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INSECTICIDE RESISTANCE IN POPULATIONS OF *ANOPHELES GAMBIAE* IN WEST AFRICA

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SYNOPSIS

A method published by Elliott in 1958 for investigation of insecticide resistance in *Anopheles* larvae has been applied to strains of *A. gambiae* from various parts of West Africa, and the results analysed to provide information as to the proportions of the three possible genotypes in the different populations.

At Kano, the gene conferring resistance to dieldrin and gamma-BHC appears to be increasing in the wild population, being present in about 50% and 42% of the population in the homozygous (RR) and heterozygous (RS) states respectively. Theoretical considerations suggest that in a situation where the male heterozygote is sterile, the gene in the minority will tend to become extinct in the absence of any specific selection. In a colony maintained in captivity for six generations without intentional selection the reverse was seen to happen, the S gene increasing its representation at the expense of the R gene.

In Sokoto Province, some figures of the same type were obtained; these are analysed and found to produce results compatible with those from Kano, especially as regards the LC_{50} level derived for the effect of gamma-BHC on the RS fraction of the population.

At Freetown, a similar analysis suggests that the factor conferring resistance to gamma-BHC has also appeared. About 66% of the sample examined appear to carry the R gene.

At Ibadan, samples of the population showed some tolerance to dieldrin, but the presence of the specific type of resistance seen elsewhere was not established. The tolerance appears to be non-specific, as some relative tolerance to DDT appeared to be present also.

The method devised by Elliott (1958) for the measurement of insecticide resistance in *Anopheles* larvae was mainly intended to allow the small tropical species to be tested under conditions minimizing control mortality and avoiding the effects of the prolonged starvation associated with the 24-hour exposures used in the more usual methods developed primarily for culicine and aëdine larvae and for those of the large temperate zone anophelines. The method consists of a one-hour exposure period to the insecticidal dilutions, followed by a five-hour recovery period in clean

water with added food.[1] Besides allowing testing of the less robust species, the method is comparable to the established techniques for adult mosquitos (Busvine & Nash, 1953, and later modifications), which also specify a short (one-hour) exposure period followed by a recovery period. An additional sphere of usefulness is the possibility of analysing the composition of populations of mixed genetic composition, such as are met with in the field in areas where insecticide resistance has appeared. This is rendered possible by the fact that closely spaced intervals over a wide range of insecticide concentrations can be employed, so that in suitable cases the whole range of resistance from the most to the least susceptible members of a species can be examined. The cases below refer to the dieldrin-resistant *A. gambiae* strain which has at several places in West Africa become an important part of the local *A. gambiae* population as a result of the use of dieldrin or BHC for public health or other purposes. Analysis of this strain, using dieldrin, is not possible in either the adult or the larval state, since the homozygous resistant adult cannot be killed by any concentration of dieldrin obtainable by the conventional method (Davidson, 1958), and in the same way the corresponding larvae are resistant to any practicable concentration of dieldrin in water. In both cases, however, homozygous susceptible and heterozygous material can be separated by the use of dieldrin. But the most useful toxicant for separating the three genotypes is gamma-BHC, and this has been used by Davidson for adult material and by Ramakrishna & Elliott (1959) for larvae. The studies to be described consist in the application of the method to some mixed laboratory and field populations.

Experimental Method

The larvae used as samples of the populations were exposed to aqueous concentrations of insecticide in the early part of the fourth instar, for an exposure period of one hour, after which a five-hour period was allowed for recovery and the development of mortality; during this period they were fed.

Interpretation of Results

In the analysis of the populations certain assumptions have been made which are probably only approximations to the truth. One is that the Ambursa and Lagos strains of *A. gambiae* are typical of homozygous resistant and susceptible members of the species, respectively, everywhere. The other is that the heterozygous resistant strain, which has not so far been directly examined in the larval state, has approximately the same

[1] The standard WHO test method utilizing a 24-hour exposure period before the mortality is assessed has in fact encountered negligible control mortality even in *A. gambiae* larvae elsewhere. — ED.

degree of resistance wherever it is found. Davidson (1958), working with adults, found that the resistance of this cross varied quite widely, according to the different origin of the parental strains. However, the use of these assumptions appears to lead to useful and reasonably consistent results, and the practical importance of the resistance phenomenon for the future of malaria eradication in West Africa seems to justify the use of even imperfect tools for its study. It may be noted that these same assumptions have been made without special comment in respect of the adults of the species; the method of distinguishing the three genotypes by the application of discriminating dosages of BHC, first used by Davidson (1958) for the Lagos-Ambursa crosses, has since been applied to field strains in several other areas in West Africa.

When the relationship between the logarithm of dosage and the probit mortality is expressed graphically, a homogeneous population of insects produces the familiar straight line. But a population containing two or more groups widely different in their degree of susceptibility will produce a curve in which the increments of mortality obtained at each increase in concentration of toxicant will not be regular. If there is no overlap between the groups there will be a section of the curve proceeding parallel with the dosage axis, caused by the mortality in the most sensitive group being complete while the more sensitive members of the next group are not yet affected. It is at these points in the curve that the discriminating dosages used by Davidson (1958) are applied. In the case of *A. gambiae* larvae exposed to BHC the three genetic types appear, if they do not actually overlap, to be separated by only a narrow gap, so that the sections of the curve parallel to the dosage axis are short or absent. The method of analysis used therefore consists of comparing the observed curves with the expected curves for susceptible (SS) and resistant (RR) material, and thus estimating the proportion of each present. When each of the pure components is estimated, the observed curve can then be corrected for them in the same way that control mortality is compensated for (Abbott's formula), and a line derived for mortality in the remainder of the population, which will be of hybrid (RS) genetic constitution. In view of the regrettable failure to produce this cross in Nigeria, although it has been made many times in London (Davidson, 1958), it is possible at present to compare only these derived lines and LC_{50} values *inter se* and not with one from an RS population of known origin.

