The impact of propoxur on *Anopheles gambiae* s.l. and some other anopheline populations, and its relationship with some pre-spraying variables

L. MOLINEAUX,[1] G. R. SHIDRAWI,[2] J. L. CLARKE,[3] R. BOULZAGUET,[4] T. ASHKAR,[4] & K. DIETZ[5]

A significant and stable difference in the residual night-biting collection (NBC, observed/expected) of A. gambiae *s.l. was observed among different villages of the West African savannah when sprayed for 2 years with propoxur. The residual mosquito density of a given village was positively associated with some of its pre-spraying characteristics: the NBC/PSC (pyrethrum spray collections) ratio, the median biting hour, and possibly the proportion of species B. It was not significantly associated with several other pre-spraying characteristics (absolute mosquito density, the ratios between NBC indoors and NBC outdoors, between ETC (exit-trap collections) and PSC, between fed and gravid in the PSC or ETC, between males and females in the PSC), and was not associated with variations in recorded coverage, with latitude, or with distance from unsprayed villages.*

In a comparison between A. gambiae *s.l.,* A. funestus, *and* A. pharoensis, *the residual NBC (observed/expected) was positively associated with the pre-spraying NBC/PSC ratio, and was negatively associated with the median biting hour.*

The pre-spraying NBC/PSC ratio thus appears to be a predictor of the variation, between villages or species, in the reduction of the NBC by residual spraying, and may assist in forecasting the effect of a residual insecticide. The limitations of any absolute prediction must, however, be kept in mind.

Residual spraying with propoxur was used during the intervention phase of the research project on the epidemiology and control of malaria in the African savannah, conducted by the Government of the Federal Republic of Nigeria and the World Health Organization in the Garki district of Kano State in 1970–1975. Under propoxur the man-biting population of *A. gambiae* remained at a relatively high level and the effect of the insecticide varied significantly between different villages; the effect also varied between different anopheline species. This paper analyses these variations and some associated variables, and discusses the possible causes and the predictive value of the associations observed.

[1] Medical Officer, Malaria and Other Parasitic Diseases, World Health Organization, Geneva, Switzerland.

[2] Entomologist, World Health Organization, Alexandria, Egypt.

[3] Entomologist, World Health Organization, Sri Lanka.

[4] Technical Officer, World Health Organization, Kano, Nigeria.

[5] Statistician, Health Statistics, World Health Organization, Geneva, Switzerland.

MATERIAL AND METHODS

A. gambiae (species A and B) and *A. funestus* are the malaria vectors in the Garki district; two other species, *A. rufipes* and *A. pharoensis*, were also collected in relatively large numbers. The densities of all species undergo a very wide seasonal variation and, in most of the study area, are hardly measurable in the dry season. The baseline period included the dry and wet seasons of 1971 and the dry season of 1972.

Spraying with propoxur was carried out in 165 villages, both compact and scattered, situated in an area of approximately 875 km^2 during the wet seasons of 1972 and 1973. Spraying was done 3–4 times at interval of 2 months, at 2 g of technical propoxur per m^2. The intervention period included these two wet seasons and the intervening dry season. Intensive entomological evaluation was conducted in eight compact villages, 6 sprayed and 2 unsprayed (Fig. 1). During the wet season, the following studies were carried out at fortnightly

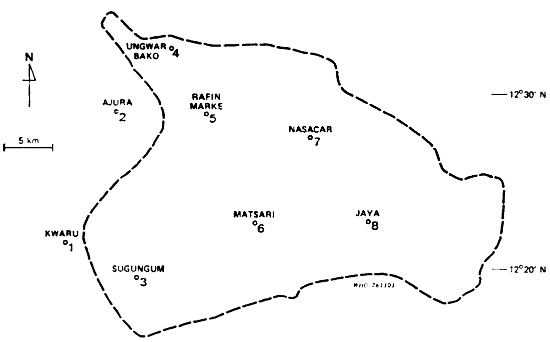

Fig. 1. The area within which all (165) villages were treated with propoxur and the eight villages in which night-biting collections were carried out.

intervals: (*a*) night-biting collections (NBC) with 2 man-nights indoors and 2 man-nights outdoors, each at 2 stations; collector-baits were changed systematically among the indoor and outdoor positions, stations, and villages, so as to avoid any bias resulting from differential attraction and/or ability; (*b*) pyrethrum spray collections (PSC) in 5–12 huts; and (*c*) exit-trap collections (ETC) in 3 huts during the baseline period and in 5 huts during the intervention period. During the dry season, NBC and ETC were performed every 5 weeks only. The collections were conducted at fixed stations, which were different for each type of collection. In the sprayed villages, the huts used as collection stations were, in principle, sprayed like any other hut; collections were also made in artificial pit-shelters but these were very unproductive and the relationship of the yield to the populations of anophelines resting outdoors is unknown. There was no systematic search for larvae. Identification of species A and B of *A. gambiae* was performed only in a fraction of the PSC.

RESULTS

The effect of propoxur on the number of A. gambiae *s.l. collected by NBC and its variation between villages*

As regards the different species and the various methods applied, only the NBC of *A. gambiae* s.l. produced sufficient numbers for a comparison between the different sprayed villages.

