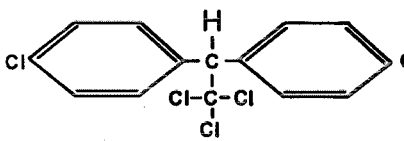
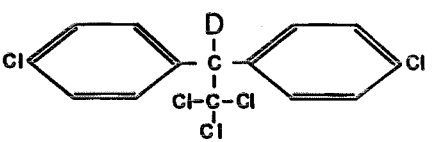
DEUTERATED ANALOGUES AS REMEDIAL INSECTICIDES AGAINST DDT-RESISTANT *AEDES AEGYPTI*

M. K. K. PILLAI,[1] D. J. HENNESSY [2] AND A. W. A. BROWN [1]

It has become evident that a modification of the DDT molecule which would render it less open to detoxication is a desirable countermeasure for DDT-resistant insects; such a modification is offered in its deuterated analogue. The structure of the compound 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane-2-d (DDT-2-d) differs from that of DDT only in having an atom of deuterium in place of an atom of hydrogen at the tertiary carbon, C(2). The physical properties of DDT and DDT-2-d are identical except for their



DDT



Deutero-DDT

infrared and proton magnetic resonance spectra (Dachauer *et al.*, 1963). But DDT-2-d reacts more slowly than DDT in the processes involving the production of DDE and HCl induced by alcoholic KOH, or by bromine and light in the absence of oxygen (Dachauer, 1961). These reactions are particular examples of the deuterium isotope rate effect (Wiberg, 1955) which is explained by a zero point energy lower for a bond to deuterium than for a bond to hydrogen, otherwise identical. Thus the reactions in which the C-D bond rather than the equivalent C-H bond is broken have a higher activation energy and proceed at a slower rate.

When DDT-2-d (deutero-DDT) was tested on house flies, it proved to be only slightly more toxic than DDT to susceptible and to resistant strains (Moorefield, Weiden and Hennessy, 1962); nor was there any significant difference between DDT and deutero-DDT in the rates at which they were dehydrochlorinated to DDE *in vivo* by resistant house flies (Barker, 1960). Moreover, deutero-DDT proved almost as ineffective as DDT against a DDT-resistant strain of *Culex tarsalis* (Plapp, 1963).

The purpose of the following studies was to ascertain whether deutero-DDT was insecticidal to DDT-resistant strains of *Aedes aegypti*. Deuterated analogues of compounds to which there was no cross-resistance (such as prolan), or slight cross-resistance (such as o-chloro-DDT), depending upon their liability to dehydrochlorination, were also compared for toxicity. The second step was to submit a normal strain of *A. aegypti* to selection with deutero-DDT to find out whether resistance would develop to deutero-DDT or to DDT. A parallel selection experiment was also performed with deutero-prolan for comparison with prolan selection.

MATERIAL AND METHODS. A total of 11 resistant strains and 3 susceptible strains were used in the tests with deutero-DDT. The susceptible strains consisted of the following: Guelph S, developed from a single Orlando female; Penang S, obtained from Penang, Malaya; Trinidad S, an isolate from Trinidad R material.

[1] Department of Zoology, University of Western Ontario, London, Canada.
[2] Department of Chemistry, Fordham University, New York 58, N. Y.

The resistant strains were from only 3 ultimate sources. The 4 Trinidad R strains were those under different degrees of DDT selection. The 5 Penang R strains had been selected with both DDT (1), malathion (2) and a mixture of Butyl-antiresistant and DDT in 1:1 ratio (2 strains, see previous paper). The 2 Isla Verde strains, originated from Puerto Rico and kept under different degrees of dieldrin and DDT selection, were resistant to dieldrin as well as DDT.

All susceptibility tests were performed on larvae in the late 3rd or early 4th stadium, using the WHO standard method for mosquito larvae. Tests were made with DDT and deutero-DDT on 15 strains, with prolan and deutero-prolan on 4 strains, and with o-chloro-DDT and deutero-o-chloro-DDT on 4 strains.

