RESISTANCE TO ANALOGS OF DDT IN LARVAE OF SUSCEPTIBLE AND RESISTANT STRAINS OF *CULEX TARSALIS* COQUILLETT [1]

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Among the methods suggested for circumventing resistance to DDT in insects are the use of combinations of DDT with synergists which block dehydrochlorination of the insecticide or the use of analogs of DDT which either cannot be dehydrochlorinated or else can be dehydrochlorinated only with considerable difficulty. Neither approach has proved satisfactory, primarily because many insect populations possess DDT resistance factors which do not involve dehydrochlorination of the insecticide.

Brown and associates (Brown 1964) demonstrated that in *Aedes aegypti* (L.) dehydrochlorination of DDT is the major mechanism of resistance to DDT and that deutero-DDT is an effective substitute for DDT against resistant strains of this species. However, more recently, Pillai and Brown (1965) showed that *A. aegypti* has other mechanisms of resistance to DDT as well.

Lipke and Chalkeley (1964) found evidence that DDT resistance in several species of anophelines is not related to ability to dehydrochlorinate DDT. Similar results were obtained with a strain of *Culex tarsalis* Coquillett that is highly resistant to DDT (Plapp *et al.* 1965). In contrast, Kimura *et al.* (1965) reported a higher level of DDT dehydrochlorinase in a moderately resistant than in a susceptible strain of the same species.

Recently, many analogs of DDT have been synthesized in the Chemistry Department of Fordham University. Preparation and properties of many of these materials have been described by Hennessy *et al.* (1961) and by Dachauer *et al.* (1963). This report presents the results of bioassays of some of these materials against larvae susceptible and resistant strains of *C. tarsalis*. We hoped to find materials related to DDT which might block resistance in *C. tarsalis* or which could serve as synergists for DDT. Although we failed to achieve our intended purpose, we are reporting our results in the hope that they may lead to a better understanding of the problem of resistance to DDT.

MATERIALS AND METHODS

INSECTS. The origin of the susceptible (S) and DDT-resistant (DDT-R) strains of *C. tarsalis* used in our experiments was recently described (Plapp *et al.* 1965). At the present time, the difference in response of larvae of the two strains to DDT is greater than 5,000-fold. This wide separation in response has been accomplished both by selection for greater resistance in the DDT-R strain and selection for greater susceptibility in the S strain. Selection in the DDT-R strain was achieved by exposing fourth instar larvae to DDT for 24–48 hours and continuing the strain from the survivors. Selection in the S strain was achieved by exposing a portion of the larvae from individual egg masses to low concentrations of DDT and saving the siblings of the most susceptible progenies to continue the strain.

INSECTICIDES. The DDT standard used was a sample of 99.3 percent *p,p'* obtained from the Geigy Chemical Co., Yonkers, N. Y. Most of the analogs tested were prepared at Fordham University, as previously described. Others were available in the Corvallis laboratory as received

[1] Mention of a company or its product does not necessarily imply endorsement of this company or products by the U. S. Department of Agriculture.

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TABLE 1.—Toxicity of DDT and a number of analogs to fourth instar larvae of susceptible (S) and DDT-resistant (DDT-R) strains of *Culex tarsalis*.