In measuring the effect of propoxur on the NBC, both the pre-spraying data and the data from the unsprayed controls should be taken into account. It was assumed that, without propoxur, the treated villages would have shown in 1972 and 1973 the same proportional changes as the unsprayed controls; under this assumption, the expected total numbers of *A. gambiae* collected by NBC indoors (IN) and outdoors (OUT) in the wet seasons of 1972 and 1973 in a given village were, respectively, 0.42 and 1.33 times the 1971 figure. The corresponding factors for the indoor and outdoor collections were calculated separately; Table 1 shows the numbers actually collected per village and per wet season and, in the case of the sprayed villages, the ratio of the number observed to the number expected. The numbers collected by night-biting collection (IN, OUT, or total (TOT)) are directly comparable since they represent, for each year and village, the total yield obtained during a period starting with very low densities at the end of a dry season, rising to high or relatively high densities during the wet season, and returning to very low densities at the end of the wet season, using a fixed number of baits and a fixed sampling frequency. A few collections were missed (see footnote *a* to Table 1), but only at times of low density, so that the total number collected is very little affected. The analysis, therefore, starts from the actual numbers collected; the replacement of the few missing NBCs by interpolation would have no effect on the conclusions. On average in the sprayed follow-up units, the density of *A. gambiae*, as estimated by NBC, was reduced to 10% and 13% of its expected value (wet seasons of 1972 and 1973, respectively). The reduction was greater indoors than outdoors, but there was a relatively strong association between the indoor and outdoor reductions in the same village (see Table 1).

Table 1 shows that the ratio between the observed and expected numbers varied from village to village. In the six sprayed villages the distribution of *A. gambiae* collected in 1972 and 1973 was significantly different from that expected on the basis of the 1971 figures, as related to indoor, outdoor, and total collections (chi-square test, $P<0.001$). The differences were also relatively stable: there was a strong positive correlation between the 1972 and the 1973 findings (observed/expected) in the same village (correlation coefficients significantly different from zero at the 5% level for IN, OUT, and IN + OUT; rank correlation coefficients significantly different from zero at the 5% level for IN and IN + OUT).

While the NBCs in the six sprayed villages were higher in 1973 than in 1972, the effect of spraying, in terms of reduction of the expected NBC yield, was

Table 1. The number[a] of *A. gambiae* s.l. collected by night-biting collection (NBC) and, in the sprayed villages, the ratio of that number to the number expected

Village	Treatment	1971 (21 June-7 Nov.)			1972 (22 May-22 Oct.)			1973 (18 June-4 Nov.)		
		IN	OUT	TOT	IN	OUT	TOT	IN	OUT	TOT
Kwaru	none	359	275	634	95	138	233	386	689	1075
2. Ajura		495	344	839	187	204	391	438	445	883
Subtotal		854	619	1473	282	342	624	824	1134	1958
3. Sugungum	propoxur in 1972 and 1973	2439	2939	5378	61 (0.076)[b]	186 (0.11)	247 (0.11)	289 (0.12)	804 (0.15)	1093 (0.15)
4. Ungwar Bako		335	442	777	4 (0.036)	12 (0.049)	16 (0.049)	15 (0.046)	35 (0.043)	50 (0.048)
6. Matsari		943	522	1465	11 (0.035)	42 (0.15)	53 (0.085)	44 (0.048)	153 (0.16)	197 (0.10)
8. Jaya		670	678	1348	19 (0.086)	37 (0.099)	56 (0.098)	79 (0.12)	239 (0.19)	318 (0.18)
5. Rafin Marke		288	153	441	16 (0.17)	38 (0.45)	54 (0.29)	42 (0.15)	85 (0.30)	127 (0.22)
7. Nasakar		495	933	1428	5 (0.031)	27 (0.052)	32 (0.053)	10 (0.021)	64 (0.037)	74 (0.039)
Subtotal		5170	5667	10 837	116 (0.068)	342 (0.11)	458 (0.10)	479 (0.096)	1380 (0.13)	1859 (0.13)

[a] The actual numbers collected are shown. The number of collections was nearly but not exactly constant for each village and wet season. In 1971, villages 1–3 had 10 NBCs, villages 4–8 had 9; in 1972, village 4 had 9, the others 10; in 1973, village 4 had 8, the others 10.

[b] Ratio of the observed to the expected, i.e. 61/805 = 0.076. The number expected was computed on the assumption that villages 3–8 would, in the absence of propoxur, have undergone the same natural change as villages 1 and 2; e.g., the expected No. IN, in village 3, in 1972 = 2439 × (282/854) = 805.

on average only slightly (and probably not significantly) smaller in 1973; in individual villages this effect appears to be smaller in three cases and greater in the remaining three.

Variables associated with inter-village differences

In looking for variables possibly associated with the significant and relatively stable differences between villages, the following factors were considered: the coverage achieved by the spraying operations; the risk of immigration of vectors; and the baseline entomological observations.

The recorded spray coverage was very high (99%); it varied very little between villages and the variations in 1972 and 1973 were not significantly related. There was also no significant association between spray coverage and the effect of propoxur.