The selection procedure also involved larvae of similar age, and was performed in the 16-oz. wide-mouth glass jars with 250 c.c. water similar to those used for the susceptibility tests, with a 24-hour exposure period. The dosage was adjusted in each generation to give a selection pressure of approximately 90 percent mortality. Each generation was tested for its susceptibility levels. The strain submitted to selection was the Trinidad S, a susceptible strain bearing the genetic marker *black-tarsus* and *yellow* (larva) derived as an isolate from the Trinidad resistant strain. Selections were made with deutero-DDT for 5 generations, and a parallel series with DDT for 6 generations. In another experiment, selections were made with deutero-prolan for 6 generations, being paralleled with a similar series of selection with prolan.

The deutero-DDT [2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane-2-d] was prepared according to the method of Dachauer *et al.* (1963), and had a melting point of 108–109.5° C., as compared with 109–110° C. for p,p'-DDT. The deutero-prolan (Salomone, 1963) and deutero-o-chloro-DDT (Hartigan, 1963) were made by similar methods.

Dehydrochlorination of deutero-DDT *in vivo* was determined by exposing 100 larvae to 1 p.p.m. deutero-DDT in four 16-oz. glass jars for 24 hours. After being allowed to clean themselves in fresh water for 20 minutes, they were ground with Na_2SO_4 and sand and extracted in 50 ml. CCl_4. The extract was passed through a column of Woelm aluminum oxide (cationotropic) and the eluate was freed of solvent by evaporation. Determination of DDE and deutero-DDT by ultra-violet spectroscopy was made in cyclohexanone solution at 241 and 260 millimicrons (Brown, 1956; Sternburg, Kearns and Moorefield, 1954); the absorption maximum for deutero-DDT had been found to be 236 millimicrons, as with DDT (Barker, 1960). Determination of these compounds by the Schechter-Haller method proceeded according to the operations employed for mosquito larvae by Chattoraj and Brown (1960). Dehydrochlorination of DDT *in vivo* was determined by using ring-labelled C^{14}-DDT (obtained from Tracerlab, Inc.), the larval extracts then being submitted to paper chromatography according to the method of Robbins as employed for *A. aegypti* by Abedi, Duffy and Brown (1963), and scanned for radioactivity in the two fractions (DDT and DDE) obtained. Dehydrochlorination of deutero-DDT and DDT *in vitro* was investigated by incubating homogenates according to the method of Brown (1956)—these homogenates having been prepared at 0° C. and with added glutathione—and determining the metabolites by the Robbins chromatographic method.

RESULTS. Deutero-DDT proved to be 1.5 to 2 times as toxic as DDT to the Trinidad S and Guelph S strains, and 6 times as toxic as DDT to the somewhat-tolerant Penang S strain (Table 1). The striking discovery was that deutero-DDT was 50 to 100 times as toxic as DDT to the 9 DDT-resistant strains. To the 2 strains resistant to Antiresistant-DDT mixture, deutero-DDT was 20 times as toxic as DDT. The susceptibility levels for the 9 DDT-resistant strains to deutero-DDT show close correlation with those of DDT ($r=0.97$) when plotted on logarith-

TABLE 1.—LC_{50} levels in p.p.m. of resistant and susceptible strains to DDT and to deutero-DDT.

Strain	DDT	Deutero-DDT
Trinidad R_4	31.0	0.40
Trinidad R_3	9.0	0.12
Trinidad R_2	5.0	0.11
Trinidad R_1	2.1	0.041
Trinidad S	0.013	0.008
Penang R	3.7	0.075
Penang Malathion-R_2	3.0	0.037
Penang Malathion-R_1	1.9	0.030
Penang S	0.065	0.011
Antiresistant R from Penang R	5.0	0.21
Antiresistant R from Penang S	1.35	0.07
Isla Verde R_2	1.3	0.019
Isla Verde R_1	0.8	0.016
Guelph S	0.012	0.004

mic paper (Fig. 1); those for the 2 Antiresistant R strains show a parallel regression, but with the LC_{50} for deutero-DDT at a higher level.