Analysis of a Mixed Population from Kano, Northern Nigeria

Soon after the discovery of the resistant strain of *A. gambiae* in Western Sokoto Province (Elliott & Ramakrishna, 1956), a similar strain resistant to dieldrin and BHC was reported from Kano, some 300 miles eastward

(Davidson, 1958). At Kano the use of insecticides for public health purposes had taken place on an extremely limited scale only, being confined to protection of the international airport with DDT and BHC, but in the immediate post-war years quantities of BHC amounting to several tons were used to protect the " pyramids " of ground-nuts awaiting transport.

The first report of the resistant strain at Kano was based on the evidence provided by the offspring of a few females which survived air transport to London; the identical single dominant gene previously isolated from Ambursa in Sokoto Province was found to be responsible. A second investigation by Armstrong (Elliott & Armstrong, 1956) was carried out using wild-caught female *A. gambiae* collected in the dormitories of a school several miles from the airport and from the railway sidings where the ground-nuts were stored. These two investigations produced the following estimates of the various proportions of homozygous resistant, heterozygous, and homozygous susceptible material in the population:

	RR	*RS*	*SS*
Davidson (1958)	39%	45%	16%
Elliott & Armstrong (1956) . .	30%	20%	50%

Davidson's result quoted above comes reasonably close to a distribution according to the Hardy-Weinberg law, the expression $A^2+2AB+B^2$ being satisfied by the distribution 36 %: 48 % : 16 %. As will be seen later, however, there is reason to suppose that in mixed populations of resistant and susceptible *A. gambiae* the distribution of the three genotypes will not be according to the Hardy-Weinberg law.

The results now to be described refer to a colony of *A. gambiae* derived from about two hundred females collected in the same place as Armstrong's material, and transported by air to Lagos in the cabin of the aircraft. On the journey they suffered less than 10% mortality, in contrast with Davidson's material, which had experienced the long journey to London in the freight compartment and had suffered a much higher casualty rate. In the present colony, then, the natural proportions of the genotypes in the Kano wild population may have been relatively unaffected by selection for vigour by exposure to adverse travelling conditions.

The results of tests with dieldrin and BHC against the larvae of this colony are given in Table 1. The results for dieldrin against the first generation show that below a toxicant concentration of 4 parts per million (p.p.m.), probit mortality increased regularly with the logarithm of the concentration. Above this concentration, however, further increments of concentration produced no increase in mortality. The break in the line occurs at the 50% point, and it seems clear that the rising part of the curve represents the RS and SS fractions of the population, while the remaining 50% represent RR material which is tolerant of even higher concentrations of dieldrin at a one-hour exposure period.

The influence of the RR fraction may be removed by recalculating the mortality figures to a base of 50, thus leaving a regression line referring to RS and SS material only. If these were present in equal amounts, another break in the line might be expected, caused by the absence of further mortality between the upper limit of SS mortality and the lower limit of RS mortality. Such a break does not appear, and if the line is compared with the expected mortality in SS larvae, for which the results for the Lagos colony (Elliott, 1958) may be used, the reason seems to be the presence

TABLE 1. MORTALITY IN *A. GAMBIAE* LARVAE FROM KANO : GENERATION 1

Concentration of toxicant (p.p.m.)	Dieldrin		Gamma-BHC	
	percentage mortality	number exposed	percentage mortality	number exposed
20	48	37	100	20
10	52	85	93	73
6	47	72	86	78
4	45	80	79	82
2	36	94	50	104
1	28	74	45	111
0.6	14	92	29	80
0.4	7	55	11	72
0.2	7	100	6	69
0.1	2	38	0	55

of SS material in very small amount in the sample. For example, at a concentration of 0.2 p.p.m. of dieldrin, where 100% of SS material is expected to die, only 14% of the sample died; and at 0.1 p.p.m., where 97% SS mortality is expected, only 4% died. This suggests that an average of 9% of the 50%, or about 5% of the total population, is SS genetically. Correcting the figures for RS and SS together for the SS fraction, treating it as control mortality in Abbott's formula, a set of values for the RS fraction alone may be deduced. From this set of values the LC_{50} of the RS material may be estimated as 1.2 p.p.m. It is not at present possible to check this estimate against observations on RS material, as the RS cross has not yet produced viable eggs at Yaba.

