *Culex quinquefasciatus* Say. Mosquito species may also therefore be a factor governing the importance of irritancy of ageing insecticide deposits.

The escape behavior of mosquitoes exposed to an insecticide consists of independent components with generally increased activity not necessarily leading to specific passage through an exit hole (Gerold and Laarman 1967). Sole re-

Table 3. Mean ± SE arcsine-transformed proportion exiting-survivors of ER boxes. Values sharing the same letter at each dose rate are not significantly different ($P > 0.01$). (No. of mosquitoes placed into boxes).

	Dose		
Treatment	Field rate	One-third field rate	One-tenth field rate
Distilled water control	9.27 ± 1.97a (136)	0 ± 0a (142)	5.42 ± 4.69a (139)
Bendiocarb	0 ± 0b (141)	0 ± 0a (140)	3.32 ± 5.76a (135)
Lambda-cyhalothrin	22.64 ± 3.65c (134)	44.68 ± 4.30b (138)	45.82 ± 8.03b (144)
DDT	35.04 ± 5.26d (143)	42.12 ± 6.06b (134)	37.88 ± 7.53b (133)

liance on conducting observations of mosquitoes in conical exposure chambers should therefore be avoided as this methodology does not give data on the exiting process and may not provide a realistic indication of likely house-leaving behavior in the field. In addition to excito-repellency test boxes, other methods for measuring the exiting response of mosquitoes exposed to insecticides have been proposed. Gerold (1970, 1977) developed methodologies for measuring flight activity and escape reaction involving modifications to the standard WHO bioassay test kits and use of a twin-funnel apparatus. Georghiou et al. (1972) reported an apparatus that could be used to assess simultaneously the behavior of mosquitoes in both the presence and absence of insecticide, a situation more analogous to that occurring in the field. In conducting laboratory evaluations of insecticide irritancy, choice of methodology and bloodfeeding status of the mosquitoes should be carefully considered. Unfed mosquitoes may show more pronounced irritant behavior (Qutubuddin 1967) and may therefore require extra experimenters to record the data.

Although laboratory studies of irritancy to mosquitoes may provide useful data on the behavioral effects of insecticides, data from the field are necessary to determine whether these effects are important under operational conditions. A number of studies have assessed insecticide irritancy in the field. Bown et al. (1987) observed *An. albimanus* Wiedemann to have greater contact with the insecticide in bendiocarb-treated houses, resulting in higher mortality than occurred in deltamethrin-treated houses. Similarly, Mpofu et al. (1991) reported very few *An. arabiensis* Patton to escape from bendiocarb-treated huts and concluded that the lethal effect of bendiocarb was more pronounced than any irritant effect. Insecticide irritancy may also be observed as a reduced entrance of treated houses. In the study of Bown et al. (1987), mosquitoes attempted to enter houses treated with bendiocarb in higher densities than those treated with deltamethrin. Reduced entrance of houses treated with DDT has been reported by Rozendaal et al. (1989) and Suwonkerd et al. (1990). Mosquitoes may also show significantly reduced entrance of houses containing permethrin-impregnated nets (Darriet et al. 1984).

Insecticide irritancy has on occasion been implicated as a factor resulting in the failure to control mosquito populations in the field. The irritancy of both permethrin and deltamethrin was seen as a primary factor leading to the escape and survival of many *An. gambiae* and *An. funestus* Giles from treated huts, and an unsatisfactory impact on population levels was recorded (Rishikesh et al. 1978). Similarly, Bondareva et al. (1986) and Sharp et al. (1990) recommended the cessation of use of DDT due to its high irritancy in Soviet Central Asia and South Africa, respectively. However, other researchers have viewed insecticide irritancy as a favorable means of providing protection against indoor man–vector contact. Roberts and Alecrim (1991) believed the strongly reduced entrance of houses by *An. darlingi* Root following spraying of DDT in Brazil would provide considerable protection from malaria transmission.

There is as yet no widespread agreement about the importance of insecticide irritancy for the control of malaria, although there is a consensus that insecticide nonirritancy is desirable for reducing mosquito numbers. The key to the prevention of malaria transmission, however, relies on reducing the level of man–vector contact, which may not be directly related to mosquito population size. Until more field studies are performed that directly compare the impact of irritant and nonirritant insecticides on malaria transmission, this subject is likely to remain open and controversial.

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