The proportions of deutero-DDT dehydrochlorinated to DDE *in vivo* (Table 2) were approximately 10 percent for 2 susceptible strains, and approximately 20 percent for the 2 resistant and the Penang S strains; DDE was evidently the only metabolite produced judging from the paper chromatograms obtained from the exracts by the Robbins method. The dehydrochlorination of DDT as compared to deutero-DDT accomplished in 24 hours by the last 3 strains was determined by the use of C^{14}-DDT and paper chromatography to be as follows:

	Penang S	Penang R	Trinidad R
Percent DDE from DDT	44.9	47.6	41.7
Percent DDE from Deutero-DDT	20.3	18.9	18.2

The dehydrochlorination of deutero-DDT to DDE *in vitro* was determined for DDT-dehydrochlorinase preparations from the Trinidad R strain by Mr. T. Kimura; he found that the amount of deutero-DDT converted to DDE in three hours was only 1.6 percent, while the amount of DDT converted by similar preparations under identical conditions was 19.3 percent.

Deutero-prolan (Table 3) proved to be only slightly more toxic than prolan to the susceptible and DDT-resistant strains. Indeed the DDT-resistant strains were only slightly (3 to 5 times) more resistant to prolan than their susceptible counterparts. With o-chloro-DDT the situation was different; here the DDT-resistant strains were appreciably (20 to 40 times) cross-resistant to o-chloro-DDT, and to these strains deutero-o-chloro-DDT proved to be definitely more toxic than o-chloro-DDT itself.

Deutero-DDT selection of the Trinidad S strain (Fig. 2) increased the tolerance by only 4 times, a stable level which was virtually reached in the 1st generation of selection; the selecting dosage (Table 4) in fact had to be decreased in the F_4, but even then it allowed insufficient survivors to proceed from the F_5 to the F_6. When tested for its cross-resistance to DDT, this deutero-DDT strain was found to have reached a stable level of 70-fold DDT-resistance. Parallel selection of the Trinidad S with DDT itself, on the other hand, increased the DDT-resistance by over 1000 times.

Deutero-prolan selection of the Trinidad S strain (Fig. 3) increased the tolerance by only 3 times, and the level reached was stable. The cross-resistance thus developed to DDT reached a stable level of 14-fold DDT-resistance. Prolan selection of the Trinidad S strain increased the prolan-tolerance by 4 times, but the cross-resistance to DDT increased by 35 times.

DISCUSSION. The close correlation between the degree of DDT-resistance and the LC_{50} for deutero-DDT in the various strains of *A. aegypti* tested suggests that one and the same detoxication mechanism is involved, which is far weaker for deutero-DDT than for DDT. This mechanism is logically dehydrochlorination, which would determine the far greater susceptibility of resistant strains to deutero-

TABLE 2.—DDE production per 100 larvae of susceptible and resistant strains from exposure to 1 p.p.m. Deutero-DDT for 24 hours.

Strain	LC_{50} (p.p.m.) Deutero-DDT	Micrograms DDE produced			Av. DDT micrograms	Percent conversion
		U.-V.	Sch.-Haller	Average		
Guelph S	0.004	0.97	2.43	1.70	12.82	11.7
Trinidad S	0.008	0.37	0.52	0.40	5.95	6.3
Penang S	0.011	1.45	6.21	.83	15.02	20.3
Penang R	0.10	8.45	2.34	.40	23.18	18.9
Trinidad R	0.40	2.28	1.60	1.94	8.71	18.2

