TESTS FOR TYPE OF ACTION OF HYDROCARBON INSECTICIDES APPLIED JOINTLY

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The development of strains of insects resistant to DDT, and subsequently to other chlorinated insecticides, is a major problem in insect control. In the past insects which developed resistance to an insecticide were controlled by using a different insecticide. Thus, San Jose scale which developed resistance to lime sulfur in Washington (Melander, 1914) was controlled by oil emulsions. Similarly, citrus scales in California which were resistant to HCN (Quayle, 1932) were controlled by oil emulsions.

Unfortunately, house flies which developed resistance to DDT soon developed resistance to methoxychlor, chlordane, lindane, and dieldrin. It is obvious that changing insecticides is not always a successful solution to the resistance problem.

Demerec (1952) has pointed out a way to prevent development of resistance of bacteria to antibiotics. He suggests use of a mixture of two effective antibiotics independent of each other as regards resistance. Under such conditions, mutations to resistance to the antibiotics occur independently, and the only survivors would be those bacteria undergoing mutations for resistance to both antibiotics simultaneously. In bacteria, single mutations occur at the rate of 10^{-8}, and double mutations should occur at the rate of 10^{-16}. Demerec also points out the possible benefits of synergistic action in mixtures of poisons acting differently.

However, Crow (1952) calculated a greater increase in resistance of insects following use of mixtures of poisons than following alternate use. The calculations were based on independent action of the insecticides, multiple factors for resistance, and normal distribution of resistance.

It is obvious that a knowledge of the mode of action of the various insecticides would be of real value in meeting the problem of resistance. If the development of resistance in insects is comparable with that in bacteria, mixtures of poisons having different modes of toxic action would be valuable. If the hypothesis of Crow (1952) is correct, alternating insecticides having different modes of action would be highly desirable.

Unfortunately the exact mode of action of most insecticides in current use is unknown. This is true more particularly of the relatively new

[1]Entomology Department. The laboratory work was done by Mrs. Nancy W. Wheeler and Mrs. Joan T. Curtiss, and is acknowledged with thanks.

materials which have the qualities of toxicity to insects and persistence needed for efficient control of pests.

The action of mixtures of chemicals as determined by their toxicity has been studied for many years. Macht (1929), working with drugs, proposed the term synergism for toxicity larger than expected from a combination of materials. Gnadinger and Corl (1932) and LePelley and Sullivan (1936) investigated the joint action of nicotine and pyrethrum. Bliss (1939) published a comprehensive study in which he recognized independent action, similar action, and synergism and proposed statistical methods for their separation. Finney (1952) extended the statistical methods and accepted the Bliss classification.

Horsfall (1945) clarified the subject and introduced the idea of subtractive and potentiated antagonism. He proposed the "titration" design for testing for synergism, in which the median lethal dose of each ingredient is determined, and mixtures of the two were tested in the ratio of 90 per cent — 10 per cent, 80 per cent — 20 per cent, etc., of the m.l.d. Sakai *et al.* (1951) made use of this technique in studies of insecticides.

More recently joint action has been considered thoroughly by Plackett and Hewlett (1952). They have introduced a new classification and new statistics. Similar joint action, in which the response is induced "by causing the same physiological system to react or fail" may be either (a) simple or (b) complex (the amount of one ingredient or the response from one ingredient is affected by the other). Dissimilar joint action results when the materials cause different or distinct physiological systems to react or fail, and may be independent or dependent (physiological interaction present). This is more than a rearrangement of Bliss' (1939) types. Plackett and Hewlett (1952) include materials producing non-parallel dosage response curves as acting similarly. Both Bliss (1939) and Finney (1952) exclude such a type of response from similar action. Furthermore, it is difficult to visualize physiological interactions even under Plackett and Hewlett's (1952) definition of similar action.

A simplified method of computation of data in testing for type of joint action, based on the work of Bliss (1939) and Finney (1952) has been proposed by Wadley (1945) (1949). Wadley devised a graphical test for similar action. If the toxicity was substantially less, independent action would be indicated; if significantly more, synergism would be suggested. Turner and Bliss (1953) have found one case in which mortality on the assumption of independent action exceeded slightly that expected from similar action. With this exception, the graphical test has proved to be very convenient for preliminary examination of data.

Methods and Procedure

Application of the insecticides was by the injection technique, using adult milkweed bugs (*Oncopeltus fasciatus*). The bugs were reared in the laboratory, and had been in the adult stage for 5 to 7 days before injection. The material was injected into the abdominal cavity, the puncture being made at the juncture of the median abdominal segments.

The injection apparatus delivered 3μl. of the diluted insecticide through a standard 1 ml. tuberculin syringe and a 27 guage hypodermic needle.

Liquid insecticides not water soluble were used as emulsions by addition of 15 per cent of *Triton X-100*. Water-insoluble solid insecticides were dissolved in xylene which was emulsified by 15 per cent *Triton X-100*.

The insects were anesthetized by carbon dioxide, injected and placed in four lots of 12 or three lots of 16 in petri dishes, with moist cotton plugs for water and a small supply of milkweed seed for food. They were held at a constant temperature of 80°F. and a relative humidity of 60 per cent for 48 hours before determining mortality. Those insects incapable of coordinated movement were classed as dead, and insects slightly affected but capable of coordinated locomotion as alive. At intervals during the study representatives of these two groups were saved for several days to verify the accuracy of the separation. The fate of these samples was invariably as expected. One group of insects was injected with distilled water in each test. There was seldom any mortality, and if substantial mortality occurred, the results of the entire test were discarded.

