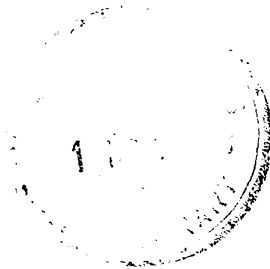


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THE EFFECTS OF SOME MIXTURES OF INSECTICIDES ON MOSQUITOS

(A preliminary investigation using Culex fatigans larvae)

by

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Introduction

It has lately been suggested that there may be a case for the employment against malaria vectors of two insecticides combined in a single treatment. It is visualized that the members of each pair would fall into separate categories as to their physiological action on the insect; thus insects capable of producing strains resistant to one would continue to be controlled by the other. Possible combinations in the chlorinated hydrocarbon group would be, for example, DDT/dieldrin or DDT/BHC, but not dieldrin/BHC, since insects resistant to one of the latter usually show cross-resistance to the other. Or alternatively, a member of either section of the chlorinated hydrocarbon group might be combined with an organo-phosphorus compound.

At present our knowledge of the combined action of two insecticides is rather limited; theoretical treatments of certain types of interaction are available (Plackett & Hewlett, 1948; Hewlett & Plackett, 1950, 1952), supported by some experimental data, but there is clearly room for further experimental work, not only to establish the physiological processes by which different toxicants may interact, but also to determine on a more practical basis which toxicants may be expected to be mutually compatible for control of various insects.

Before any conclusions may be reached as to the combinations and relative proportions of imagicidal formulations which may offer hopes of successful control of malaria vectors in the field, a knowledge of the effects of combined toxicants on the adult insect in the laboratory is needed. Tests using practical formulations sprayed onto simulated field surfaces would be most informative, but tests under more artificial conditions may also give clear indications. The specific case of a DDT/dieldrin mixture in the proportion of 10:1 has been dealt with by Macdonald (1959). It may be noted that when working with adult mosquitos the experimental work involved in testing the toxicants in different proportions would be extremely time-consuming, and as this point may be of considerable interest it was felt that the approach through study of the reactions of mosquito larvae might be of some value. Larvae are more amenable experimental subjects, and there is some evidence suggesting that in some cases at least the physiology of insecticide action is identical in larvae and in the corresponding adults (Elliott, 1959). This is indeed a further long step away from the practical problems of imagicidal control, but the information may also be of some use in providing indications for the larvicidal use of insecticide mixtures.<sup>1</sup>

The trials here described are confined to one species, Culex fatigans; both Anopheles gambiae and Aedes aegypti have given rather similar results, but these have not yet been accumulated in sufficient quantity to justify further mention.

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<sup>1</sup> The evaluation of combinations of two insecticides based on larval reactions could not always tell of their possible utility in adult control for the following reasons:

(a) The well recognized irritability of DDT, for example, would reduce markedly the exposure time permissible for another chemical combined with it.

(b) The larval tests are based on continuous exposures, whereas in adult tests where the exposure is for a limited period only, the amount of absorbed dose of the two insecticides might differ markedly.

[Editor's remark]

### Design of experiments

The toxicant dilutions to which the larvae were exposed were arranged in the form of a 6 x 6 square, with five dilutions of each toxicant arranged on each axis, and the sixth place on each axis occupied by one of the toxicants only. Of the 36 positions, therefore, 25 were occupied by different proportions of the two toxicants, 10 (five of each) by different dilutions of the single toxicants, and one served as an untreated control.

Experimental conditions approximated closely to the standard WHO test for susceptibility to insecticides in larval mosquitos (Brown, 1958), the only departure being the use of small containers, these being 75-ml porcelain dishes, which kept the layout within a reasonable space.

Two rows each of six beakers were set out at right angles, each containing a row of five serial dilutions of insecticide prepared from alcoholic solution, followed by one of water. The dilutions were double those of a range expected to produce mortalities from 90% to 10% approximately. The square of 36 exposure containers was then filled from the beakers by automatic pipette, each receiving half its contents from one row of beakers and half from the other.