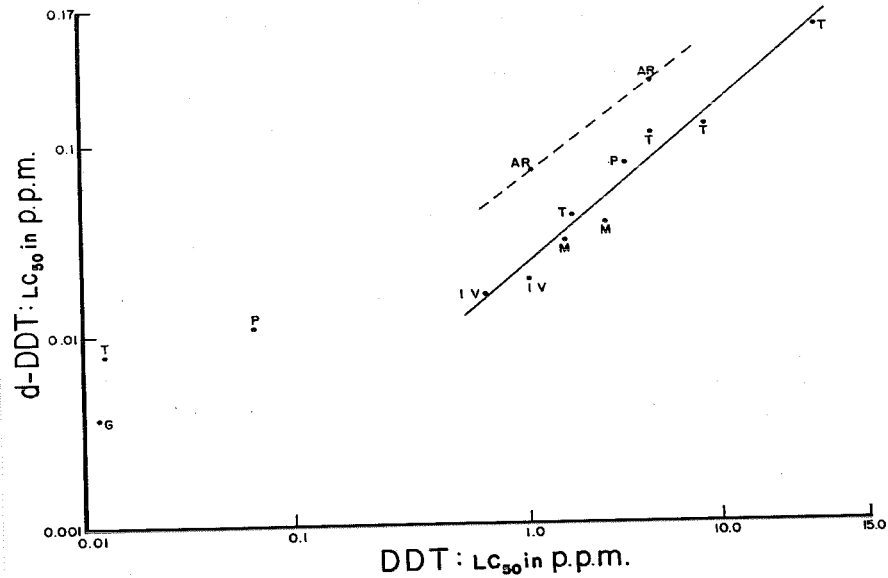


FIG. 1.—LC_{50} levels for deutero-DDT plotted against those for DDT, for the Trinidad (T), Penang (P), Malathion (M), Isla Verde (I.V.), Guelph (G) and Antiresistant (AR) strains mentioned in Table 1.

TABLE 3.—LC_{50} levels in p.p.m. of resistant and susceptible strains to prolan and o-chloro-DDT, and their Deutero analogues.

	o-Chloro-DDT	Deutero-o-Chloro-DDT	Prolan	Deutero-prolan
Trinidad R	2.4	1.6	0.15	0.14
Trinidad S	0.062	0.054	0.042	0.038
Penang R	2.7	1.8	0.195	0.175
Penang S	0.125	0.05	0.036	0.031

TABLE 4.—Susceptibility levels and selecting dosages in p.p.m. for successive generations selected by DDT and prolan and their Deutero analogues.

	DDT		Deutero-DDT		Prolan		Deutro-Prolan	
Generation	Selecting dosage	LC_{50}	Selecting dosage	LC_{50}	Selecting dosage	LC_{50}	Selecting dosage	LC_{50}
P	..	0.013	..	0.008	..	0.042	..	0.038
	0.07	..	0.04	..	0.12	..	0.10	..
F_1	..	0.11	..	0.026	..	0.062	..	0.045
	0.64	..	0.10	..	0.15	..	0.12	..
F_2	..	1.1	..	0.030	..	0.11	..	0.094
	6.0	..	0.12	..	0.25	..	0.24	..
F_3	..	4.5	..	0.035	..	0.14	..	0.12
	20.0	..	0.14	..	0.64	..	0.40	..
F_4	..	12.5	..	0.037	..	0.17	..	0.13
	41.0	..	0.10	..	0.50	..	0.32	..
F_5	..	14.4	..	0.036	..	0.17	..	0.12
	50.0	..	..	..	0.40	..	0.25	..
F_6	..	15.0	..	..	..	0.16	..	0.13