The principal objective of the tests was to determine which type of action resulted from combinations of the insecticides. The dosage range of each material was established, and each material was used at four dosages differing by $\sqrt{2}$. Since the first test was actually directed at similar action, the dosage range of the mixtures was selected to provide mortality between 10 and 90 per cent from the four dosages. If mortality exceeded the expectancy, a second complete test was made. In a majority of cases a single test consisted of four dosages of the two materials used alone, and four dosages of mixtures of the two materials in two different ratios.

Insecticides

The insecticides used were DDT, TDE, DFDT[1], methoxychlor, *Perthane* (di(p-ethyl phenyl) dichloroethane), *Dilan* (a mixture of 2-nitro -1 : 1 - bis (p - chlorophenyl)) butane and propane, chlordane. heptachlor, aldrin, dieldrin, endrin, isodrin, lindane and *Strobane* (commercial product, chlorinated terpenes).

DDT and related compounds. DDT, TDE, DFDT, methoxychlor and *Perthane* are obviously closely related chemically. *Dilan* has been considered with this group because of the similarity of basic molecular structure. It differs from DDT by the substitution of NO_2 group and a propane or butane radical for two of the chlorine atoms attached to the ethane carbon. The third chlorine is replaced by hydrogen.

[1]Sample furnished by the Pennsylvania Salt Mfg. Co.

Calculation of Results

The results of the tests were calculated by the graphical method of Wadley (1949). The results of the two materials used alone were plotted on logarithmic probability paper and the curves fitted by eye. The toxicity of the mixtures was compared with the toxicity expected on the basis of similar action. The expected toxicity was determined by calculating the dosage of the mixture in terms of one of the ingredients. Referring to Table 1, the mortality expected from .05 per cent *Dilan* was determined on the *Dilan* curve. The amount of methoxychlor which would produce this same mortality was interpolated from the methoxychlor curve. This was added to the amount of methoxychlor used in the mixture, and the expected mortality from the total methoxychlor calculated.

Classification of Results

The results could be classified in five groups.

Group 1. Materials producing parallel dosage-response curves, with toxicity consistent with similar action. Table 1 gives a summary of the results of two tests using methoxychlor and *Dilan,* and Fig. 1 dosage-response curves for the materials and mixtures. Expected toxicity from similar action averaged about the same in one test and about 10 per cent more (on the basis of dosage for equal control) than was observed. The other combinations giving this type of result are as listed below. The

TABLE 1.

RESULTS OF TWO TESTS WITH METHOXYCHLOR AND DILAN.

Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality			
		3/25/52		6/24/53	
Methoxychlor	Dilan	Observed	Expected*	Observed	Expected*
.14	—	92		94	
.1	—	67		67	
.07	—	29		25	
.05	—	25		15	
—	.14	92		96	
—	.1	65		73	
—	.07	27		40	
—	.05	8		17	
.07	.05	77	80	73	86
.05	.035	48	48	54	60
.035	.025	35	15	18	28
.025	.0175	6	2	6	8
.05	.05	49	66	39	73
.035	.035	25	28	31	40
.025	.025	8	7	6	15
.0175	.0175	6	1	2	3

*Similar action.

figures are L.D. 50's in terms of percentage composition as determined from dosage-response curves fitted by inspection. In all cases the L.D. 50 of the mixture is in terms of the insecticide in the second column.

DFDT	.45	*Perthane*	.135	mixture	.14
DDT	.035	*Perthane*	.11	mixture	.09
Dilan	.07	DDT	.05	mixture	.05
Dilan	.07	*Perthane*	.09	mixture	.10
Perthane	.11	TDE	.25	mixture	.35
DFDT	.25	*Dilan*	.06	mixture	.05

TABLE 2.

RESULTS OF APPLICATION OF METHOXYCHLOR AND DFDT.

Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Methoxychlor	DFDT	Observed	Expected*
.14	—	98	
.1	—	87	
.07	—	46	
.05	—	19	
—	.56	58	
—	.4	37	
—	.28	14	
—	.2	10	
.07	.2	77	92
.05	.14	67	70
.035	.1	27	38
.025	.07	10	13
.05	.2	56	78
.035	.14	33	48
.025	.1	29	22
.0175	.07	9	5

*Similar action.

Group 2. Materials with non-parallel dosage-response curves, with toxicity consistent with similar action. Table 2 gives a summary of the results using methoxychlor and DFDT and Fig. 2 dosage-response curves. DDT and methoxychlor produced like results. (L.D. 50's — DDT .06, methoxychlor .066, mixtures .064)

Group 3. Materials with non-parallel dosage-response curves, with toxicity 23 per cent lower than consistent with similar action, and much higher than can be accounted for by independent action: DDT and DFDT (Table 3).

Group 4. Materials with parallel dosage-response curves, with curves for mixtures much less steep than for the materials themselves: DDT and TDE (Table 4, Fig. 3), and the following combinations:

Methoxychlor	.056	TDE	.23	mixture	.23
TDE	.27	DFDT	.27	mixture	.23
Dilan	.088	TDE	.35	mixture	.4

TABLE 3. RESULTS OF APPLICATION OF DDT AND DFDT.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration DDT	DFDT	Per cent mortality Observed	Expected*
.14	—	100	
.1	—	98	
.07	—	75	
.05	—	28	
—	.56	56	
—	.4	56	
—	.28	15	
—	.2	12	
.05	.28	77	97
.035	.20	52	83
.025	.14	17	53
.0175	.10	4	20
.05	.2	44	95
.035	.14	48	88
.025	.1	15	60
.0175	.07	2	10

*Similar action.