The calculations above are set out in Table 2 and in Fig. 1. The same processes can be applied to the BHC results, with the difference that all three genetic types are susceptible to the toxicant (Table 3 and Fig. 2). In

this case it is most convenient to correct first for the proportion of SS material estimated to be present by comparing the observed mortalities with those expected for SS. Once again the fraction of SS present seems to be very low; 0%, 6%, and 11% being the apparent proportions at 0.1, 0.2 and 0.4 p.p.m. The figure of 6% may be accepted as an average of the three estimates, and the observed figures corrected as if for 6% of control mortality. The set of figures thus derived may now be transformed to

TABLE 2. ANALYSIS OF KANO *A. GAMBIAE* DIELDRIN DATA : GENERATION 1

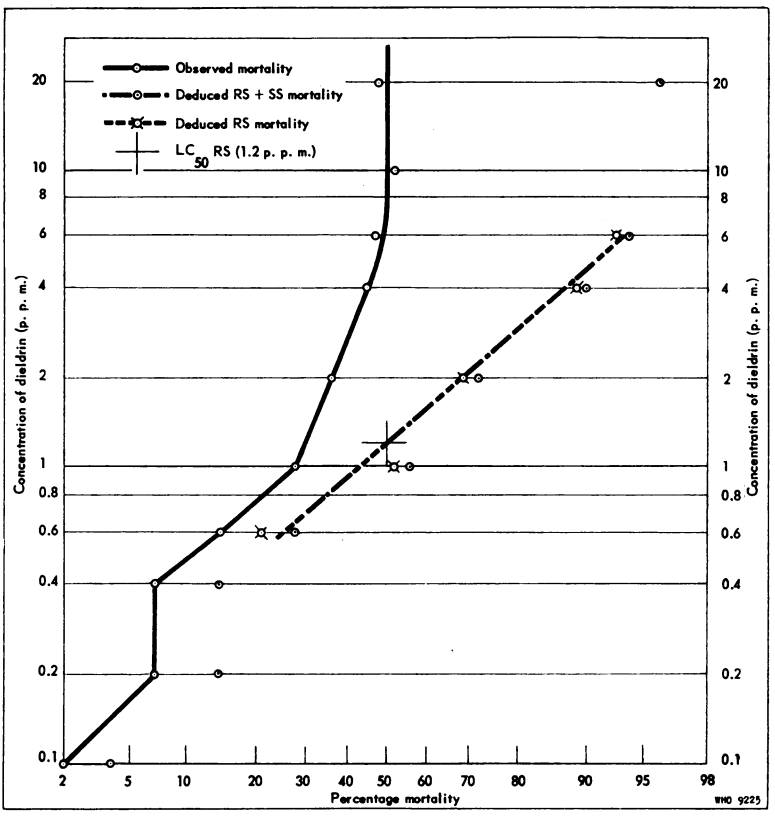
Concentration of dieldrin (p.p.m.)	Observed mortality (%)	RS and SS mortality (base 50) (%)	Expected SS mortality (%)	Percentage SS (base 50)	Corrected RS mortality (%)
20	48	96			
10	52	100			100
6	47	94			93
4	45	90			89
2	36	72			69
1	28	56			52
0.6	14	28			21
0.4	7	14			5
0.2	7	14	100	14	5
0.1	2	4	97	4	0
				Average = 9%	

percentages of survival and compared with the expected survival of RR material. The three lower figures so obtained—52%, 55%, and 59%—agree among themselves and with the estimate of RR obtained by the use of dieldrin, so their average may be accepted as the percentage of RR material present. This is a percentage of the 94% remaining after removal of the SS fraction, giving 52% in the total population. Finally, a corrected set of figures may be extracted from the combined RR and RS mortality figures, giving the estimated mortalities in RS material alone. This gives an LC_{50} value of 0.62 p.p.m. of BHC for the RS fraction.

Considering the dieldrin and BHC figures together, the proportions of the three genetic types in the population may be estimated as follows:

	Dieldrin	*BHC*
RR	50%	52%
RS	45%	42%
SS	5%	6%
LC_{50} for RS	1.2 p.p.m.	0.62 p.p.m.

FIG. 1. ANALYSIS OF KANO *A. GAMBIAE* DIELDRIN DATA : GENERATION 1



Distributions according to the Hardy-Weinberg law which fall fairly close to these are:

A^2	$2AB$	B^2
56	38	6
49	42	9

But in view of the fact that most of the males of RS genetic type have been observed by Davidson (1956) to be sterile, the application of this rule to this type of population appears to be inappropriate. This is brought out by consideration of the progeny of a population constituted as above, in conditions of no selection, under three sets of conditions, namely:

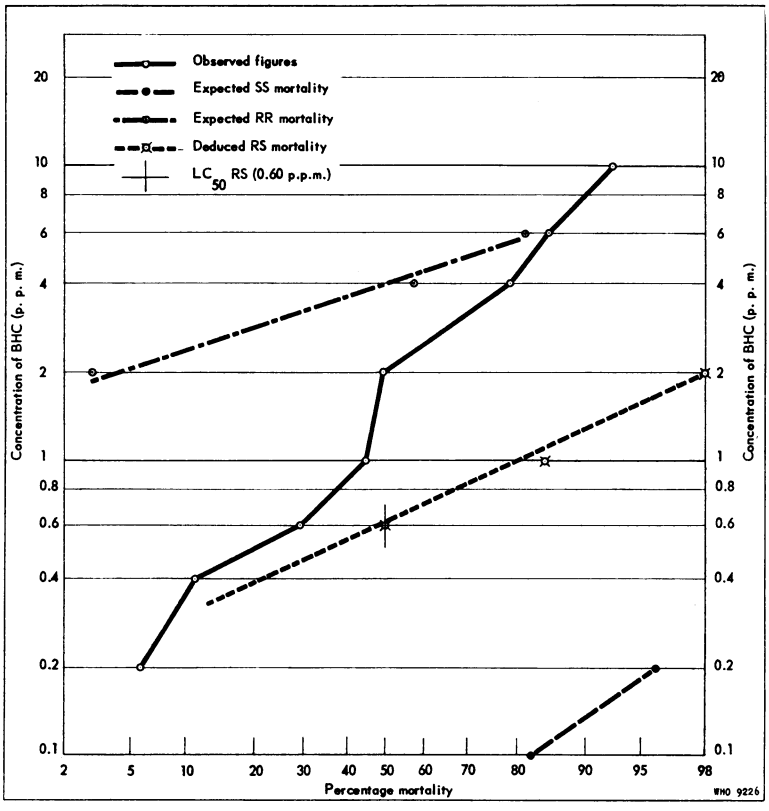
1. *No male sterility*

The original proportions are repeated indefinitely in each generation (Hardy-Weinberg law).