The latitude of sprayed villages and their proximity to unsprayed villages were taken as possible indicators of the risk of immigration of vectors. Neither was regularly associated with the variation of the effect of propoxur, as can be seen from Fig. 1 and Table 1. For instance, the two northernmost villages that are closest to unsprayed villages (Ungwar Bako and Rafin Marke) were at the extremes of the range of response to propoxur.

Among the baseline entomological observations considered, some related to the proportions of species A and B in the different villages but most related to *A. gambiae* s.l. and in particular to possible indicators of its behaviour.

The ratio of NBC to PSC (man-fed component) yields is possibly an index of exophily (see discussion), and its potential association with the variation in response to propoxur was investigated. The results are given in Table 2 and Fig. 2 and 3.

With respect to the pre-spraying NBC/PSC ratio, the observed variation between villages was relatively wide and significantly associated with the variation in the response to propoxur, i.e., the higher

Table 2. The pre-spraying ratio of the man-biting rate of *A. gambiae* s.l. to its man-fed indoor resting density, in the wet season of 1971, and the residual NBC (observed/expected) in the wet seasons of 1972 and 1973

Village	Pre-spraying NBC (IN)/man-night / (PSC/hut) (HBI) [a]	Residual NBC (IN) observed/expected [b]		Pre-spraying NBC (IN + OUT)/man-night / (PSC/hut) (HBI)	Residual NBC (IN + OUT) observed/expected [b]	
	1971	1972	1973	1971	1972	1973
5. Rafin Marke	$\frac{288/36}{(115/39)\ (50/51)} = 2.8$	0.17	0.15	$\frac{441/72}{(115/39)\ (50/51)} = 2.1$	0.29	0.22
8. Jaya	$\frac{670/36}{(810/81)\ (104/117)} = 2.1$	0.086	0.12	$\frac{1348/72}{(810/81)\ (104/117)} = 2.1$	0.098	0.18
3. Sugungum	$\frac{2439/40}{(5066/69)\ (49/80)} = 1.4$	0.076	0.12	$\frac{5378/80}{(5066/69)\ (49/80)} = 1.5$	0.11	0.15
6. Matsari	$\frac{943/36}{(1422/66)\ (123/132)} = 1.3$	0.035	0.048	$\frac{1465/72}{(1422/66)\ (123/132)} = 1.0$	0.085	0.10
4. Ungwar Bako	$\frac{335/36}{(1125/79)\ (119/132)} = 0.72$	0.036	0.046	$\frac{777/72}{(1125/79)\ (119/132)} = 0.84$	0.049	0.048
7. Nasakar	$\frac{495/36}{(2019/47)\ (99/112)} = 0.36$	0.031	0.021	$\frac{1428/72}{(2019/47)\ (99/112)} = 0.52$	0.053	0.039

Residual NBC (IN), 1971 vs 1972: $r = +0.91$ [c], $R = +0.94$ [c]; 1971 vs 1973: $r = +0.90$ [c], $R = +0.94$ [c]

Residual NBC (IN + OUT), 1971 vs 1972: $r = +0.72$, $R = +0.89$ [c]; 1971 vs 1973: $r = +0.97$ [d], $R = +1.00$ [d]

[a] HBI = human blood index (No. positive for man/No. examined in the PSC).
[b] From Table 1.
[c] Significantly different from zero at the 5% level.
[d] significantly different from zero at the 1% level.

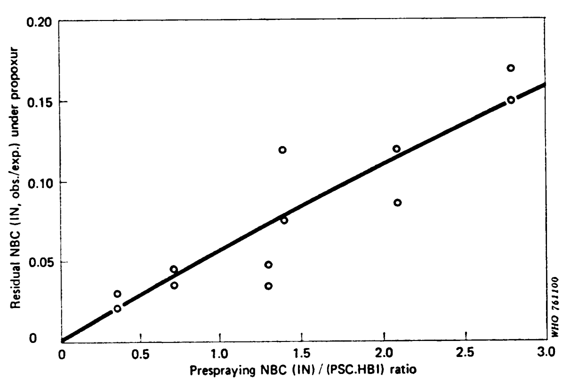

Fig. 2. The prespraying NBC (IN)/(PSC.HBI) ratio of *A. gambiae* s.l. and its residual NBC (IN) under propoxur, by village.

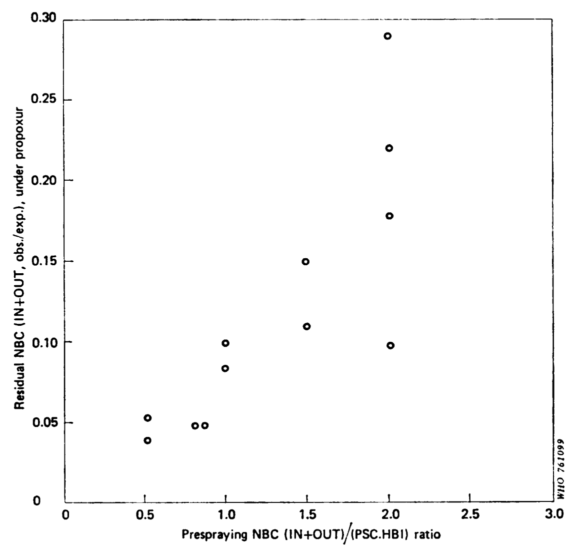

Fig. 3. The prespraying NBC (IN + OUT)/(PSC.HBI) ratio of *A. gambiae* s.l. and its residual NBC (IN + OUT) under propoxur, by village.