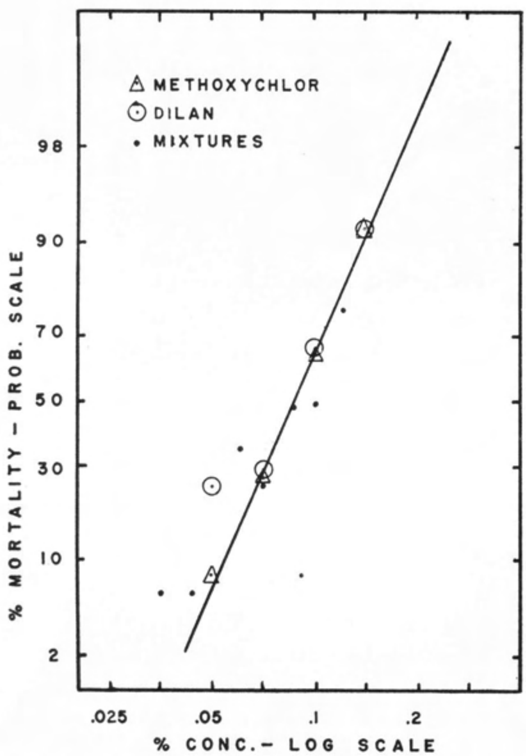

Figure 1. Dosage-response curves for methoxychlor, *Dilan,* and mixtures.

All of the combinations in group 1 meet the requirements for similar action. Plackett and Hewlett (1952) have included DDT and methoxychlor in their classification of similar action, in spite of the fact that the dosage-response curves are not parallel. It has been generally accepted that differences in the slope of the curves means a different distribution of susceptibility and, therefore, a different mode of action, in the absence of differences in the samples of insects and in methods of application (See Horsfall, 1945, and Dimond *et al.*, 1944). Obviously Bliss (1939) and Finney (1952) held this view in their consideration of similar action.

Metcalf (1948) summarizes the work of Welsh and Gordon, which showed that DDT, DFDT, TDE, and methoxychlor all produced similar bursts of impulses when applied to nerve axons.

Finally, the toxicity of methoxychlor to flies and potato flea beetles resistant to DDT might imply a different mode of action. However, these insects developed a resistance to methoxychlor relatively rapidly. Obviously, the two do not act on the same locus and in an identical manner. Perhaps the suggestion of Plackett and Hewlett (1952) that they may act on the same physiological system at different loci is the answer.

For the purposes of this study, the insecticides acting in this way may be considered as acting similarly.

TABLE 4. RESULTS OF APPLICATION OF DDT AND TDE.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
DDT	TDE	Observed	Expected*
.14	—	79	
.1	—	81	
.07	—	42	
.05	—	15	
—	.56	94	
—	.4	77	
—	.28	56	
—	.2	23	
.07	.2	75	84
.05	.14	53	59
.035	.1	26	31
.025	.07	19	11
.05	.2	56	74
.035	.14	33	45
.025	.1	31	20
.0175	.07	17	4

*Similar action.

The materials in group 4 produced parallel dosage-response curves, but the curves for the mixtures were not parallel with curves for the materials (on the basis of similar action). This is more contradictory than the lack of parallelism in the curves for the insecticides in group 2.

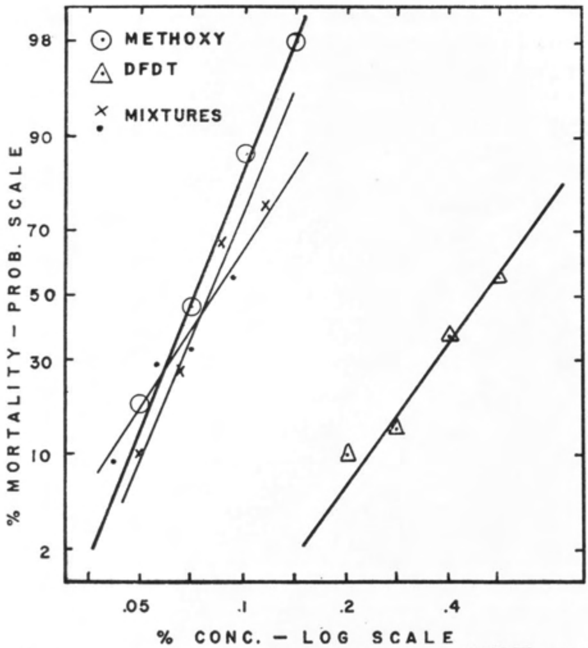

Figure 2. Dosage-response curves for methoxychlor, DFDT, and mixtures (plotted on the basis of dosage of methoxychlor equivalent to DFDT.)

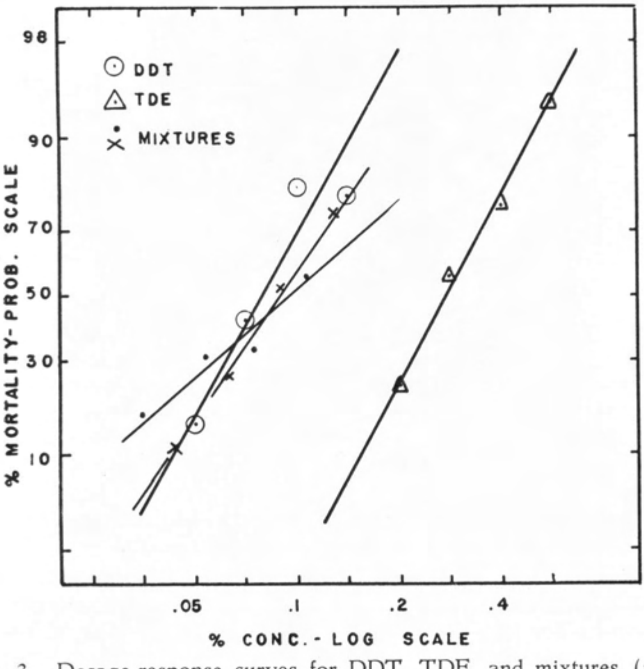

Figure 3. Dosage-response curves for DDT, TDE, and mixtures (plotted on the basis of dosage of DDT equivalent to TDE).

