CONVERSION OF DDT INTO DDE BY THE LARVAE OF AEDES AEGYPTI (L.) STRAIN PCSIR. DIPTERA—CULICIDAE

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Different strains of *Aedes aegypti* are known to convert DDT into lower homologue DDE. studies were made to assess how much known amount of DDT is converted into DDE by *Aedes aegypti* strain PCSIR Karachi. Third or fourth instar larvae of *Aedes aegypti* strain PCSIR collected from Clifton area were exposed to 1 ppm. DDT for 24 hours. Conversion of DDT into DDE in this strain was found higher than the reported ones.

Introduction

Several workers have reported the internal conversion of DDT into DDE by different strains of *Aedes aegypti* larvae. This fact was first investigated by Brown and Perry.[1] They reported that the larvae of DDT resistant *Aedes aegypti* and *Aedes taeniorhynchus* (Wied) produced more DDE from DDT, while normal larvae of these species produced little amount of DDE. It is also reported by Tahori and Hoskins [2] that certain strains of DDT resistant flies are able to change absorbed DDT into the relatively nontoxic compound DDE, presumably by a process of dehydrohalogenation. In addition Sternburg, Kearns and Moorefield [3] have reported that all DDT-resistant flies possess an enzyme which was not found in measurable quantities in susceptible strains. According to them this enzyme is responsible for DDT-resistance. Hoskins, Miskus and Eldefrawi[4] have also reported that larvae of normal *Culex quinquefasciatus* produced a certain amount of DDE, while resistant larvae almost completely metabolized the DDT dose to DDE. Bami, Sharma and Kalra[5] have reported that resistant adults of *Culex fatigans* (Wied) produced more DDE than normal adults. Frontali and Carta[6] also reported that adults of DDT-resistant strain of *Anopheles atroparvus* van Thiel developed by laboratory pressure produced twice as much DDE as the normal strain. Perry[7] found that adults of a DDT-resistant Turkish strain of *Anopheles sacharovi* Favre, produced large amounts of DDE at high doses of DDT. Uptill now no report has been published about the conversion of DDT into DDE by the strain of *Aedes aegypti* larvae found in Pakistan, therefore, it was decided to study it and the present work deals with the internal conversion of DDT into DDE by the larvae of *Aedes aegypti* found in Pakistan.

Material and Method

Aedes aegypti were collected from Clifton area of Karachi and reared in the laboratory. These mosquitoes were named as PCSIR strain of *Aedes aegypti*. The adult mosquitoes were kept in $16\frac{1}{2}'' \times 12'' \times 12\frac{1}{2}''$ wooden cages, covered with 20 mesh and one side with a long sleeve of muslin cloth for feeding purpose. The eggs were collected on a wet filter paper strip having the size $6'' \times 2''$ kept in dishes containing water. The egg strips were then transferred in big dishes (170×90mm.) and filled with 500 ml. water. The larvae hatched after 48 hours under normal temperature and humidity *i.e.*, $75 \pm 5°$F. and 70%-80% respectively. The water used was treated with streptomycin. The larvae were given a diet of Brewer's yeast and blood albumin in a ratio of 9:1. The readings were taken on a Beckman DB Spectrophotometer.

Standard Graph of DDT.—Ofner and Calvery[8] method was followed to prepare standard solutions of DDT. The concentrations of 10, 20, 30, 40 and 50 micrograms of DDT per ml. were prepared in ether. One ml. of each of the solutions was taken out in separate test tubes which was evaporated on a steam water bath. After evaporation, 2 ml. of the nitration mixture (1:1 $HNO_3 + H_2SO_4$ concentrated) were added to each tube according to Schechter and Haller's[9] method and the test tubes were placed in a hot water bath for heating. After 60 minutes of heating, the test tubes were removed from the water bath and allowed to cool. When cooled to room temperature, 20 ml. of ice-

cold distilled water were added to each tube which was then transferred to a separatory funnel. The residual content of the tube was washed into the separatory funnel with another 20 ml. of distilled water and finally washing was done three times each with 10 ml. portions of ether. The funnel was shaken vigorously so that all the nitration products were transferred in the ether layer. The lower aqueous layer was removed and discarded. Then 10 to 15 ml. of 2% sodium hydroxide solution were added in the separatory funnel. After vigorous shaking it was allowed to stand until twolayers were separated completely. The lower layer was discarded again. The washing with sodium hydroxide was repeated three times.