Table 3. The pre-spraying median biting hour of *A. gambiae* s.l. and the residual NBC under propoxur

Village	Median biting hour [a] in 1971 [c]	NBC (IN + OUT) observed/expected [b] 1972 [c]	NBC (IN + OUT) observed/expected [b] 1973 [c]
3. Sugungum	01 h 40	0.11	0.15
4. Ungwar Bako	01 h 58	0.049	0.048
6. Matsari	02 h 06	0.085	0.10
8. Jaya	02 h 24	0.098	0.18
5. Rafin Marke	03 h 09	0.29	0.22
7. Nasakar	01 h 36	0.053	0.039

$r = +0.86$ [d]; $R = +0.54$

$r = +0.76$; $R = +0.83$

[a] By interpolation from the distribution of bites by 2-hour periods, NBC (IN + OUT).

[b] From Table 1.

[c] Wet season, see Table 1.

[d] Significantly different from zero at the 5 % level.

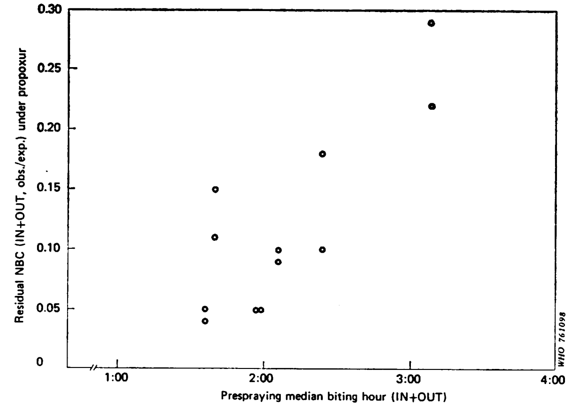

Fig. 4. The prespraying median biting hour (NBC, IN + OUT) of *A. gambiae* s.l. and its residual NBC (IN + OUT) under propoxur, by village.

the pre-spraying ratio, the higher the residual NBC yield (observed/expected) after spraying. The association holds for the indoor NBC or the average NBC but not for the outdoor NBC alone. The PSC was expressed as: (females/hut) × (proportion of fed females positive for human blood in the precipitin test); the ratio of the PSC yield in females/hut to the PSC yield in fed females/sleeper did not in fact vary significantly between times or places.

If the total PSC yield (females/hut) is used instead of its man-fed component only, the correlation between the pre-spraying NBC/PSC ratio and the residual (observed/expected) NBC remains significant but becomes somewhat weaker. The man-fed proportion of the PSC yield showed only moderate variation: 0.61 (49/80) in Sugungum and 0.88–0.98 (in samples of 51 to 132) in the other villages; by itself it showed no correlation with the residual NBC.

Some other pre-spraying variables may reflect vector behaviour, such as biting hours and the ratios between NBC (OUT) and NBC (IN), between ETC and PSC, between fed and gravid in the PSC and in the ETC, and between males and females in the PSC. Variation among villages was compared with the variation in post-spraying NBC (observed/expected). There was a significant association only in the case of the biting hours, as shown in Table 3 and Fig. 4: the later the median biting hour, the greater was the post-spraying NBC (observed/expected).

The absolute post-spraying NBC was significantly associated with the pre-spraying NBC, but the relative (observed/expected) post-spraying NBC was not.

The observed pre-spraying distribution of species A and B of *A. gambiae* s.l. captured by PSC, and its relationship with the residual NBC under propoxur, are shown in Table 4. This information was not available for all the villages, for 1971 it applies only to the month of August, and some of the ratios are based on relatively small numbers. With these restrictions in mind, it appears that the higher the pre-spraying proportion of species B, the higher was the residual NBC (observed/expected). However, the association was usually not statistically significant. The proportion of species B may vary relatively little between villages (in 1970 the variation was not statistically significant by the chi-square test) in comparison with the residual NBC.

The village characteristics that we have tried to relate to the response to propoxur may obviously be interdependent. The pre-spraying NBC (IN + OUT)/PSC ratio, for example, is strongly associated with the pre-spraying median biting hour (r = +0.90; R = +0.90, both significantly different from zero at the 5% level). However, it is not strongly associated with the pre-spraying proportion of species B (r = +0.21 and R = +0.13, using the cytotaxonomic results of 1970; r = +0.13 and R = +0.68, using the cytotaxonomic results of 1971).