Here insecticides which produce parallel curves no longer do so when they are mixed. In the example given (Table 4, Fig. 3) one of the curves for the mixtures is reasonably parallel and the other definitely flat. If this were the only case, the one flat curve might be written off as an aberration. However, too many other similar cases occurred. Furthermore, TDE was involved in all of them. For the purposes of this study, these combinations can be considered as undesirable for practical use because of the low toxicity of the higher dosages, regardless of the possible type of joint action.

Likewise the combination of DFDT and DDT, which was less toxic than expected from similar action but too toxic for independent action, will require more study. There is no other reason than toxicity to assume other than similar action.

TABLE 5.

RESULTS OF APPLICATION OF PERTHANE AND METHOXYCHLOR.

Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Perthane	**Methoxychlor**	**Observed**	**Expected***
.28	—	83	
.2	—	67	
.14	—	52	
.1	—	27	
—	.14	98	
—	.1	98	
—	.07	92	
—	.05	75	
.14	.05	87	88
.1	.035	69	69
.07	.025	52	46
.05	.0175	23	24
.1	.05	83	82
.07	.035	67	64
.05	.025	42	41
.035	.0175	.6	18

*Independent action.

The most serious limitation of this method of determining type of action is the lack of consistency. DDT and *Perthane*, and DDT and methoxychlor apparently acted similarly. However, *Perthane* and methoxychlor acted independently. If all these materials except either *Perthane* or methoxychlor acted similarly, more cases of independent action involving either *Perthane* or methoxychlor would be expected. It is obvious that *Perthane* acted similarly in the tests included in group 1. One combination containing methoxychlor was assigned to group 1, but the others are involved either in independent action or changes in slope of the curves. This seems to be a valid reason for questioning similar action when slopes are different. However, the matter cannot be settled with the data at hand.

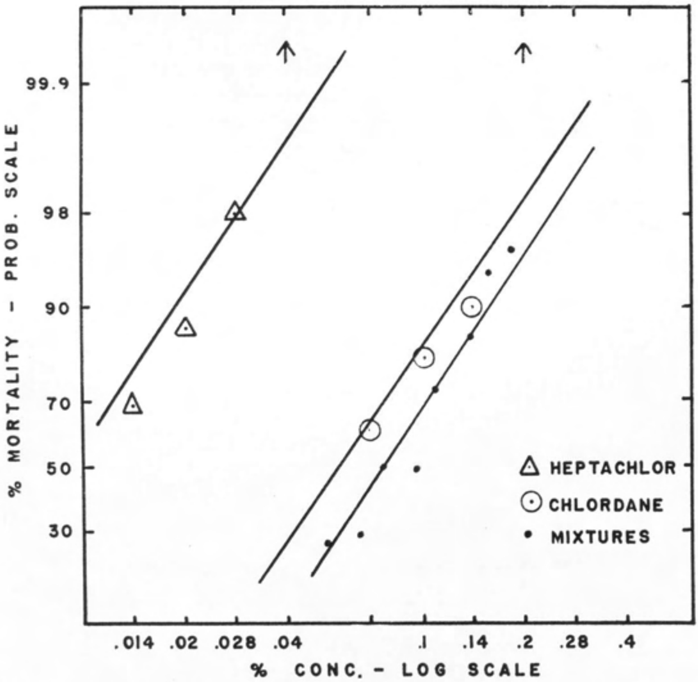

Figure 4. Dosage-response curves for heptachlor, chlordane, and mixtures (plotted on the basis of dosage of heptachlor equivalent to chlordane).

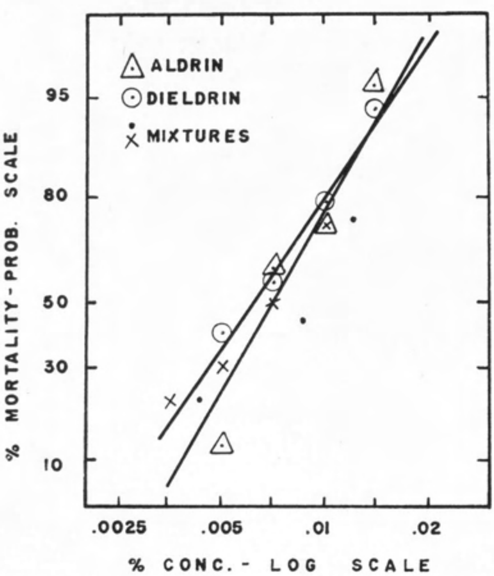

Figure 5. Dosage-response curves for aldrin, dieldrin, and mixtures.

Combinations of Materials

Chlordane and heptachlor. Chlordane has been identified as octachlorodihydrodicyclopentadiene (Martin, 1953). Technical chlordane, used in these tests, contains other chlorinated compounds including heptachlor (heptachlorodicyclopentadiene). Heptachlor is also prepared in technical form containing related compounds. Tests of the two materials produced parallel dosage-response curves (Fig. 4) and toxicity of mixtures about 20 per cent less than expected from similar action. This seems to be a rather large difference in toxicity.