The biological material consisted of the Lagos strain of Culex fatigans, a susceptible strain maintained at Yaba since 1952, exposed early in the fourth larval instar.

The following combinations of toxicants have so far been investigated:  
DDT/dieldrin; DDT/malathion; dieldrin/malathion; dieldrin/BHC.

### Results

The mortalities observed are summarized in Tables 1a-4a; in each case the effect of increasing concentrations of one toxicant in the presence of a given concentration of the other can readily be distinguished.

### Discussion

The four combinations studied comprise three in which the pair probably act on different physiological systems and one (dieldrin/BHC) in which they may be expected to act in the same way on the same system. Plackett & Hewlett (1948) give three equations relating the mortality produced by independent joint action of two poisons with the mortalities produced by the same two acting singly. Three equations cover the three different possible distributions of relative susceptibility to the two. The term "independent joint action" covers those cases where the two poisons act on different systems without modification of the effects of one by those of the other. The three distributions of susceptibility are random distribution of susceptibility to the two, complete negative correlation where the most resistant individuals to one are the most susceptible to the other, and complete positive correlation, the most resistant to one being also most resistant to the other. They are:

$$\begin{aligned} p &= p_1 + p_2 - p_1 p_2 && - (1) \text{ random distribution} \\ p &= p_1 + p_2 \text{ (maximum value 1)} && - (2) \text{ negative correlation} \\ p &= p_1 \text{ or } p = p_2 \text{ (whichever higher)} && - (3) \text{ positive correlation} \end{aligned}$$

where  $p$  is the proportion killed by the combination, while  $p_1$  and  $p_2$  are the proportions killed by the two toxicant dosages applied singly.

In Tables 1b-4b, the mortalities observed are compared with those expected, these being obtained by substituting the observed values for the single toxicants for  $p_1$ ,  $p_2$ . In no case do they correspond at all closely. The nearest fit is equation (2), above negative correlation, especially for DDT/dieldrin and dieldrin/BHC. It seems likely, then, that the slight apparent correspondence is due to this equation giving the highest figures for combined mortality, and that in fact all the pairs of toxicants interact to some degree.

Another hypothesis to be considered is that of "similar joint action". In this case the two poisons act on the same physiological system in the same way, the effect of one being equivalent to an added dose of the other. An equation expressing this relationship is:

$$\frac{z_1}{LD_1 p} + \frac{z_2}{LD_2 p} = 1 \quad (4)$$

where  $z_1$  and  $z_2$  are the dosages of the two toxicants, and  $LD_p$  and  $LD_{2p}$  are the dosages of the two acting alone which would produce a mortality  $p$ . The case of the dieldrin/BHC mixture might be expected to fall into this category, and in fact the values shown in Table 4a show a mean value close to unity, although there is a wide scatter. If a pair of toxicants act together in such a way that the mortality produced is greater than would be expected from consideration of their separate toxicities - the phenomenon of potentiation or synergism - a value of less than one would be expected from equation (4), and Tables 1a and possibly 3a show mean values below one. Table 1a, for DDT/dieldrin, shows that in 20 out of 23 results where  $p$  is less than 0.99 the value is below unity, and the mean value of the expression is 0.886. In the case of DDT/malathion, shown in Table 2a, the value is above one in 17 out of 19 observations, the mean being 1.200. This suggests that DDT/dieldrin is a combination of insecticides likely to be compatible in use, possibly showing a degree of synergism, while DDT/malathion is an incompatible pair, perhaps showing negative synergism.

An alternative explanation might be that the effects of all the pairs are physiologically additive, but that negatively-correlated relative susceptibility is producing apparent positive synergism and vice versa. This seems inherently less likely, but cannot be entirely discounted.

Whereas the results of equations (1) to (3) can readily be compared with the observations by the  $\chi^2$  test, and in all cases were significantly different, the significance of the deviations from a value of one in (4) is not so easily tested; advice is being sought on this point.

### Conclusions

These experiments<sup>1</sup> do not provide an immediate basis for action in imagicidal campaigns, but do show that one objection to the use of mixtures in general, which has been seriously put forward, is probably unsound; namely that the full single dose of each toxicant would have to be used. Indeed it is possible that certain combinations will be found to allow some economy in the expenditure of material.