2. *Complete male sterility in hybrids*

In the generation following, the percentages of R and S gametes produced will be different in males and females, and the composition of the second generation will be different, as shown in Fig. 3.

FIG. 2. ANALYSIS OF KANO *A. GAMBIAE* GAMMA-BHC DATA : GENERATION 1



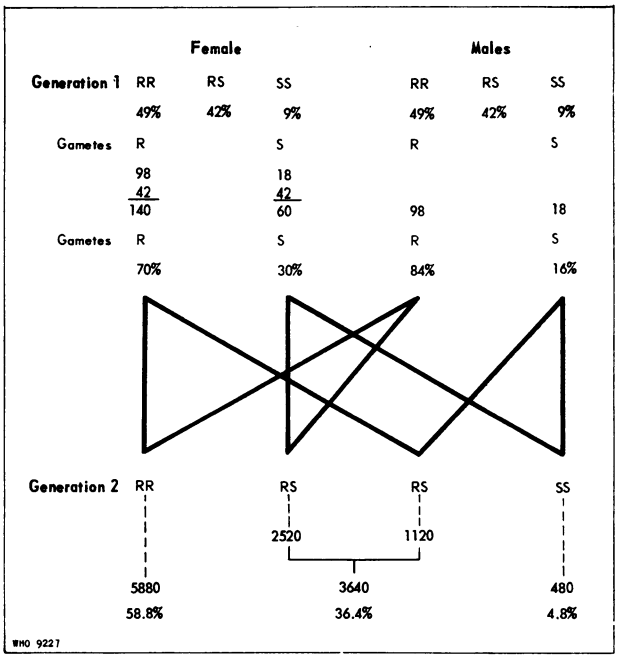
The gene which is in the minority therefore gradually disappears, without the intervention of any selection, the process for the first five generations taking place as follows:

RR	*RS*	*SS*		
49	42	9	Generation	1
59	36	5	»	2
71	27	2	»	3
82	17	0.4	»	4
93.3	6.7	0.001	»	5

TABLE 3. ANALYSIS OF KANO *A. GAMBIAE* GAMMA-BHC DATA : GENERATION 1

Concentration of BHC (p.p.m.)	Observed mortalities (%)	Expected SS mortality (%)	Percentage SS present	Corrected RR and RS mortality (%)	Survival RR and RS (%)	Expected survival RR (%)	Percentage of RR present (base 94)	Deduced mortalities RS (%)
20	100			100	0	0		
10	93			92	8	0.2	(100)	
6	86			85	15	18	(83)	
4	79			78	22	42	52	100
2	50			47	53	97	55	98
1	45			41	59	100	59	85
0.6	29			24	76			50
0.4	11	100	11	5	85			10
0.2	6	96	6	0	100			
0.1	0	83	0					
			Average = 6 %				Average = 55 %	LC_{50} = 0.6 p.p.m.

FIG. 3. COMPOSITION OF SECOND GENERATION OF *A. GAMBIAE* FOLLOWING COMPLETE MALE STERILITY IN HYBRIDS



3. *50% male sterility in hybrids*

The minority gene disappears more slowly, as follows:

RR	*RS*	*SS*		
49	42	9	Generation	1
53	40	7	»	2
57	37	6	»	3
61	34	5	»	4
65	31	4	»	5
69	28	3	»	6

Various other cases are possible, such as a situation where the male sterility is mainly due to the failure only of the gametes carrying one of the two genes. If the male heterozygote produces no R genes, but half the expected number of S genes, the decline in the S element in the population is much slower, though it still takes place.

It must be accepted, then, that any resemblance to a distribution according to the Hardy-Weinberg law is likely to be purely coincidental if observed in a population of *A. gambiae* containing members bearing the resistance gene, and subsequent generations are likely to proceed, in the absence of selection, to a point where the gene which is in a minority is extremely rare. This seems an adequate explanation of the virtual absence of the R gene in untreated populations of *A. gambiae* in spite of its observed effects on hardiness, longevity and fecundity. Although these effects might be thought to favour its spread, the associated hybrid infertility would be sufficient to keep the gene in a permanently insignificant minority, presumably maintained by mutation, until the change in the environment caused by the use of insecticides occurred. Once it has gained a majority position as a result of selection by insecticides, the gene might be expected to increase its representation to the point where the S gene in turn becomes virtually extinct, even without continued insecticide pressure. The further history of the colony derived from the first generation examined above is interesting in this connexion.