Aldrin and its relatives. Some combinations of aldrin and its isomer isodrin, and dieldrin and its isomer endrin have been tested. Dosage-response curves for the materials used were apparently parallel except in the case of dieldrin and aldrin (Fig. 5). However, in all cases results were consistent with those expected from similar action. The L.D. 50's of the materials and their mixtures were as follows:

Aldrin	.0036	Isodrin	.0014	mixture	.0012
Aldrin	.0068	Endrin	.0021	mixture	.0019
Dieldrin	.006	Endrin	.0028	mixture	.0028
Dieldrin	.006	Isodrin	.0019	mixture	.0017

Chlordane and aldrin groups. Heptachlor and dieldrin, chlordane and endrin, chlordane and isodrin and chlordane and dieldrin apparently acted similarly. However, one ratio of chlordane and dieldrin produced toxicity 22 per cent greater than expected on the basis of dosage for equal control. The L.D. 50's of these combinations were as follows:

Dieldrin	.0034	Heptachlor	.013	mixture	.012
Endrin	.004	Chlordane	.11	mixture	.095
Isodrin	.0033	Chlordane	.09	mixture	.082
Chlordane	.19	Dieldrin	.006	mixture	.055-.035

TABLE 6.

RESULTS OF APPLICATION OF CHLORDANE AND ALDRIN.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Chlordane	Aldrin	Observed	Expected*
.56	—	98	
.4	—	77	
.28	—	67	
.2	—	47	
—	.014	85	
—	.01	52	
—	.007	29	
—	.005	12	
.28	.005	94	85
.2	.0035	92	62
.14	.0025	71	33
.1	.00175	52	11
.2	.005	85	73
.1	.0035	71	43
.07	.0025	42	18
.05	.00175	25	4

*Similar action.

Chlordane and alarin were substantially more toxic in mixtures than expected on the basis of similar action according to Bliss (1939) (Table 6). The increase in toxicity, measured in dosage for equal control (Fig. 6) was 25 per cent at one ratio and 42 per cent at the other. However, this falls well within the concept of similar action of Plackett and Hewlett (1952). There is the further complication that chlordane produced similar action with other member of the aldrin group, and by analogy should act similarly with aldrin. It seems unlikely that this point can be settled conclusively by mortality data.

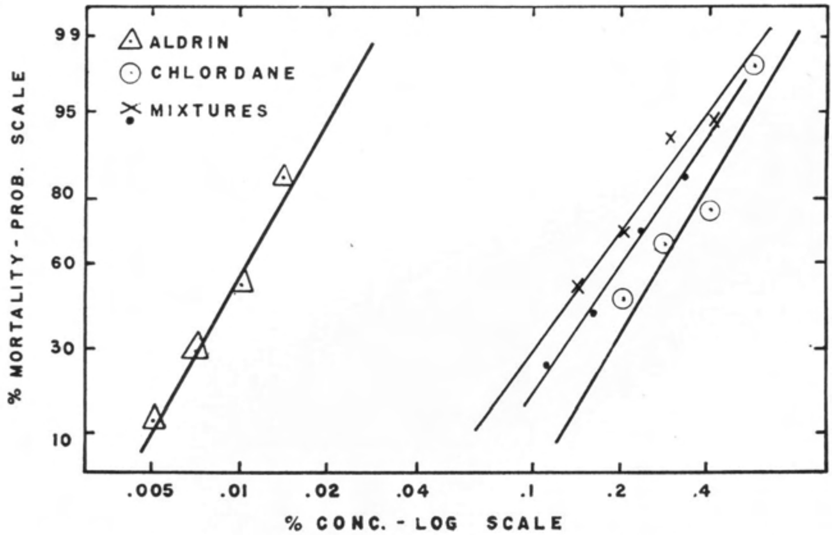

Figure 6. Dosage-response curves for aldrin, chlordane, and mixtures (plotted on the basis of dosage of chlordane equivalent to aldrin).

DDT and chlordane groups. *Dilan* and heptachlor produced results consistent with the expectancy for similar action. However, chlordane applied with members of the DDT group produced mortality substantially in excess of expectation on the basis of similar action as described by Bliss. The data for one test with chlordane and *Dilan* are summarized in Table 7 and Fig. 7.

Results from the other mixtures in the series were as follows:

						Per cent increase
Dilan	.09	Heptachlor	.018	mixture	.02	
Dilan	.11	Chlordane	.32	mixture	.19	40
Chlordane	.17	TDE	.27	mixture	.22	19
Dilan	.12	Chlordane	.25	mixture	.14-.16	40-35
Chlordane	.18	DDT	.056	mixture	.038	38
Chlordane	.28	*Perthane*	.12	mixture	.074	44
Chlordane	.24	DFDT	.34	mixture	.14	56
DFDT	.32	Chlordane	.17	mixture	.11-.08	35-56
Chlordane	.14	Methoxychlor	.1	mixture	.05	50

TABLE 7.
RESULTS OF APPLICATION OF CHLORDANE AND DILAN.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Chlordane	Dilan	Observed	Expected*
.56	—	94	
.4	—	83	
.28	—	25	
.2	—	4	
—	.2	94	
—	.14	69	
—	.1	52	
—	.07	13	
.2	.07	100	70
.14	.05	92	40
.1	.035	58	12
.07	.025	35	3
.14	.1	100	81
.1	.07	86	40
.07	.05	60	7
.05	.035	37	1

*Similar action.