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<sup>1</sup> In tests with dieldrin combinations, it might have been useful if the concentrations were modified by one degree; only 68% mortality was obtained with the highest test concentration of 0.01 dieldrin utilized in these tests, and mortalities with the two lowest concentrations (0.002 and 0.001 p.p.m.) were reduced accordingly. (Tables 1a and 1b).

Editor's remark

The results of the use of mixtures on species with the potentiality of producing resistant strains may now be considered. Let such a species be composed mainly of susceptible individuals, but let a small minority be resistant to one or other of the components of the mixture. The greatest part of the population will be susceptible and will duly be controlled. The resulting reduced residual population will contain an increased proportion of any genotype adapted to survival under the new environmental condition, i.e. the presence of insecticide. At this point the mode of inheritance of the genetic factors conferring resistance becomes the most vital factor. If both are recessive and rare, homozygotes of either will be extremely rare, and homozygotes for both almost non-existent. Homozygotes for one factor only will spend their lives in an environment containing a toxicant to which they are susceptible, and will have a lesser advantage than they would have in the presence of one toxicant only. Macdonald (1959) has shown quite clearly that recessively determined resistance must appear after a long interval, and field observations on *Anopheles* showing recessively inherited DDT resistance bear this out. In the presence of a mixture of insecticide, resistance to either toxicant will probably be effectively delayed, and resistance to both even indefinitely postponed.<sup>1</sup>

The case of dominant inheritance may now be taken. The residual population after introduction of the mixture will contain an increased proportion of individuals bearing factors for resistance, but again the concentration will not be as effective as in the presence of one toxicant only. The element in the population which can result in failure of control is the fraction bearing both factors, even in the heterozygous state. Since there is some selection against both factors, the appearance of the doubly resistant strain will take approximately the same period to arise as the appearance of a homozygous recessive resistant strain in the presence of one toxicant. This is far from a satisfactory position, but it must be better than the result of applying the toxicants seriatim; the time factor will be increased geometrically rather than arithmetically.

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<sup>1</sup> The situation with regard to *A. albimanus* in the Americas is of particular interest in view of the fact that the most complete series of resistance tests is available for this vector species. Here the widespread concurrent presence (especially as crop sprays) of DDT and dieldrin has repeatedly, though not invariably, produced strains showing dieldrin resistance (presumably dominant) plus DDT resistance (presumably recessive). The presence of dieldrin alone has produced dieldrin-resistant strains, but the presence of DDT alone has not (apparently) produced DDT-resistant strains.

In Anopheles, DDT resistance has so far been recessively inherited, resistance to BHC and dieldrin semi-dominant. In an important species, A. gambiae, the effect in West Africa of the use of DDT has been to produce a steady reduction in the seasonal peak of population over a period of a few years, this reduction being insufficient to lead to cessation of malaria transmission. Use of BHC or dieldrin in this area has caused a spectacular decrease in population for one season, any benefit resulting being immediately lost due to the rapid recovery in numbers after the appearance of the resistant strain. It seems possible that the use of a mixture would enable the shock effect of the BHC/dieldrin type of insecticide to be retained, the DDT component of the mixture slowing up both the spread of the resistance factor and the recovery of the population. A field trial on these lines is at present in progress; results to date are by no means discouraging.

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TABLE 1a. COMBINED EFFECTS OF DDT AND DIELDRIN  
 (5 replicates)

DIELDRIN

(Concentration in parts per million)