The second to fifth generations were not tested against insecticides, but were used as a source of material for malaria transmission experiments and bred on without any intentional selection. The sixth generation was tested, however, with the results summarized in Table 4. The proportions of the three genetic types which are estimated to have been present in the sixth generation in captivity are as follows:

	Dieldrin	*BHC*
RR	6%	3.5%
RS	77%	84.5%
SS	17%	12%

Tables 5 and 6 summarize the calculations on which these estimates are based. Apparently during the period of colony life some influence had been

TABLE 4. MORTALITY IN *A. GAMBIAE* LARVAE FROM KANO : GENERATION 6

Concentration of toxicant (p.p.m.)	Dieldrin		Gamma-BHC	
	percentage mortality	number exposed	percentage mortality	number exposed
10	98	43	100	34
6	—	—	100	33
4	92	134	100	37
3.5	—	—	100	71
2	—	—	100	40
1	43	46	93	46
0.6	—	—	43	56
0.4	30	46	45	144
0.2	—	—	21	42
0.1	—	—	15	52
0.06	16	123	5	37
0.04	—	—	0	47

at work which slightly increased the amount of SS material present and materially increased the RS fractions, so that the S gene, instead of dying out, had gone far towards replacing the R gene. It seems certain that some form of fortuitous selection must have occurred, either due to bias in favour of the resistant individuals in the selection of material for the transmission work, or simply due to the conditions of life in small colonies. It should be added that a seventh generation was not obtained, although plenty of

TABLE 5. ANALYSIS OF KANO *A. GAMBIAE* DIELDRIN DATA : GENERATION 6

Concentration of dieldrin (p.p.m.)	Observed mortality	Expected SS mortality	Percentage SS	Corrected RR and RS mortality	RR and RS survival	Expected RR survival	Percentage RR (of 83)
10	98			97	3	100	3
4	92			90	10	100	10
1	43			36	64		
0.4	30	100		16	84		
0.06	16	92	17 %	0	100		
							Average = 7 % (of 83) (6 % of total)

sixth generation adults were put aside to produce another generation; they fed freely but laid few eggs, which all failed to hatch. Since it would appear that four out of every five males present were sterile, this result is not entirely unexpected. What is unexpected is that the failure to reproduce should have come suddenly in a colony which had for five generations been exceedingly vigorous and prolific. It is possible that in small colonies large fluctuations in the proportions of the different elements present may occur from one generation to the next, since it seems probable that only a few of the potential parents present actually contribute to the next generation.

TABLE 6. ANALYSIS OF KANO *A. GAMBIAE* GAMMA-BHC DATA : GENERATION 6

Concentration of BHC (p.p.m.)	Observed mortality	Expected SS mortality	Percentage SS	Corrected RR and RS mortality	RR and RS survival	Expected RR survival	Percentage RR (of 88)
2	100			100	0	96	0
1	93			92	8	100	8
0.6	43			36	64		
0.4	45			38	62		
0.2	21	96	22	11	89		
0.1	15	84	17	6	94		
0.06	5	64	8	0	100		
0.04	0	44	0	0			
			Average = 12%				Average = 4% (of 88) (3.5% of total)

However this may be, it is interesting to see that the same kind of decline in resistance in captivity has shown itself in this colony as has often been observed in field strains of resistant flies and other insects brought into captivity. In the present case the mechanism at work can be seen, if not fully understood.

In the field, however, the gradual increase of the R genes and the extinction of the S gene seem to be proceeding, as the observations quoted above indicate that between 1956 and 1957 the S-bearing fraction had declined from 70% to 42% of the total population. The situation seems to be that around Kano there is an area where the R gene is in a majority and tending to increase, while outside there is the unaffected area where the gene is in an extreme minority, and tending to decrease. In between the two there must be a transition zone with a population in a highly unstable condition in respect of these two alleles.

Analysis of Mixed Populations in Sokoto Province, Northern Nigeria

The populations here studied were more fully dealt with by Ramakrishna & Elliott (1959) and are now used for comparison of the results of the method of analysis and for an independent estimate of the LC_{50} of the RS fraction of the population. The figures in Table 7 represent the mortalities obtained by exposure to BHC of wild-caught larvae collected from the neighbourhood of eight towns in Western Sokoto.