Again 10 to 15 ml. of saturated solution of sodium chloride (saturated at room temperature) were added in the separatory funnel, which was shaken vigorously and allowed to stand for the separation of two layers. The lower layer was discarded by washing it twice. The last separation was done as completely as possible without loss of the ether solution and then it was transferred through a dried cotton plug held in a small Bunsen funnel into a dry Erlenmeyer flask. The cotton should be moistened with ether before filtration. The separatory funnel was rinsed several times with ether and filtered into the flask. The Erlenmeyer flask was placed on a hot water bath to allow evaporation of the ether solution.

Now 2 ml. of benzene and 4 ml. of 10% sodium methylate solution were added to the dry residue for the development of the colour. The blue colour developed to a maximum in 10 minutes, and the readings were taken at a wavelength of 600 mμ on a Beckman DB Spectrophotometer. The reading should be taken within 10-15 minutes time before the colour fades away. Blank readings were deducted from the final readings.

MEAN READINGS FOR DDT GRAPH (Fig. 1)

Concentration	Optical density
10 Micrograms	.11
20 ,,	.20
30 ,,	.30
40 ,,	.40
50 ,,	.50

Standard Graph of DDE.—Standard solutions of DDE were prepared in ether at concentrations of 5, 10, 20, 30, 40 and 50 micrograms of DDE per ml. One ml. from each of these solutions was taken out in separate test tubes. The solutions in the tubes evaporated completely on a hot watr bath. 2 ml. of nitrating mixture were added to each tube and placed on a hot water bath for about 60 minutes. From here the rest of the DDT process was followed.

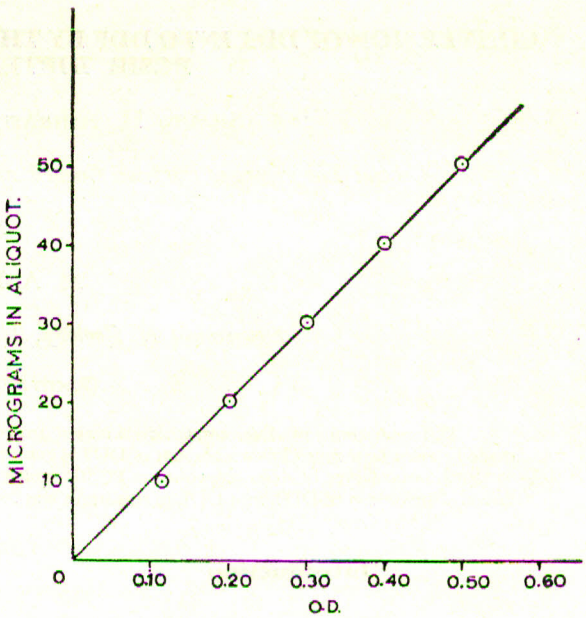

Fig. 1.—Standard graph of DDT.

The colour developed to a maximum in 5 to 10 minutes and readings were taken at a wavelength of 520 mμ on a Beckman DB Spectrophotometer. Blank readings were deducted from the final readings.

MEAN READINGS FOR DDE GRAPH (FIG. 2.)

Concentration	Optical density
5 micrograms	.03
10 ,,	.65
20 ,,	.12
30 ,,	.175
40 ,,	.24
50 ,,	.31

Experimentatal

Treatment with 1 ppm DDT.—100 larvae of *Aedes aegypti* in the late third or early fourth instar were treated with 1 ppm DDT for 24 hours at a temperature of 81°F. in 1250 ml. of distilled water. After 24 hours the treated larvae were transferred in a beaker containing 150 ml. of fresh water.