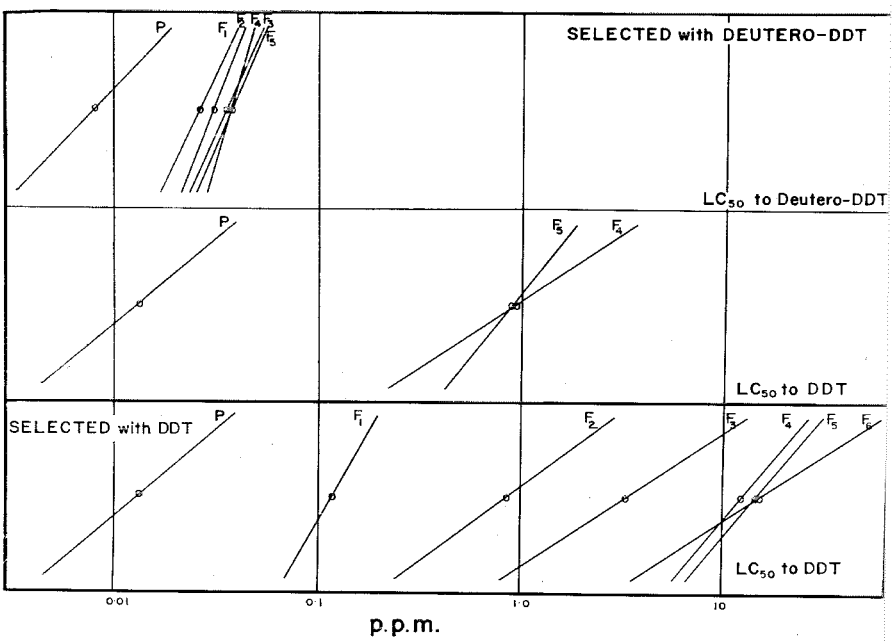


FIG. 2.—Selection of the Trinidad S strain with deutero-DDT: LC_{50} levels to deutero-DDT and the resulting cross-resistance to DDT, with results of DDT selection on bottom line for comparison.
N.B. The dosage-mortality lines extend from 20 to 80 percent mortality.

DDT than to DDT, just as it determines their level of DDT-resistance.

When the amount of dehydrochlorination *in vivo* is determined, it is found that a considerable amount of DDE is produced from deutero-DDT; but this is considerably less than that found to be produced from DDT by the same strains (Chattoraj and Brown, 1960), the amounts in micrograms being as follows:

	from Deutero-DDT	from DDT
Trinidad S	0.4	1.0
Penang S	3.7	5.2
Penang R	5.4	8.8
Trinidad R	1.9	8.0

Moreover, the percentage conversion of deutero-DDT by the two resistant strains averages less than 20 percent, whereas that of DDT averages 45 percent. This pro-

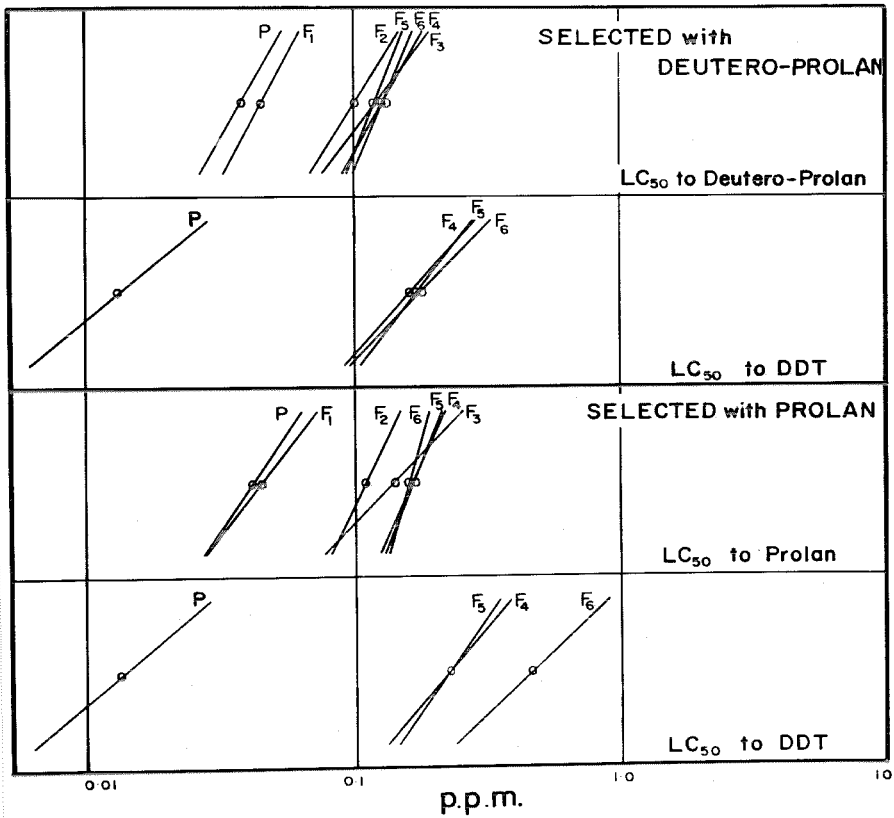


FIG. 3.—Selection of the Trinidad S strain with deutero-prolan: LC_{50} levels to deutero-prolan and the resulting cross-resistance to DDT, with results of prolan selection on lower 2 lines for comparison. N.B. The dosage-mortality lines extend from 28 to 72 percent mortality.