TABLE 7. ANALYSIS OF DATA FROM WESTERN SOKOTO : EFFECTS OF GAMMA-BHC ON *A. GAMBIAE* LARVAE

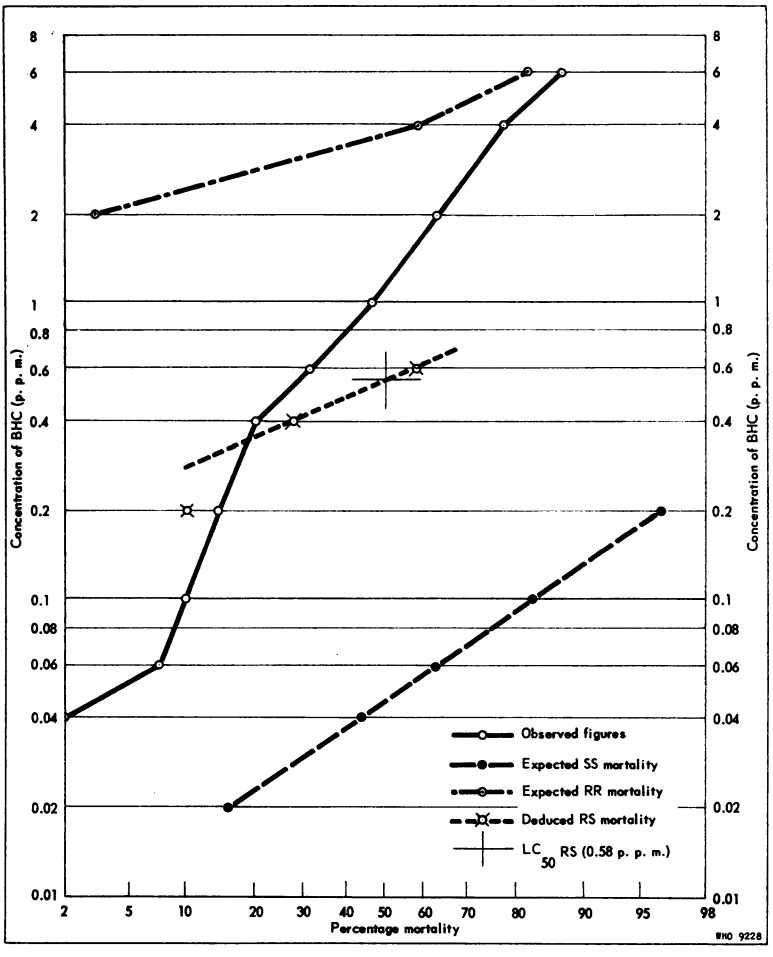
Concentration of BHC (p.p.m.)	Observed mortality	Expected SS mortality	Percentage SS present	Corrected RR and RS mortality	RR and RS survival	Expected RR survival	Percentage of RR present (base 90)	Deduced RS mortality
10	100			100	0	0.2 %		
6	87			85.5	14.5	18	89	
4	77.5			75	25	42	59	
2	62.5			58	42	97	43	
1	46			40	60	100	60	100
0.6	31			23	77			57.5
0.4	20	100	20	11	89			27.5
0.2	14	96	14.5	4	96			10
0.1	10	83	12	0	100			0
0.06	7.5	64	12	0				
0.04	2	44	4.5	0				
0.02	0	16	0	0				
			Average = 10.5 %				Average = 60 %	LC_{50} = 0.58 p.p.m.

The proportion of the three different genetic types in the population as a whole are estimated to have been:

RR 54%
RS 36%
SS 10%
LC_{50} for RS . . . . . 0.58 p.p.m.

The last figure is close to that obtained from the Kano material, and suggests that the method is capable of giving reasonably consistent results. Fig. 4 illustrates the observed, expected, and derived mortality figures.

FIG. 4. ANALYSIS OF GAMMA-BHC DATA FOR *A. GAMBIAE* FROM SOKOTO PROVINCE



Analysis of a Population near Freetown, Sierra Leone

In Freetown, DDT has been in intensive use since 1945 as a larvicide, and BHC has been used as an imagicide since 1952. The results of these control measures are sufficiently successful to render experimental material very difficult to obtain. The results summarized in Table 8 were obtained in June 1957 from larvae, some of which were wild-caught, while others were reared from eggs laid by females taken in BHC-sprayed houses. All the material came from Wellington, which is just inside the western boundary of the Freetown controlled area.

TABLE 8. ANALYSIS OF DATA FROM FREETOWN : EFFECTS OF GAMMA-BHC ON *A. GAMBIAE* LARVAE *

Concentration of BHC (p.p.m.)	Observed mortality	Expected SS mortality	Percentage SS present	Corrected RR and RS mortality	RR and RS survival	Expected RR survival	Percentage of RR present (base 66)	Deduced RS mortality
4	100			100	0	42	(0)	
2	92			88	12	96	12	100
1	79			68	32	100	(32)	77
0.6	75			62	38			70
0.4	44	100	(44)	15	85			17
0.2	35	96	36	1.5	98.5			2
0.1	14	83	17	0	100			
0.06	14	64	22					
0.04	9	44	20					
0.02	7	17	41					
0.01	5	4	(100)					
			Average = 34 %				Average = 12 %	LC_{50} = 0.62 p.p.m.

* Average number exposed at each concentration: 25

Median lethal concentrations were obtained from the two groups as follows:

LC_{50} gamma-BHC for reared larvae . . . . . 0.56 p.p.m.
(0.43 to 0.73 at 95% level)

LC_{50} gamma-BHC for wild-caught larvae . . . . . 0.18 p.p.m.
(0.07 to 0.43 at 95% level)

It is interesting to note that the degree of resistance in the offspring of the females resting in sprayed houses, which might be considered a pre-selected group, is higher, but the difference may not be significant. As the numbers in both cases were rather smaller than those dealt with hitherto they may conveniently be consolidated, giving a value for LC_{50} of 0.43 p.p.m. with 95% confidence intervals of 0.3 to 0.58 p.p.m.

At the time of this survey neither the Western Sokoto nor the Kano results were available for comparison, and it was concluded that the enhanced resistance to BHC shown by these larvae was an instance of vigour tolerance rather than a case of true resistance. However, it is now possible to examine these results on the same basis as those for the areas in which the presence of the dieldrin and BHC resistance factor is known, and when this is done the population is seen to consist in a large part of material whose reaction to BHC is similar to that of the heterozygous resistant genotype.