Table 4. The pre-spraying proportion of species B among the *A. gambiae* s.l. collected by PSC and the residual NBC (*A. gambiae* s.l., observed/expected) under propoxur

Village	Proportion of species B		NBC (IN + OUT) observed/expected	
	July-October 1970 [a]	August 1971 [b]	1972 [c]	1973 [c]
3. Sugungum	— —	0.87 (558/640)	0.11	0.15
4. Ungwar Bako	0.84 (83/99)	0.66 (19/29)	0.049	0.048
6. Matsari	0.90 (70/78)	0.76 (105/139)	0.085	0.10
8. Jaya	0.94 (116/123)	0.69 (63/91)	0.098	0.18
5. Rafin Marke	0.90 (87/97)	— (4/6)	0.29	0.22
7. Nasakar	0.93 (62/67)	0.63 (99/156)	0.053	0.039

$r = +0.82$;[d] $R = +0.80$

$r = +0.58$; $R = +0.70$

$r = +0.09$; $R = +0.43$

$r = +0.35$; $R = +0.13$

[a] From Shidrawi, unpublished document WHO/MAL/72. 776.
[b] From Coluzzi et al., unpublished document WHO/MPD/TN/75.1.
[c] Wet season, see Table 1.
[d] Significantly different from zero at the 5 % level.

Pre-spraying NBC/PSC ratio, median biting hour, and response to propoxur: comparison between species

As already mentioned, four anopheline species were collected in relatively large numbers: *A. gambiae* s.l., *A. funestus*, *A. pharoensis*, and *A. rufipes*; of these, only the first three were collected in large numbers by NBC. Table 5 shows their pre-spraying NBC/PSC ratios and their residual densities under propoxur. It should be noted that we used, for convenience, the total numbers collected by all sampling methods during the whole of the baseline and intervention periods. This allowed comparisons between species, even though the NBC/PSC ratio tended to be higher owing to the inclusion of the dry season (when PSC is more productive than NBC), and even though the two periods differed in their seasonal composition so that the " expected " was more arbitrary than that in Table 1.

For the three species that were observed to bite man in relatively large numbers, there was a clear positive association between the pre-spraying NBC/PSC ratio (1.17, 0.82, and 43.34 for *A. gambiae*, *A. funestus*, and *A. pharoensis*, respectively) and the residual density under propoxur (0.07, 0.02, and 0.79, respectively).

The median biting hour (IN + OUT) in the wet season of 1971 (baseline), in all villages combined, was: for *A. gambiae* s.l., 01 h 55; for *A. funestus*, 03 h 18; and for *A. pharoensis*, 21 h 55.

Among these three species, therefore, there is a negative association between the pre-spraying median biting hour and the residual NBC under propoxur, whereas between villages the association was positive (see above).

NBC/PSC ratio : variation with time

In the course of the wet season and the early dry season of 1971 (baseline), the NBC/PSC ratio of *A. gambiae* s.l. increased progressively, as shown in Table 6 and Fig. 5. There was no corresponding systematic change in the ETC/PSC ratio, in the fed/gravid ratio in either ETC or PSC, or in the man-fed proportion of the PSC yield. The last was studied only in September, October, and November, i.e., the second half of the period considered.

The NBC/PSC ratios of the two untreated villages, which were studied in detail in 3 successive years, are

Table 5. The pre-spraying NBC/PSC ratios of different anopheline species and their residual densities under propoxur

Period	Villages	Collections	Variable	Species			
				A. gambiae s.l.	*A. funestus*	*A. rufipes*	*A. pharoensis*
Baseline (B) (26 October 1970 to 21 May 1972)	all	NBC (1592 man-nights); PSC (3070 hut collections)	No. collected by NBC	12 948	2648	10	809
			No. collected by PSC	21 363	6190	992	36
			NBC (No./man-night) / PSC (No. females/hut)	1.17	0.82	0.02	43.34
	controls (C)	all	number, N(B,C)	6551	1542	131	94
	to be sprayed (S)	all	number, N(B,S)	30 194	8444	1044	780
Intervention (I) (22 May 1972 to 4 November 1973)	controls (C)	all	number, N(I,C)	8407	532	210	79
			N(I,C)/N(B,C) [a]	1.28	0.35	1.60	0.84
	sprayed (S)	all	number, N(I,S)	2525	48	152	519
			N(I,S)/N(B,S) [b]	0.08	0.01	0.15	0.67
			N(I,S)/N(B,S) [c] / N(I,C)/N(B,C)	0.07	0.02	0.09	0.79

[a] The change expected in the absence of spraying.
[b] The change observed in the presence of spraying.
[c] Observed/expected.