The two figures for *Dilan* and chlordane, and DFDT and chlordane, refer to two complete tests. As in the case of chlordane and aldrin, these increases fall well within the limits of the similar action of Plackett and Hewlett (1952). In one respect at least these data are consistent. Chlordane acted in the same way with each material in the DDT group.

DDT and aldrin groups. The following combinations produced results consistent with similar action, with the exception of the increase in toxicity from combinations of *Dilan* and endrin, and DFDT and dieldrin.

						Per cent increase
Dilan	.08	Isodrin	.0024	mixture	.0024	
Dilan	.068	Dieldrin	.006	mixture	.007	
Aldrin	.007	Methoxychlor	.1	mixture	.12	
Methoxychlor	.09	Dieldrin	.0058	mixture	.006	
Dilan	.11	Aldrin	.015	mixture	.013	
Dilan	.13	Endrin	.004	mixture	.0028	30
DFDT	.22	Dieldrin	.0066	mixture	.0051-.004	22-40

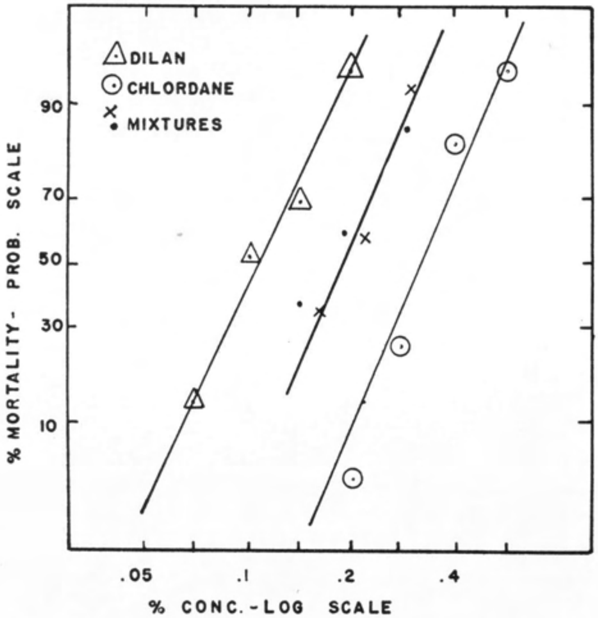

Figure 7. Dosage-response curves for *Dilan*, chlordane, and mixtures (plotted on the basis of dosage of chlordane equivalent to *Dilan*).

The remaining combinations gave the following results:

Combination	Per cent increase in toxicity	Slope of curve for mixtures
Dieldrin - *Dilan*	?	converging
Aldrin - DFDT	15-33	converging
TDE - aldrin	10-15	diverging
DDT - dieldrin	29	diverging
DDT - aldrin	25-25-38	diverging
TDE - dieldrin	37	diverging
Dieldrin - *Perthane*	24	diverging
Aldrin - *Perthane*	20	diverging

Aside from the increase in toxicity, the dosage-response curves for the mixtures present a new problem. The curves for the mixtures of aldrin and DFDT converge toward the curves for the individual materials. The curves for six of the combinations diverge (Fig. 8). One such result might be written off, but six tests involving 13 combinations cannot be ignored.

Under certain conditions, a change in slope of the dosage-response curve is evidence that the distribution of susceptibility in the test insects has been changed. One condition is that the dosage is known. In the case of these tests, the dosage of the two materials applied is known. When the computation to test similiar action is made, the dosage acting is computed on the basis of similar action. These divergent curves could

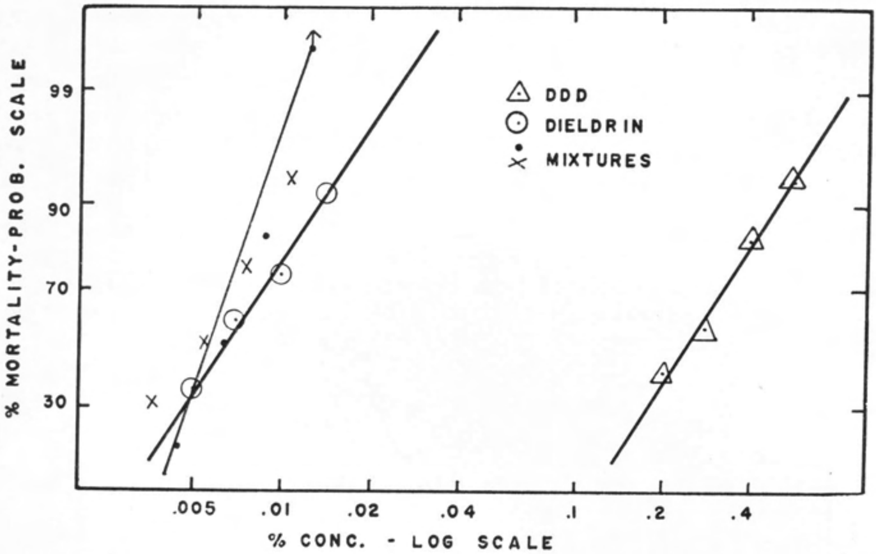

Figure 8. Dosage-response curves for TDE (DDD), dieldrin, and for mixtures (on the basis of dosage of dieldrin equivalent to TDE).

result if the hypothesis of similar action underestimated the dosage acting in the relatively high concentrations, or overestimated the dosage acting in the lower concentrations. However, since the *ratio* of the ingredients in the mixture remained constant, a bias in results because of one

TABLE 8.
RESULTS OF APPLICATION OF METHOXYCHLOR AND LINDANE.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Methoxychlor	Lindane	Observed	Expected*
.14	—	94	
.1	—	77	
.07	—	52	
.05	—	25	
—	.01	81	
—	.007	54	
—	.005	21	
—	.0035	12	
.07	.0035	98	82
.05	.0025	85	56
.035	.00175	60	26
.025	.00125	23	5
.05	.0035	90	67
.035	.0025	79	38
.025	.00175	60	13
.0175	.00125	15	4

*Similar action.

ingredient in the mixture should also remain constant. Futhermore, if the calculation for similar action were basically in error, a majority of these tests should show the error, rather than a few.