vides evidence that the isotopic effect of deuterium substitution in DDT does decrease its dehydrochlorination, but the difference seems insufficient to account for the unusual susceptibility of DDT-resistant strains to deutero-DDT. However there is a real difference in the lability of deutero-DDT, as compared with DDT, to dehydrochlorination by enzyme preparations *in vitro* from DDT-resistant larvae.

Further evidence that stability to dehydrochlorination is the factor involved in the superior toxicity of deutero-DDT is given by the data with deutero-prolan. Here is a non-dehydrochlorinatable insecticide, and here the isotopic effect of deuterium substitution is naturally non-operative. On the other hand, with o-chloro-DDT which is partially labile to dehydrochlorination (Abedi, Duffy and Brown, 1963), deuterium substitution does increase the toxicity by about 50 percent to the resistant strains with their significant cross-resistance to o-chloro-DDT. With DDD, which is freely dehydrochlorinatable, deutero substitution was found to increase the toxicity by 5 times, the LC_{50} to the Penang Malathion-R_1 strain being 2.0 p.p.m. DDD and 0.40 p.p.m. deutero-DDD.

It is remarkable that deutero-DDT shows such high toxicity to DDT-resistant *A. aegypti* mosquitoes, while it does not show it to DDT-resistant house flies. In house flies, the addition of Cl or Br in the ortho position greatly increased the toxicity of DDT to resistant strains, but the toxicity of the o-Cl-DDT or o-Br-DDT is not further increased when these compounds are deuterated. The DDT-resistant larvae of *A. aegypti* present the remarkable circumstance of retaining considerable resistance to o-Cl-DDT and o-Cl-DDT-2-d but of being quite unresistant to DDT-2-d. The steric effect of the ortho-chlorine somewhat increased lethality to the resistant strains and the added introduction of deuterium at C(2) increased the lethality still more. DDT-2-d with deuterium at C(2) but with no ortho-chlorine is however by far the most effective material.

In house flies, the resistant strains appear to dehydrochlorinate deutero-DDT to DDE as readily as DDT itself (Barker, 1960); this is not the case with resistant *A. aegypti* larvae *in vivo*, and certainly not with their enzyme preparations *in vitro*. Already a number of differences are known between the properties of *A. aegypti* dehydrochlorinase and that of the house fly: the mosquito enzyme is far more sensitive to lack of glutathione in the preparatory processes than that of the house fly; the mosquito DDT-dehydrochlorinase is inhibited by TPNH whereas that of the house fly is not; and the mosquito enzyme can dehydrochlorinate o-chloro-DDT, giving a cross-resistance to this DDT analogue which is not found in the house fly (Hennessy *et al.*, 1961). It would seem not unlikely that a fourth difference could be the comparative inability of the mosquito enzyme to overcome the isotopic effect of deuterium and dehydrochlorinate deutero-DDT.

In view of the finding of Plapp (1963) that deutero-DDT was scarcely toxic to DDT-resistant *Culex tarsalis*, tests were performed in our laboratory on the Oakridge DDT-resistant strain of this mosquito. They revealed that deutero-DDT had an LC_{50} of 0.21 p.p.m. for the resistant larvae, as compared to an LC_{50} of 3.0 p.p.m. for DDT. The normal Corvallis strain of *C. tarsalis* was so susceptible to DDT that the larval LC_{50} was only 0.000045 p.p.m.; even then, the LC_{50} of deutero-DDT was even lower, approximately 0.000025 p.p.m. The studies of Mr. T. Kimura indicate that in *C. tarsalis* larvae the *in vitro* DDT-dehydrochlorination is proportional to the DDT-resistance to much the same extent as in *A. aegypti*.