Table 6. The NBC/PSC ratios of *A. gambiae* s.l. per 4-week period during the wet season of 1971 [a]

4-week period	$\frac{\text{NBC(IN)/man-night}}{\text{PSC/hut}}$	$\frac{\text{NBC(IN + OUT)/man-night}}{\text{PSC/hut}}$
21 June–18 July	$\frac{93/24}{227/34} = 0.58$	$\frac{171/48}{227/34} = 0.53$
19 July–15 Aug.	$\frac{953/24}{2552/36} = 0.56$	$\frac{1765/48}{2552/36} = 0.52$
16 Aug.–12 Sept.	$\frac{1357/24}{2602/33} = 0.72$	$\frac{2822/48}{2602/33} = 0.75$
13 Sept.–10 Oct.	$\frac{798/24}{1342/35} = 0.87$	$\frac{1821/48}{1342/35} = 0.99$
11 Oct.–7 Nov.	$\frac{92/24}{121/34} = 1.08$	$\frac{272/48}{121/34} = 1.59$

[a] In villages 1–3, i.e. those examined every fortnight by NBC and PSC, throughout the 20–week period; see Table 1, footnote [a].

shown in Table 7. The ratios varied from year to year, and in any one year were higher in village 2 than in village 1. The NBC (IN)/PSC ratio was less variable than the NBC (IN + OUT)/PSC ratio; replacing the total PSC by its man-fed component reduced this variation somewhat.

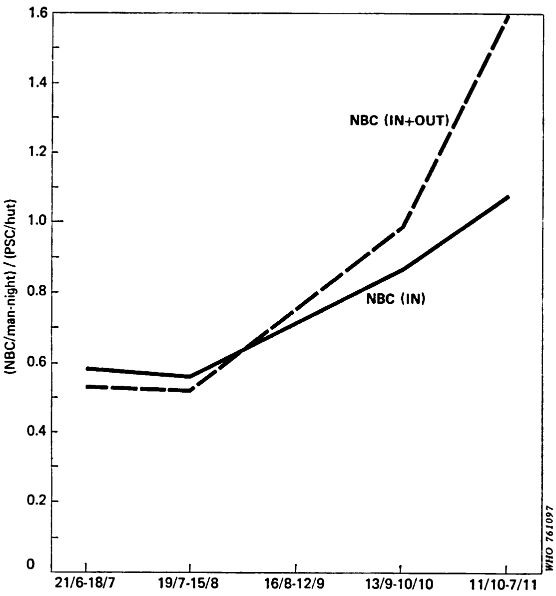

Fig. 5. The NBC/PSC ratios of *A. gambiae* s.l., per 4-week period, during the wet season of 1971.

DISCUSSION

Sampling error

Sampling error in the pre-spraying NBC and/or PSC would, by itself, produce variation in the NBC/PSC ratio. However, if the variation in the ratio were due to error in the PSC, no association would be expected with the residual NBC (observed/expected). If the variation in the ratio were due to error in the NBC, a negative association would be expected with the residual NBC (observed/expected), since overestimation of the pre-spraying NBC (and of the NBC/PSC ratio) would result in an overestimate of the effect of the insecticide, and vice-versa.

Resting behaviour

The NBC/PSC ratio must reflect both the resting behaviour of an anopheline mosquito and the relative bias of the two sampling methods. In the case of *A. gambiae* s.l. in the West African savannah, a relatively high ratio (i.e., higher than expected under the hypothesis of complete endophily) has often been noted and attributed either to the relative bias of the sampling methods (*2*) or to relative exophily (*4*). The observation that the pre-spraying NBC/PSC ratio of *A. gambiae* s.l. in a given village is positively associated with its residual (observed/expected) NBC under propoxur would be explained, at least in part, by exophily. The positive association of the pre-spraying NBC/PSC ratio of a given man-biting species with its residual (observed/expected) NBC during propoxur treatment again suggests that the

Table 7. The NBC/PSC ratio of *A. gambiae* s.l. in two untreated villages, in the wet season, in 3 successive years

Village	Year	$\frac{\text{NBC(IN)/man-nights}}{\text{PSC/huts}}$	$\frac{\text{NBC(IN)/man-nights}}{\text{(PSC/huts)(HBI)}^{a}}$	$\frac{\text{NBC(IN+OUT)/man-nights}}{\text{PSC/huts}}$	$\frac{\text{NBC(IN)/man-nights}}{\text{(PSC/huts)(HBI)}^{a}}$
1 Kwaru	1971	$\frac{359/40}{636/40} = 0.56$	$\frac{359/40}{(636/40)(97/103)} = 0.60$	$\frac{634/80}{636/40} = 0.50$	$\frac{634/80}{(636/40)(97/103)} = 0.53$
	1972	$\frac{95/40}{151/52} = 0.82$	—	$\frac{233/80}{151/52} = 1.0$	—
	1973	$\frac{386/40}{605/45} = 0.72$	$\frac{386/40}{(605/45)(202/210)} = 0.75$	$\frac{1075/80}{605/45} = 1.0$	$\frac{1075/80}{(605/45)(202/210)} = 1.0$
2 Ajura	1971	$\frac{495/40}{1142/63} = 0.68$	$\frac{495/40}{(1142/63)(81/91)} = 0.77$	$\frac{839/80}{1142/63} = 0.58$	$\frac{839/80}{(1142/63)(81/91)} = 0.65$
	1972	$\frac{187/40}{323/74} = 1.1$	—	$\frac{391/80}{323/74} = 1.1$	—
	1973	$\frac{438/40}{548/69} = 1.4$	$\frac{438/40}{(548/69)(121/125)} = 1.4$	$\frac{883/80}{548/69} = 1.4$	$\frac{883/80}{(548/69)(121/125)} = 1.4$

[a] HBI = human blood index (No. positive for man/No. examined in the PSC).

ratio is an indicator of exophily; this seems obvious in the extreme case of *A. pharoensis*, but it may also be true for the species of which large numbers are collected indoors (*A. gambiae* s.l. and *A. funestus*). It also seems more plausible to explain differences in the NBC/PSC ratios between species by differences in the degree of exophily rather than by species-specificity in the relative bias of the two sampling methods.