The only conclusion possible under the evidence is that these cases represent a something distinctly different from the general pattern of results and, therefore, merit additional study.

Lindane combinations. Lindane was tested with each material in the DDT group and with dieldrin. The results showed toxicity somewhat greater than expected from Bliss similar action. Results from lindane and methoxychlor are given in Table 8 and Fig. 9. The increase in toxicity was as follows:

						Per cent increase
Lindane	.0064	DFDT	.5	mixture	.33	31
Lindane	.0037	TDE	.2	mixture	.18	40
Lindane	.003	*Perthane*	.11	mixture	.084-.066	24-40
Lindane	.0056	*Dilan*	.1	mixture	.072	28
Lindane	.0054	DDT	.08	mixture	.066	17
Lindane	.006	Dieldrin	.0074	mixture	.0048-.0032	35-56
Lindane	.0064	Chlordane	.2	mixture	.23	—

With the exception of chlordane, the increasees in toxicity were substantial. Furthermore, the entire DDT group produced the same type of result when tested with lindane. Similar reesults followed application of lindane with dieldrin.

TABLE 9.

RESULTS OF APPLICATION OF PERTHANE AND STROBANE.

Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Perthane	Strobane	Observed	Expected*
.28	—	100	
.2	—	77	
.14	—	44	
.1	—	23	
—	.4	98	
—	.28	90	
—	.2	73	
—	.14	33	
.14	.14	98	93
.1	.1	73	75
.07	.07	37	44
.05	.05	15	15
.1	.14	85	85
.07	.1	48	60
.05	.07	23	28
.035	.05	8	6

*Similar action.

Lindane and chlordane were definitely similar in action, at one ratio. At the other ratio tested, the results agreed with the expected for independent action better than for similar action.

Strobane combinations. The following were the results of combinations containing *Strobane*.

						Per cent increase
Strobane	.16	*Perthane*	.14	mixture	.15	
Methoxychlor	.05	*Strobane*	.23	mixture	.23	
Dilan	.1	*Strobane*	.18	mixture	.18	
DDT	.078	*Strobane*	.21	mixture	.22	
Strobane	.19	TDE	.27	mixture	.19	
DFDT	.35	*Strobane*	.24	mixture	.13	
Strobane	.15	Chlordane	.24	mixture	.25	30
Strobane	.22	Dieldrin	.0074	mixture	.008	46

The type of action was obviously similar with the exception of combinations with TDE and DFDT. *Strobane* and aldrin were much less toxic than expected from the *Strobane* in the mixtures, and antaganism must have occurred (Table 10).

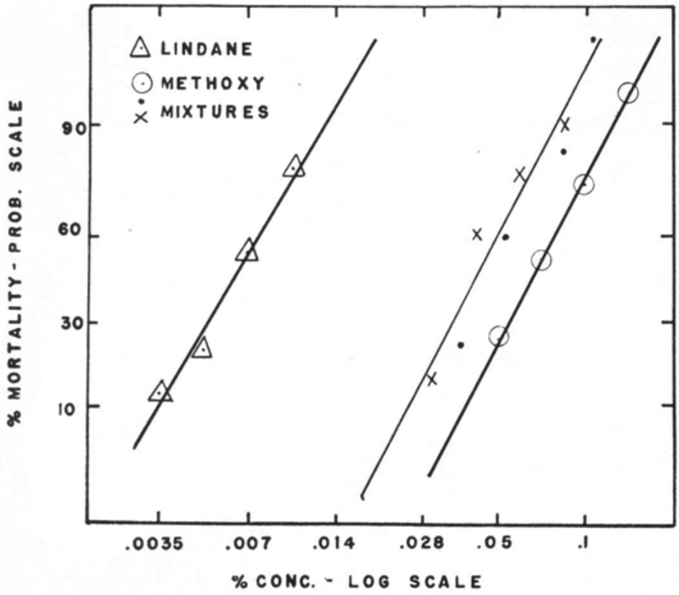

Figure 9. Dosage-response curves for lindane, methoxychlor, and mixtures (on the basis of dosage of methoxychlor equivalent to lindane).

TABLE 10.
RESULTS OF APPLICATION OF STROBANE AND ALDRIN.
Each Percentage of Mortality Is Taken From 48 Bugs.

Per cent concentration		Per cent mortality	
Strobane	Aldrin	Observed	Expected*
.4	—	98	
.28	—	90	
.2	—	79	
.14	—	56	
—	.014	83	
—	.01	67	
—	.007	42	
—	.005	23	
.2	.005	51	79
.14	.0035	23	56
.1	.0025	12	34
.07	.00175	4	15
.14	.005	31	56
.1	.0035	10	34
.07	.0025	17	15
.05	.00175	6	5

*Strobane only.

DISCUSSION

In this study, an attempt was made to determine the type of joint action of chlorinated insecticides by applying them alone and in mixtures. A large proportion of the materials used unquestionably acted similarly. These materials would be of little use in controlling insects which have developed resistance to any one of them.