DDT (Concentration in parts per million)	0.01	0.006	0.004	0.002	0.001	0
	0.2	267 265 99% -	226 219 97% 0.936	275 271 99% -	274 256 93% 1.004	246 226 92% 1.002
0.1	250 239 96% 0.784	307 289 94% 0.706	256 210 82% 0.912	262 189 72% 0.969	267 223 84% 0.687	300 194 65%
0.06	269 244 91% 0.712	319 229 72% 0.950	248 184 74% 0.778	253 141 56% 0.850	287 126 44% 0.895	395 103 26%
0.04	205 160 78% 0.999	258 166 64% 0.942	219 114 52% 0.908	257 80 31% 0.968	277 84 33% 0.928	291 44 15%
0.02	228 175 77% 0.889	268 120 45% 1.006	240 67 28% 0.993	217 41 19% 0.800	226 25 11% 0.755	258 11 4%
0	277 188 68%	289 68 24%	296 40 14%	244 2 1%	255 0 0%	234 0 0%

Figures in squares: First pair: number exposed and number dead

Second line: percentage mortality

Third line:  $\frac{z_1}{LD_{1p}} + \frac{z_2}{LD_{2p}}$

TABLE 1b. COMBINED EFFECTS OF DDT AND DIELDRIN

DIELDRIN

(Concentration in parts per million)

DDT (Concentration in parts per million)	DIELDRIN (Concentration in parts per million)					
	0.01	0.006	0.004	0.002	0.001	0
0.2	99	97	99	93	92	89
	97	92	91	89	89	
	100	100	100	90	89	
	89	89	89	89	89	
0.1	96	94	82	72	84	65
	89	73	70	65	65	
	100	89	79	66	65	
	68	65	65	65	65	
0.06	91	72	74	56	44	26
	76	44	36	27	26	
	94	50	40	27	26	
	68	26	26	26	26	
0.04	78	64	52	31	33	15
	73	35	27	16	15	
	83	39	29	16	15	
	68	24	15	15	15	
0.02	77	45	28	19	11	4
	69	27	17	5	4	
	72	28	18	5	4	
	68	24	14	4	4	
0	68	24	14	1	0	0

Figures in squares: First: percentage mortality  
 Second:  $p_1 + p_2 - p_1 p_2$   
 Third:  $p_1 + p_2$  (maximum 100%)  
 Fourth:  $p_1$  or  $p_2$  (whichever greater)

TABLE 2a. COMBINED EFFECTS OF DDT AND MALATHION  
 (3 replicates)

MALATHION

(Concentration in parts per million).

		0.1	0.06	0.04	0.02	0.01	0
		DDT (Concentration in parts per million)	0.1	119 119 100% -	138 136 99% -	144 132 92% 1.300	167 152 91% 1.089
0.06	137 136 99% -		123 117 95% 1.133	146 92 63% 1.510	150 85 57% 1.239	145 68 47% 1.368	153 65 42% -
0.04	134 134 100% -		165 141 85% 1.213	160 47 29% 1.702	140 35 25% 1.332	82 19 23% 1.136	132 24 18% -
0.02	134 133 99% -		153 122 80% 1.081	182 43 24% 1.363	149 9 6% 1.285	75 3 4% 1.057	127 1 1% -
0.01	92 91 99% -		128 86 67% 1.128	105 25 24% 1.136	84 4 5% 0.945	87 2 2% 0.749	109 0 0% -
0	160 159 99% -		191 128 67% -	164 30 18% -	138 6 4% -	93 0 0% -	169 0 0% -

Figures in squares: As in Table 1a

TABLE 2b. COMBINED EFFECTS OF DDT AND MALATHION

MALATHION

(Concentration in parts per million)

DDT (Concentration in parts per million)	0.1	0.06	0.04	0.02	0.01	0
	0.1	100 100 100 99	99 94 100 83	92 86 100 83	91 84 87 83	87 83 83 83
0.06	99 99 100 99	95 81 100 67	63 52 60 42	57 44 46 42	47 42 42 42	42
0.04	100 99 100 99	85 73 85 67	29 33 36 18	25 21 22 18	23 18 18 18	18
0.02	99 99 100 99	80 67 68 67	24 19 19 18	6 5 5 4	4 1 1 1	1
0.01	99 99 100 99	67 67 67 67	24 18 18 18	5 4 4 4	2 0 0 0	0
0	99	67	18	4	0	0

Figures in squares: As in Table 1b

TABLE 3a. COMBINED EFFECTS OF DIELDRIN AND MALATHION  
 (5 replicates)