The NBC/PSC ratio of *A. gambiae* s.l. increases progressively in the course of the wet season. In theory, this could be due to a progressive increase in anthropophily (i.e., a decreasing contribution of animal-fed mosquitos to the PSC), to a decrease in the time spent indoors, to a decrease in the proportion resting indoors after feeding, or to a change in the relative bias of the two sampling methods. There was no systematic change in the results of the precipitin test, which rules out the first possibility; there were also no systematic changes in the ETC/PSC ratio, or in the fed/gravid ratio in either ETC or PSC, which rules out the second possibility. Of the last two possible explanations, a progressive increase in the proportion resting outdoors, possibly related to the simultaneous marked increase in vegetation (*1*, *3*) is more plausible than a systematic change in the relative bias of the two sampling methods.

Predictive value of the NBC/PSC ratio

If the NBC/PSC ratio is indeed an indicator of exophily and a predictor of the effect of a residual insecticide, it would be interesting to know whether it applies to other anopheline species, environments, and insecticides. So far, a comparison has been made with observations in Kisumu, Kenya, where fenitrothion produced a much greater reduction in *A. gambiae* s.l. than the one produced by propoxur in Garki. The recorded spray coverage was equally high in both places and the entomological sampling methods were essentially the same. In Kisumu, the pre-spraying NBC (IN)/PSC ratio was available from 6 locations for 5 months; it averaged 0.37 with a range of 0.24–0.47, compared with 0.32–2.7 in Garki (see Table 2). Total PSC was used in both studies. The ratio of persons to huts was 2 in both places, so that the lower NBC/PSC ratio suggests that *A. gambiae* s.l. in Kisumu is more endophilic; this could explain, at least in part, the differences between the results. In Kisumu, with fenitrothion, there was no significant correlation between the residual NBC of a given village and its pre-spraying NBC/PSC ratio, but the numbers of mosquitos collected after spraying were very small and the variation in the pre-spraying NBC/PSC ratio was also very small.

If the NBC/PSC ratio is a qualitative predictor of the effect of a residual insecticide, it would be interesting to know its value for a quantitative prediction also. This obviously depends on the importance of the other factors involved, such as the model applied for the factor, the quality of the spraying operations, and the measurement error. If an exponential model is fitted to the data included in Fig. 2, the following relationship is obtained:

$$y = 1-\exp(-0.058\ x)$$

where y is the residual NBC and x the pre-spraying NBC(IN)/PSC (man-fed) ratio. The fitting was made by weighting the points by their pre-spraying NBC(IN)/PSC ratio. The corresponding curve has been included in Fig. 2. According to the model, the residual density (observed/expected) increases with the pre-spraying NBC/PSC ratio, from zero for a ratio of zero (i.e., when the pre-spraying NBC is zero, the residual NBC is also zero) to a maximum of 1, when the ratio tends to infinity (i.e., when the pre-spraying PSC is negligible in relation to the NBC, the latter is not affected by spraying). In Kisumu, the average pre-spraying man-fed proportion in the PSC was 0.95, the average pre-spraying NBC(IN)/PSC (man-fed) ratio was 0.37/0.95 = 0.39, and the residual NBC (IN, observed/expected), as expected from the above model, was 1–exp (0.058) (0.39) = 0.022. The residual NBC (IN, observed/expected), estimated from the actual observations of the last baseline year and the first intervention year by the same method as that used in Garki, was 0.007.

Degree of error (prediction)

In practice, the spontaneous changes estimated in the comparison villages and used in the above " predictions " are only known *a posteriori* and are therefore not available for a prediction in the true sense. If they are ignored, the best estimate of the expected NBC, in the absence of insecticide, is the pre-spraying NBC in the same village. The correlation between pre-spraying characteristics (NBC/PSC ratio or median biting hour) and residual NBC (observed/expected, where " expected " is redefined as the uncorrected pre-spraying value) in a particular year (1972 or 1973) is not affected because it involves only the multiplication of the residual NBC by a constant, which does not affect its correlation coefficient or rank correlation coefficient with any other variable. However, a large error is introduced in the prediction of the actual NBC from the NBC/PSC ratio or, for that matter, from any other predictor: the prediction would, by definition, be the same for 1972 and 1973, while the outcome is very different (by a factor of 1859/458 = 4.1 on average, see Table 1). This is a simple illustration of the relatively large error that may be involved in making absolute predictions.