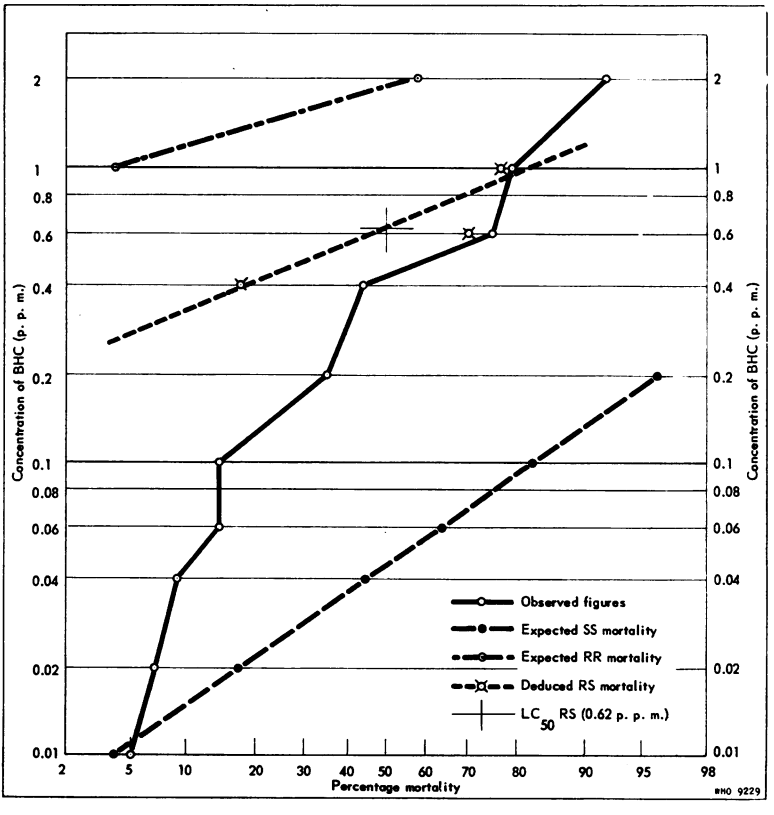
The proportions of the three genotypes estimated to be present are:

RR	8%
RS	58%
SS	34%
LC_{50} of RS	0.6 p.p.m.

The close correspondence of the LC_{50} values deduced for the RS fraction of the three populations studied after removal of the two fractions whose reactions to the insecticide are approximately known (0.62, 0.58, and 0.6 p.p.m.) suggests that the preliminary assumption—i.e., that the RR, RS, and SS genetic constitutions confer about the same degree of resistance wherever they may occur—is justifiable at least as a working hypothesis.

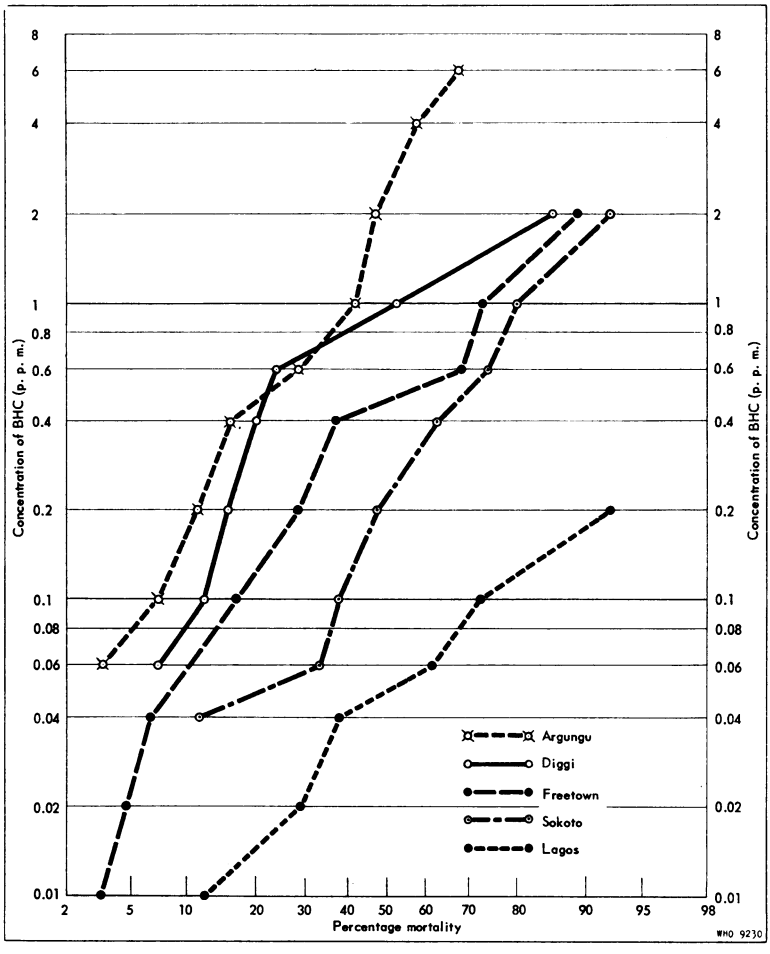
The appropriate calculations are set out in Table 8 and are illustrated in Fig. 5. In Fig. 6 are shown the reactions of larvae from Lagos and from three Western Sokoto towns alongside those of the Freetown larvae; Argungu has had two biennial treatments with BHC, Diggi has had one,

FIG. 5. ANALYSIS OF GAMMA-BHC DATA FOR *A. GAMBIAE* FROM FREETOWN



and Sokoto has had none, but is influenced by the proximity of sprayed territory 15 miles (25 km) away. At Lagos the resistance factor is absent. It will be seen that at Freetown the replacement of the normal by the

FIG. 6. FREETOWN GAMMA-BHC DATA FOR *A. GAMBIAE* COMPARED WITH THOSE FOR FOUR NIGERIAN TOWNS



•resistant strain has not proceeded so far as in the two sprayed towns in Nigeria; no doubt the presence of a nearby area in which a reservoir of the resistant strain had been produced by some years of selection by dieldrin accounts for the faster progress of change in the latter.