DIELDRIN

(Concentration in parts per million)

		0.01	0.006	0.004	0.002	0.001	0	
		MALATHION (Concentration in parts per million)		0.06	214 214 100% -	225 223 99% 0.661	243 226 93% 0.902	174 151 87% 0.927
0.04	245 245 100% -			240 226 94% 0.763	223 184 83% 0.896	242 140 58% 0.961	196 101 52% 0.004	164 49 30%
0.02	134 126 94% 0.770			222 152 68% 1.047	249 118 48% 1.100	261 69 26% 1.071	255 26 10% 1.153	205 9 5%
0.01	191 168 87% 0.869			183 121 66% 0.900	219 104 47% 0.885	202 33 16% 0.958	244 12 5% 1.000	234 5 2%
0.006	279 234 84% 0.902			238 150 63% 0.880	249 75 30% 0.071	209 26 12% 0.936	169 7 4% 0.804	167 2 1%
0	276 211 76%			181 91 50%	205 53 26%	165 9 5%	162 4 2%	196 0 0%

Figures in squares: As in Table 1a

TABLE 3b. COMBINED EFFECTS OF DIELDRIN AND MALATHION

DIELDRIN

(Concentration in parts per million)

		0.01	0.006	0.004	0.002	0.001	0
		MALATHION (Concentration in parts per million)	0.06	100 96 100 83	99 92 100 83	93 87 100 83	87 84 88 83
0.04	100 83 100 76		94 78 80 50	83 48 56 30	58 33 35 30	52 31 32 30	30
0.02	94 77 81 76		68 52 55 50	48 30 31 26	26 10 10 5	10 7 7 5	5
0.01	87 77 78 76		66 51 52 50	47 27 28 26	16 7 7 5	5 4 4 2	2
0.006	84 76 77 76		63 51 51 50	30 27 27 26	12 6 6 5	4 3 3 2	1
0	76		50	26	5	2	0

Figures in squares: As in Table 1b

TABLE 4a. COMBINED EFFECTS OF DIELDRIN AND BHC  
 (4 replicates)

DIELDRIN

(Concentration in parts per million)

		0.02	0.01	0.006	0.004	0.002	0
		BHC (Concentration in parts per million)	0.01	222 222 100% -	207 207 100% -	198 198 100% -	186 186 100% -
0.006	193 193 100% -		189 187 99% 0.977	160 132 83% 1.038	222 185 83% 0.936	216 168 78% 0.927	198 130 66% -
0.004	197 197 100% -		254 209 82% 1.026	254 182 72% 1.025	265 175 66% 1.002	226 119 53% 1.040	230 96 42% -
0.002	132 128 98% 0.502		236 183 78% 0.861	245 110 45% 1.244	266 101 38% 1.125	276 43 16% 1.316	226 18 8% -
0.001	184 142 83% 1.012		200 134 67% 0.967	243 102 42% 1.065	204 38 19% 1.363	212 17 8% 1.300	231 9 4% -
0	200 164 82% -		209 119 57% -	247 92 37% -	233 39 17% -	217 13 6% -	220 0 0% -

Figures in squares: As in Table 1a

TABLE 4b. COMBINED EFFECTS OF DIELDRIN AND BHC

DIELDRIN

(Concentration in parts per million)

BHC (Concentration in parts per million)	0.02	0.01	0.006	0.004	0.002	0
	0.01	100 98 100 90	100 96 100 90	100 94 100 90	100 92 100 90	98 91 96 90
0.006	100 94 100 82	99 85 100 66	83 79 100 66	83 72 83 66	78 68 72 66	66
0.004	100 88 100 82	82 75 99 57	72 63 79 42	66 52 59 42	53 45 48 42	42
0.002	98 84 90 82	78 70 75 57	45 42 45 42	38 24 25 17	16 14 14 8	8
0.001	83 83 86 82	67 59 61 57	42 40 41 37	19 20 21 17	8 10 10 6	4
0	82	57	37	17	6	0

Figures in squares: As in Table 1b