The results of the selection experiments on *A. aegypti* suggest that whereas deutero-DDT is capable of selecting for some degree of DDT-resistance, it is incapable of selecting for a resistance to deutero-DDT. Selection with deutero-prolan is unable to select for DDT-resistance, but selection

with prolan can increase the level of DDT-resistance, as Dilan selection can in house flies (Brown, 1956). It is tempting to hope that deutero-DDT cannot select for resistance to itself because the mechanism for its dehydrochlorination against its isotopic effect lies outside the genotype of *Aedes aegypti*. At present it appears to be one remedial insecticide for DDT, with much the same physical and chemical properties as DDT for ease of formulation, to which resistance will probably not develop.

SUMMARY. Deutero-DDT, an insecticide in which the hydrogen on the tertiary carbon atom of DDT has been replaced by deuterium, was found to be highly toxic to larvae of all the 11 DDT-resistant strains of *Aedes aegypti* tested. It proved to be less readily dehydrochlorinated *in vivo*, and much less readily dehydrochlorinated *in vitro*, than DDT; this stability can be attributed to the isotopic effect of the deuterium substitution. Selection of a susceptible strain with deutero-DDT for 5 generations caused a slight increase in DDT-tolerance in the F_1, but thereafter no resistance developed. Experiments with deutero-prolan and deutero-o-chloro-DDT supported the idea that DDT-resistance depends on a dehydrochlorination to which deuterated analogues are more stable.

This work was performed with the financial assistance of the Canadian Commonwealth Fellowships Fund, which supported the senior author, and the National Institutes of Health, which supported the research facilities utilized.

References

ABEDI, Z. H., DUFFY, J. R., and BROWN, A. W. A. 1963. Dehydrochlorination and DDT-resistance in *Aedes aegypti*. Jour. Econ. Ent. 56:(in press).

BARKER, R. J. 1960. Syntheses of the aliphatic deuterium analogs of DDT and TDE and their toxicity and degration when applied to adult houseflies. Jour. Econ. Ent. 53:35–41.

BROWN, A. W. A. 1956. DDT-dehydrochlorinase activity in resistant houseflies and mosquitoes. Bull. Wld. Hlth. Org. 14:807–12.

CHATTORAJ, A. N., and BROWN, A. W. A. 1960. Internal DDE production by normal and DDT-resistant larvae of *Aedes aegypti*. Jour. Econ. Ent. 53:1049–1051.

DACHAUER, A. C. 1961. The deuterium isotope rate effect in free reactions of t-carbon-deuterated DDT and related compounds. Ph.D. Dissertation, Fordham University.

DACHAUER, A. C., COCHEO, B., SOLOMON, M. G., and HENNESSY, D. J. 1963. The synthesis of tertiary carbon deuterated DDT and DDT analogs. Agric. Food Chem. 11:47–50.

HARTIGAN, J. 1963. The synthesis and toxicity to houseflies of ortho substituted analogs of DDT. Ph.D. Dissertation, Fordham University.

HENNESSY, D. J., FRATANTONI, J., HARTIGAN, J., MOOREFIELD, H. H., and WEIDEN, M. H. J. 1961. Toxicity of 2-(2-halogen-4-chlorophenyl)-2-(4-chlorophenyl)-1, 1, 1-trichloroethanes to normal and to DDT-resistant house flies. Nature 190:341.

MOOREFIELD, H. H., WEIDEN, M. H. J., and HENNESSY, D. J. 1962. Relationship of the insecticidal and the free radical activities of DDT. Contrib. Boyce Thompson Inst. 21:481–486.

PLAPP, F. W. 1963. Personal communication to D. J. H., January 18.

SALOMONE, R. A. 1963. The synthesis and toxicity of some prolan analogs. Ph.D. Dissertation, Fordham University.

STERNBURG, J., KEARNS, C. W., and MOOREFIELD, H. H. 1954. DDT-dehydrochlorinase, an enzyme found in DDT-resistant flies. Agric. Food Chem. 2:1125–1130.

WIBERG, K. B. 1955. The deuterium isotope effect. Chem. Rev. 55:713–743.