No.	Name [1]	Source [2]	24 hr LC_{50} (ppm) S	24 hr LC_{50} (ppm) DDT-R	Resistance ratio ($LC_{50}R \div LC_{50}S$)
1	DDT	A	0.0015	>10	>6667
2	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane-2-d (DDT-2d)	B	.0012	>10	>8333
3	Dicofol	C	>10	>10	...
4	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl-2-fluoroethane (DDT fluoride)	B	.2	>10	>50
5	1,1,1,2-tetrachloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT-chloride)	B	>10	>10	...
6	1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)-2-bromoethane (DDT bromide)	B	>10	>10	...
7	1,1,1-trichloro-2-(*p*-chlorophenyl-2-(4-chloro-2-fluorophenyl)ethane (*o*-fluoro DDT)	B	0.01	>10	>1000
8	1,1,1-trichloro-2-(*p*-chlorophenyl-2-(4-chloro-2-fluorophenyl)ethane-2-*d* (*o*-fluoro DDT-2d)	B	.008	>10	>1250
9	1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(2,4-dichlorophenyl)ethane (*o*-chloro DDT)	B	.008	>10	>1250
10	1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(2,4-dichlorophenyl)ethane-2-*d* (*o*-chloro DDT-2d)	B	.005	>10	>2000
11	1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)ethane (DFDT)	C	.03	>1	>33
12	1,1,1-trichloro-2,2-bis(*p*-bromophenyl)ethane (DBrDT)	C	.02	>1	>50
13	1,1,1-trichloro-2,2-bis(*p*-bromophenyl)ethane-2-*d* (DBrDT-2d)	B	.01	>1	>100
14	Methoxychlor	C	.01	>10	>1000
15	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane-2-*d* (Methoxychlor-2d)	B	.008	>10	>1250
16	1,1,1-trichloro-2-(2-chloro-4-methoxyphenyl)-2-(*p*-methoxyphenyl)ethane (*o*-chloro Methoxychlor)	B	.01	>10	>1000
17	TDE	C	.001	>10	>10,000
18	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane-1-*d* (TDE-1d)	B	.003	>10	>3333
19	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane-2-*d* (TDE-2d)	B	.0008	>10	>12,500
20	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane-1,2-d_2 (TDE-1,2-di-d)	B	.001	>10	>10,000
21	1,1-dichloro-2-(*p*-chlorophenyl)-2-(2,4-dichlorophenyl)ethane (*o*-chloro TDE)	B	.002	>10	>5000
22	4,4′-dichloro-*alpha*-dichloromethyl)benzhydrol (FW-152)	C	.6	>10	>16
23	1,1-dichloro-2,2-bis(*p*-chlorophenyl)-2-fluoroethane (TDE fluoride)	B	.006	1	>167
24	1,1-dichloro-2,2-bis(*p*-chlorophenyl)-2-fluoroethane-1-*d* TDE fluoride-1d	B	.003	>10	>3333
25	1,1,2-trichloro-2,2-bis(*p*-chlorophenyl)ethane (TDE chloride)	B	.03	>10	>333
26	4,4′-dichloro-*alpha*-(dichloromethyl)benzhydrol formate (2-formoxy TDE)	B	.2	1	5
27	4,4′dichloro-*alpha*-(dichloromethyl)benzhydrol acetate (2-acetoxy TDE)	B	>10	>10	...
28	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethyl hydroperoxide (TDE hydroperoxide)	B	0.2	0.4	2

TABLE 1.—Continued

No.	Name [1]	Source [2]	24 hr LC_{50} (ppm) S	24 hr LC_{50} (ppm) DDT-R	Resistance ratio ($LC_{50}R \div LC_{50}S$)
29	1,1-dichloro-2,2-bis(*p*-bromophenyl)ethyl hydroperoxide (Dibromo TDE hydroperoxide)	B	0.2	>10	>50
30	4,4′-dichloro-*alpha*-(chlorofluoromethyl)-benzhydrol (Fluoro TDE hydroxide)	B	6	6	1
31	1,2-dichloro-2,2-bis(*p*-chlorophenyl)-1-fluoroethane (Fluoro TDE chloride)	B	3	>10	>3
32	4,4′-dichloro-*alpha*-(bromochloromethyl)-benzhydrol (Bromo TDE hydroxide)	B	2	>10	>5
33	1-bromo-1-chloro-2,2-bis(*p*-chlorophenyl)-2-fluoroethane (Bromo TDE fluoride)	B	.002	>10	>5000
34	1-bromo-1,2-dichloro-2,2-bis(*p*-chlorophenyl)-ethane (Bromo TDE chloride)	B	2	>10	>5
35	1,2-dibromo-1-chloro-2,2-(*p*-chlorophenyl) ethane (Bromo TDE bromide)	B	2	>10	>5
		B	.08	>10	>125
36	1,2-dichloro-1,1-bis(*p*-chlorophenyl)propane (Methyl TDE chloride)	B	.08	>10	>125
37	2-chloro-1,1-bis(*p*-chlorophenyl)ethyl methyl ether (Methyl TDE methoxide)	B	.1	>10	>100
38	K3926® (1,1-bis(*p*-chlorophenyl)ethane)	C	3	>3	>1
39	Dimite® (4,4′-dichloro-*alpha*-methylbenzhydrol)	C	>1	>1	...
40	Prolan® (1,1-bis(*p*-chlorophenyl)-2-nitropropane)	B	.006	3	500
41	1,1-bis(*p*-chlorophenyl)-2-nitropropane-1-*d* (Prolan-1d)	B	.003	4	1333
42	1,1-bis(*p*-chlorophenyl)-2-nitropropane-2-*d* (Prolan-2d)	B	.006	>10	>1667
43	1,1-bis(*p*-chlorophenyl)-2-nitropropane-1,2-d_2 Prolan-1,2 di-d)	B	.006	>10	>1667
44	1,1,1-trifluoro-2,2-bis(*p*-chlorophenyl)ethane (TFDDT)	A	.6	6	10
45	1,1,1-trifluoro-2,2-bis(*p*-chlorophenyl)ethane-2-*d* (TFDDT-2d)	B	.6	6	10
46	1,1-bis(*p*-methoxyphenyl)-2,2-dimethyl propane (DANP)	B	.06	6	100

[1] Approved common proprietary names are followed by chemical definitions; when no approved short name is available, the chemical name is followed by a short form in parenthesis used only for discussion in the text.