Analysis of Some Samples from the Population at Ibadan, Western Nigeria

In Ibadan relatively limited use has been made of DDT, almost entirely as a larvicide. *A. gambiae* from the Ibadan area have been shown by Bruce-Chwatt & Service (1957) to consist of two distinct populations, one distinguished by the pattern of the egg, which resembles that of *A. gambiae melas*. Attempts to raise colonies of either type have been uniformly unsuccessful, and all the results of tests with insecticides so far obtained refer to wild-caught material: principally wild-caught larvae, but a few larvae from eggs laid by wild-caught females. The various batches tested at different times have shown great variation in their response to dieldrin. Results are shown in Table 9, and values for median lethal concentrations are summarized as follows:

Batch	*Origin*	*LC_{50} of dieldrin*
A	All offspring of one female (*A. gambiae* type eggs), March 1957	0.16 p.p.m. (0.10-0.24)
B	Wild-caught larvae, April 1957	0.038 p.p.m. (0.032-0.045)
C	Wild-caught larvae, May 1957	0.060 p.p.m. (0.050-0.072)
D & E	Wild-caught larvae November 1957 and January 1958	0.015 p.p.m. (0.013-0.017)

TABLE 9. EFFECTS OF DIELDRIN ON SEVERAL BATCHES OF *A. GAMBIAE* LARVAE FROM IBADAN

Concentration of dieldrin (p.p.m.)	Percentage mortality in fourth-stage larvae from Ibadan							
	Batch A		Batch B		Batch C		Batches D and E	
	mortality	number exposed	mortality	number exposed	mortality	number exposed	mortality	number exposed
0.4	100	9	100	22	100	37	100	58
0.2	60	7	84	101	82	41	—	—
0.1	0	11	77	88	74	58	100	58
0.06	5	21	63	92	47	59	—	—
0.04	0	26	52	93	37	96	93	56
0.02	0	12	22	103	15	88	—	—
0.01	0	14	16	95	13	53	30	64
0.006	0	13	7	70	5	58	—	—
0.004	—	—	—	—	—	—	19	47
0.002	0	15	—	—	—	—	—	—
0.001	0	12	—	—	—	—	0	20

The wide variation in resistance between different batches contrasts very markedly with the situation in the Lagos area, where successive batches of larvae reared from wild-caught females have shown very uniform reactions over more than a year of periodical testing.

Batch A, though small in numbers, is of the most interest from the point of view of deciding whether the resistance factor is present at Ibadan; assuming that some of the group do carry this gene it is most likely that one parent was heterozygote, while the other was a pure susceptible. Half the offspring would then be heterozygous resistant with an LC_{50} value of about 1 p.p.m. (suggested by the Kano figures), while the rest would be pure susceptible with the LC_{50} at about 0.012 p.p.m. The expected mortalities for a population of this composition and the observed mortalities may then be compared:

	Expected	*Observed*
0.4 p.p.m. dieldrin	70%	100%
0.2 » »	58%	60%
0.1 » »	52%	0%
0.06 » »	44%	5%
0.02 » »	32%	0%

In view of the very small numbers available for testing (the majority of the batch were expended on tests with DDT) the case for the presence of the gene for dieldrin resistance in this batch must be regarded as not proven. It may also be noted that the batches apparently showing some dieldrin resistance were also relatively tolerant of DDT, so that the tolerance is apparently non-specific.

If the figures for batches B and C are compared with the expected mortalities for SS material, they may be considered to refer to a population containing 37% and 57% of material with a greater resistance than true SS. But the figures when corrected for 63% and 43% of SS material do not suggest that this more resistant fraction corresponds to the RS fraction seen at Kano, Western Sokoto and Freetown. The apparent LC_{50} figures are much lower than the estimates for RS material.

Further investigations, using BHC, are called for, but the presence at Ibadan of dieldrin-BHC resistance of the Northern Nigeria type is certainly not established on the present information.

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RÉSUMÉ

La méthode préconisée par Elliott en 1958 pour l'étude génétique de la résistance des anophèles aux insecticides a été appliquée à des souches de *A. gambiae* en Afrique occidentale. Les larves — échantillons de la population anophélienne — au début du stade IV étaient exposées pendant 1 heure à des solutions aqueuses d'insecticides, puis mises durant 5 heures dans l'eau claire et nourries, avant que l'on évalue la mortalité.

A Kano, le gène conférant la résistance à la dieldrine et au HCH-gamma semble augmenter de fréquence dans la population (50% de la population homozygote (RR) et 42% de la population hétérozygote (RS) le possèdent). Théoriquement, on admet que, lorsque le mâle hétérozygote est stérile, le gène présent dans la minorité tend à disparaître, en l'absence d'une sélection spécifique. Or, dans une colonie maintenue en captivité pendant 6 générations, sans sélection dirigée, c'est le contraire qui s'est produit, le gène S s'accroissant aux dépens du gène R.

Dans la province de Sokoto, les résultats ont été du même ordre, et comparables à ceux de Kano, en particulier la DL_{50} calculée d'après l'effet du HCH-gamma sur la fraction RS de la population.

A Freetown, il semble, d'après une analyse du même genre, que le facteur correspondant à la résistance au HCH-gamma se soit manifesté. Environ 66% de l'échantillon examiné étaient porteurs du gène R.

A Ibadan, des échantillons de populations montraient une certaine résistance à la dieldrine, mais la résistance spécifique, telle qu'on l'a observée ailleurs ne paraît pas y exister. La tolérance semble être non spécifique, car elle s'étend aussi, dans une certaine mesure, au DDT.

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