Some combinations produced higher mortality than expected on the basis of similar action as defined by Bliss (1939). These are (1) chlordane with DDT, DFDT, TDE, methoxychlor, *Dilan, Perthane,* and aldrin; (2) lindane with DDT, DFDT, TDE, methoxychlor, *Dilan, Perthane,* and dieldrin; (3) aldrin or dieldrin with TDE, DDT, and *Perthane;* and (4) *Strobane* with TDE and DFDT. For most of these combinations, the only evidence of action other than similar is the increase in toxicity. However. aldrin and dieldrin with TDE, DDT and *Perthane* produced diverging dosage-mortality curves, which might be accepted as evidence for an interaction.

Chlordane and lindane occurred in 14 of the 22 combinations. TDE occurred in 5, DDT in 4, and DFDT, *Perthane,* aldrin and dieldrin in 3. This sort of result was reported by Sakai *et al.* (1951) in tests of 13 insecticides used in all possible combinations. In tests on *Drosophila,* all combinations more toxic than expected for similar action contained dieldrin, nicotine, TEPP, or BHC. In tests on the turnip aphid, nicotine, TEPP, and the pyrethrins occurred in the more toxic combinations.

This type of result might be good evidence regarding mode of action if there were not so many inconsistencies. Thus, methoxychlor and *Perthane* acted independently, but both produced the same type of result with chlordane and lindane. The members of the DDT group which were similar in action did not all act alike when used with *Strobane*. Chlordane produced high toxicity when combined with aldrin, but not when used with other members of the aldrin group. The same was true of lindane and dieldrin.

Many of the materials used were highly toxic to the milkweed bug. Lindane, aldrin, dieldrin, endrin, and isodrin killed 50 per cent of the bugs in concentrations ranging from .0019 per cent to about .008 per cent. Heptachlor was about one-tenth as toxic, and DDT about one-twentieth. The other materials, in order of descending toxicity, were methoxychlor, *Dilan, Perthane,* chlordane, *Strobane,* TDE, and DFDT. The two last materials were about one-one hundredth as toxic as lindane, aldrin, dieldrin, endrin, and isodrin. What effect this large difference in toxicity might have on joint action is unknown. Use of the single test insect certainly did not eliminate either the compounds of low or high toxicity. TDE occurred in five combinations producing substantial mortality, and aldrin in only four.

One possibility of distinguishing modes of toxic action has been the use of strains resistant to an insecticide. Thus, house flies resistant to DDT were found to be susceptible to methoxychlor by King and Gahan (1949), Gilbert, Wilson and Coarsey (1950), and Hansens (1950), among others. Later Hansens (1953), Goodwin and Schwardt (1953), March, Metcalf and Lewallen (1952), and Gilbert, Couch and McDuffie (1953) reported that flies in barns had developed resistance to methoxychlor, and in at least one instance to each of the following insecticides: TDE, lindane, toxaphene, chlordane, dieldrin, and *Dilan*. In some cases, flies were resistant to insecticides to which they had supposedly not been exposed, but in most instances the resistance was developed by use of the insecticide. Thus, whether or not the insecticides acted in the same way as DDT, resistance developed relatively rapidly.

Bruce and Decker (1950) developed nine strains of house flies by exposure to one or two insecticides for each strain. The susceptibility of each strain to seven insecticides was then determined. The DDT strain showed an enormous increase in resistance to DDT, a 14-fold increase in resistance to methoxychlor, and a small increase in resistance to lindane, chlordane, dieldrin, pyrethrum, and toxaphene. The methoxychlor strain was susceptible to DDT. Relatively slight resistance (about two-fold) was developed in the chlordane strain, and slightly more to lindane, dieldrin, and pyrethrum.

Busvine (1954) studied the toxicity of a group of chlorinated hydrocarbons to five strains of house flies, three of which were obtained from foreign countries. A record of the materials used to control the flies was available. Flies exposed to BHC and DDT (three locations) were resistant to chlordane, aldrin, and dieldrin. Those exposed to DDT and chlordane were also somewhat resistant to aldrin and dieldrin. He concluded that DDT, chlordane, aldrin, and dieldrin must act similarly because of cross-resistance. Cross-resistance of DDT and chlordane-treated flies to lindane was only moderately high. Isodrin and endrin showed even less cross-resistance.

Gilbert, Couch and McDuffie (1953) also (among others) found resistance to insecticides which had not been used on the population of resistant flies.

It is obvious that a test for cross-resistance may indicate whether or not the insecticides have a similar mode of toxic action. However, flies resistant to DDT did develop resistance to other chlorinated hydrocarbons very quickly. This could mean (1) that the modes of action of these insecticides are so closely related that development of resistance to one is a partial step in development of resistance to others, or (2) that development of a resistant strain also segregates the individuals which may develop resistance to other poisons quickly.

Lalonde and Brown (1954) studied the effects of these insecticides on the action potentials of insect nerves. DDT, TDE, and methoxychlor had generic similarities. Aldrin and dieldrin produced effects somewhat like DDT, but with a delayed action. Action from chlordane was even more delayed. Lindane produced a different type of effect. These observations are not inconsistent with the results of the tests reported here.

It seems obvious that determination of the type of toxic action by application of materials alone and in combination has not been successful in this study. A few combinations of materials appeared to produce higher toxicity than expected from similar action, as defined by Bliss (1939). However, if these combinations are to be used to prevent development of resistance, reinforcement of this information by physiological evidence is highly desirable.

The tests completed do not eliminate the possibility that the mode of action of these chlorinated insecticides is too close to similar to permit their use to prevent development of resistance.

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