[2] Compounds obtained as follows: A—analytical sample from Geigy Co., Yonkers, N. Y.; B—prepared in laboratory of D. J. Hennessy, Fordham University, New York; C—obtained from Pesticides Chemicals Research Branch, USDA, Beltsville, Maryland.

from the manufacturer or were obtained from the Pesticide Chemicals Research Branch of the Entomology Research Division, Agricultural Research Service, Beltsville, Maryland.

MEASUREMENT OF TOXICITY. We measured the toxicity of the various analogs of DDT to larvae of the S and DDT-R strains of *C. tarsalis*. Also, 1:1 combinations of DDT and the various analogs were tested against the DDT-R strain to see whether any of the analogs were synergistic in combination with DDT. Desired amounts of the various insecticides in acetone solution were pipetted into ½-liter (one-pint) glass jars, and the solvent was evaporated. Two hundred and fifty ml of distilled water was then poured into the jars, and 20–25 fourth instar larvae of the appropriate strain were added. The jars were

held at 21–25° C., and mortality was determined 24 hours after initial exposure. Usually in the initial tests we tried several concentrations and one replicate per dose. If a material was toxic to larvae of either strain, we conducted further tests at lower concentrations with two or more replicates per test. The LC_{50} values were determined from plots of mortality data on LDP paper.

RESULTS

About 100 materials were tested. Names, structures, toxicity of DDT and 45 analogs, and resistance ratios (LC_{50} DDT-R/LC_{50} S), are shown in Table 1. The materials listed are representative of all types of analogs tested and are a complete series of several types.

Many materials were effective as insecticides, that is, they were highly toxic to larvae of the S strain. All effective materials possessed a minimum of 1 hydrogen or 1 deuterium on the central ethane bridge. TDE, 2-deutero TDE, and 1,2-dideutero TDE were the only materials more toxic than DDT. In general, the substitution of deuterium for hydrogen on carbon #2 of DDT and several related materials caused a distinct, but usually less than 2-fold, increase in toxicity. Similar increases in toxicity for the same materials have been reported previously (Barker 1960, Dachauer *et al.* 1963).

There was almost no correlation in response to the various toxicants between the S and DDT-R strains. With nearly all analogs, the LC_{50} values for the DDT-R larvae were greater than the highest concentration of analog tested, one ppm in some cases and 10 ppm in others. "Resistance," as measured by the ratio of the response of the two strains, ranged to >10,000-fold though there were several exceptions. Thus, the Prolan series, (compounds 40–43), all of which were highly toxic to S larvae, were in several instances measurably toxic to DDT-R larvae. TFDDT and TFDDT-2d, though they had relatively low toxicity to S larvae, produced a measurable response in DDT-R larvae, as did compound 31, the hydroxide of a fluoro-substituted TDE.

The only materials tested which were of similar and measurable toxicity to larvae of the S and DDT-R strains were the several analogs of TDE in which the hydrogen atom on carbon #2 was replaced by more polar substituents. For example, 2-formoxy TDE (compound 26) was moderately toxic to S larvae and one-fifth as toxic to DDT-R larvae, and TDE hydroperoxide (compound 28) was half as toxic to DDT-R as to S larvae. However, these materials were not sufficiently toxic for use as practical substitutes for DDT.

The results obtained when we measured the toxicity of 1:1 combinations of DDT and the various analogs are not listed because no synergism was ever observed. Even with TDE hydroperoxide, combinations of this material and DDT at 1:1 ratios or at other ratios failed to give any evidence of synergism. Similarly, tests with piperonyl butoxide and other methylenedioxyphenyl synergists in combination with DDT also gave no evidence of synergism. To date, we have found no chemicals which increase the toxicity of DDT to larvae of the DDT-R strain.

DISCUSSION

Our results strongly suggest that resistance to DDT in the strain of *C. tarsalis* studied is caused by an unknown mechanism or mechanisms and does not involve dehydrochlorination of the insecticide. In many instances, resistance was as high to nondehydrochlorinatible analogs as to DDT itself. The lack of synergism with any combination of DDT and its analogs or, for that matter with other synergists, suggests a type of resistance not directly associated with an increased rate of detoxication of DDT.

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