Median biting hour

The median biting hour may be determined in part by the distance from the breeding sites if the mosquito re-feeds on the night of oviposition, which is probably usual for *A. gambiae* s.l. in Garki. (Thirty-five specimens collected by NBC at different times were classified by the condition of their ovariolar sacs; all had uncontracted sacs.) Based on the comparison between villages with respect to *A. gambiae* s.l. it could be suggested that the median biting hour be used to predict the effect of a residual insecticide since it determines the potential exposure time. However, this hypothesis is probably too simple in view of the fast action of propoxur and the comparison between species. The median biting hour of *A. gambiae* s.l. is strongly related to the NBC/PSC ratio; it may be determined by the same genetic and environmental factors (see below) and may be only indirectly related to the response to a residual insecticide. This response probably varies, not with the exposure time for exposed mosquitos but with the proportion of mosquitos not exposed at all.

Species composition of A. gambiae

In this study, the pre-spraying proportions of species A and B were less reliable predictors of the effect of propoxur than the pre-spraying NBC/PSC ratio. Also, the variation in A/B ratio between villages does not appear important enough to explain their variation in the residual NBC under propoxur: the two pre-spraying years gave inconsistent results.

The relationship between inversion polymorphism and response to the residual insecticide has not yet been analysed.

Genetic and environmental factors

The resting behaviour of a mosquito is the result of genetic factors, environmental factors, and chance. Detailed genetic and environmental studies would be required to understand and predict behaviour. However, to predict response to a residual insecticide it may be preferable and, when only limited resources are available, more reliable to measure behaviour itself. This approach integrates the effects of genetic and environmental factors. A ratio between numbers collected by different standard sampling methods, e.g., the NBC/PSC ratio, may be a useful indicator of behaviour; even though it is an indirect and imperfectly understood indicator, it may have some predictive value.

Test of hypothesis

There are obvious limitations to the interpretation of associations demonstrated retrospectively. It may be argued that the hypothesis of the predictive value of the NBC/PSC ratio has, in fact, been tested prospectively. On the one hand, the study was explicitly designed to allow the detection of differences between villages, including differences in their response to the insecticide. On the other hand, the NBC/PSC ratio has been used previously as an indicator of exophily (*4*). Admittedly, this is not a prospective approach, but a true experiment ls not feasible since the pre-spraying degrees of exophiiy and/or NBC/PSC ratio do not lend themselves to manipulation. It may, however, be desirable to include the testing of the hypothesis in projects designed for the evaluation of insecticides.

ACKNOWLEDGEMENTS

The authors thank Dr A. R. Zahar, Dr C. Pant, and Mr C. Garrett-Jones for their comments on the manuscript.

RÉSUMÉ

EFFET DU PROPOXUR SUR *ANOPHELES GAMBIAE* S.L. AINSI QUE SUR D'AUTRES POPULATIONS ANOPHÉLIENNES, ET SA RELATION AVEC CERTAINES VARIABLES ANTÉRIEURES AU TRAITEMENT

Une différence significative et stable dans la collecte de spécimens piquant la nuit (CSPN, observée/prévue) d'*A. gambiae* s.1. a été observée entre divers villages de la savane d'Afrique occidentale traités pendant deux ans par pulvérisations de propoxur. On a mis en évidence une association positive entre, d'une part, la densité anophélienne résiduelle d'un village donné et, d'autre part, certaines de ses caractéristiques antérieures au traitement: rapport CSPN/CPP,[a] heure médiane de piqûre et, peut-être, proportion de l'espèce B; par contre, on n'a pas découvert d'association significative avec plusieurs autres caractéristiques antérieures au traitement (densité absolue, rapports entre CSPN (air libre) et CSPN (habitations), entre CPS[b] et CPP, entre spécimens gorgés et gravides dans la CPP ou la CPS, entre mâles et femelles dans la CPP) ni avec des variations dans la couverture insecticide, avec la latitude ou avec la distance par rapport aux villages non traités.

Dans la comparaison entre les espèces *A. gambiae* s.1., *A. funestus* et *A. pharoensis* la CSPN résiduelle (observée/prévue) était associée positivement avec le rapport CSPN/CPP antérieur au traitement, et négativement avec l'heure médiane de piqûre.

Le rapport CSPN/CPP antérieur au traitement paraît ainsi permettre de prédire la variation, entre villages ou espèces, de la réduction de la CSPN du fait de pulvérisations d'insecticides à effet rémanent. Ainsi, il aurait assez bien permis de prédire la différence entre la réponse d'*A. gambiae* s.1. au propoxur à Garki et au fénitrothion à Kisumu. Il peut donc aider à prévoir l'action d'un insecticide à effet rémanent, mais il ne faut pas perdre de vue les limitations inhérentes à toute prédiction absolue, en particulier eu égard aux modifications spontanées imprévisibles de la situation initiale.

[a] CPP: collecte après pulvérisation de pyrèthre.

[b] CPS: collecte sans piège de sortie.

REFERENCES

1. Foll, C. V. et al. *Bulletin of the World Health Organization*, **32**: 531-550 (1965).
2. Garrett-Jones, C. & Shidrawi, G. R. *Bulletin of the World Health Organization*, **40**: 531-545 (1969).
3. Hamon, J. *Bulletin of the World Health Organization*, **28**: 83-109 (1963).
4. Hamon, J. et al. *Cahiers de l'ORSTOM*, No. 1, pp. 37-61 (1959).