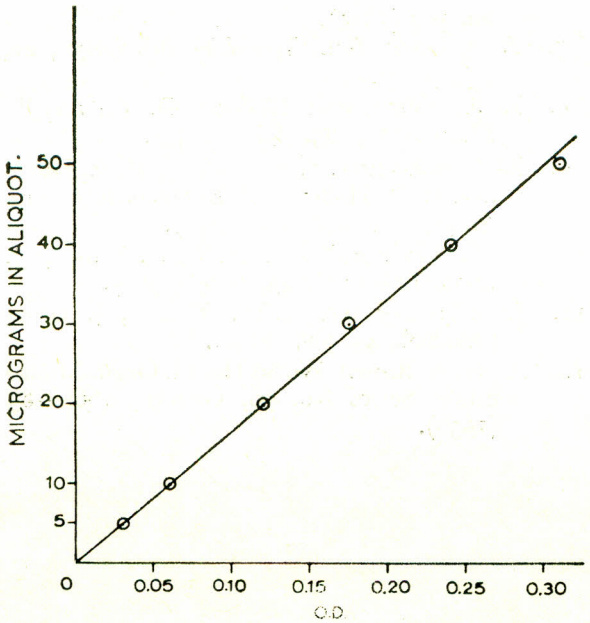

Fig. 2.—Standard graph of DDE.

Again larvae were rinsed with 100 ml. of fresh water and they were put on an absorbent paper for drying. After drying the larvae were ground in a powder form in a mortar by adding some sea sand and anhydrous sodium sulphate. The powder was extracted with a total of 50 ml. of carbon tetrachloride which was poured through a column of adsorption alumina (Fisher) 6 cm. long and 13 mm. in diameter. [10,11]

The extracted carbon tetrachloride was collected in a conical flask for evaporation on a steam bath for drying the extract. 5 ml. of nitrating mixture were added to the dry residue which was kept on a hot water bath for the nitration process upto 3 hours. After nitration 25 ml. of ice-cold distilled water were added in the conical flask and again 25 ml. of distilled water were added to the above mixture collected in a separatory funnel. Finally the conical flask was washed three times with 50 ml. of ether which was also collected in the same separatory funnel. Funnel was shaken vigorously and allowed to stand for separating the two layers. Out of the two, the lower aqueous layer was drained off and discarded. The remaining process was followed as described for DDT and DDE determination.

Colour intensities were measured at 520 and 600 mμ on a Beckman DB Spectrophotometer. Blank readings were deducted from the final readings.

	MEAN	
	DDE/520 mμ 0.12 O.D	DDT/600 mμ 0.25 O.D

TABLE 1.—DDT AND DDE CONTENT IN MICROGRAMS PER 100 LARVAE EXPOSED TO 1 PPM DDT FOR 24 HOURS IN 1250 ml. WATER.

Strain	LC_{50} ppm DDT	No. of samples	DDT	DDE
PCSIR *Aedes aegypti*	.05	4	25.0	20.0

Discussion

The results have indicated clearly that the conversion of DDT into DDE is higher. The larvae which were exposed to 1 ppm DDT showed 50% to 55% mortality in 24 hours exposure period, therefore, the DDE conversion was higher due to the number of survival.

Chattoraj and Brown[10] in their paper have also reported that the susceptible strains at the concentration of 1 ppm were knocked down in 1 hour and showed 100% mortality in 6 to 10 hours, had less DDE production than those strains which were resistant to DDT and showed 20% to 40% mortality. Tahori and Hoskins[2] have reported that absorption is greatly reduced, but not stopped on knock down by DDT, Brown[12] also reported that absorption rate increases with temperature which effects the conversion of DDT into DDE.

The larvae of *Aedes aegypti*, PCSIR strain produced considerable amount of DDE, and was found higher than the reported ones.

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