THE EFFECT ON MOSQUITOES OF SUBLETHAL EXPOSURE TO INSECTICIDES. II. DDT METABOLISM

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It has previously been reported that insecticides given in sublethal amounts during the larval stage can induce an increase in the reproductive potential of *Aedes aegypti* (L.) (Sutherland, Beam and Gupta, 1967) and in *Culex pipiens* L. (Zaghloul and Brown, 1968). This response appears to be general and not specific for the insecticide, since ethanol acts similarly. The only apparent requirement is that the treatment or stimulus be close to the maximum sublethal level. The importance of this stress in insect hormoligosis has been recently discussed (Luckey, 1968) and the possible effect on reproduction suggested.

In the continued investigation to determine the mechanism by which stress induces an increase in reproductive potential, it becomes important to quantitate the responsible external stimulus and its duration. This paper reports on the uptake and metabolism of one such responsible chemical, DDT, by larvae exposed to sublethal concentrations.

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MATERIALS AND METHODS. The history of the laboratory strain of *A. aegypti* and its susceptibility to insecticides has been previously reported (Sutherland, 1964). Eight ml of pp' DDT solution in 95 percent ethanol were added under the surface of 2000 ml of distilled water in enameled pans (27 x 45 cm) and mixing was accomplished by blowing air through the pipette while stirring. An estimated 800 larvae, within one hour after egghatch, were added and approximately 0.8 gram of ground Purina Laboratory Food (of less than 177 microns mmd) was added each day. The pans were covered loosely with aluminum foil and a temperature of 30.5° C maintained. On days 3 and 5 respectively, total larval or pupal populations of individual pans were counted, recovered by filtration, rinsed with fresh water and homogenized in 5–10 ml acetone. Adult populations were trapped within 24 hours of emergence, counted, and frozen to be eventually combined into a single sample from each pan and homogenized in acetone.

Each homogenate was filtered with acetone washings through a 2 ml sintered glass funnel, and the acetone was evaporated on a rotary flash evaporator at room temperature. The remaining water-acetone solution was extracted three times with 5 ml portions of hexane, and the com-

bined hexane layers were concentrated to about 2 ml. The extract sample was passed through Florisil (Mills, 1961) previously activated and standardized by the procedure of Moddes (1961). The sample was eluted with 50 ml of 6 percent ethyl ether in hexane, and the eluate was concentrated to 2–4 ml and adjusted to 5 ml volume. Samples of 5 μl were injected into a Micro Tek MT 220 gas chromatograph equipped with a Ni-63 electron capture detector. The columns and conditions for the separation and quantitation of DDT and 12 of its metabolites have been previously described (Siewierski and Helrich, 1967). Recovery samples were subjected to the entire procedure and all values were corrected for losses encountered in extraction and analysis. Recovery values were: DDE 90.1 percent, DDD 84.8 percent, DDT 81.7 percent.

In experiments involving DDT disappearance from aqueous systems not including larvae, 1 ml of DDT solution in 95 percent ethanol (1.25 μg/ml) was added to 1000 ml freshly distilled water in 1-liter Pyrex beakers. These systems were tightly covered with plastic wrap, stored at 30° C, and at 24, 48 and 72 hours thereafter, extracted and analyzed for DDT and DDE. The total water samples were extracted for 5 hours by continuous liquid:liquid extraction with hexane. The hexane was concentrated on a steam bath and analyzed as above.

RESULTS AND DISCUSSION. The amounts of DDT, DDE and DDD recovered from larvae, pupae and adults after exposure during the larval instars to DDT suspended in water are given in Table 1. The other ten possible DDT reaction products assayable by the method (Siewierski and Helrich, 1967) were not detected. Also included are the percent mortalities experienced above those of the control groups during the various stages. The data from two such experiments are presented, and to fully indicate variability in results all values rather than averages are reported.

The greatest variation encountered in these studies was the inconsistent appearance of significant amounts of DDD in larval and pupal tissues (Experiment I versus II). This compound has not been reported in previous studies as a metabolite of DDT in the mosquito (Abedi, Duffy and Brown, 1963), and the DDD production associated with microflora, e.g. *Proteus vulgaris* and occurring in certain aqueous systems (Miskus *et al.*, 1965), suggests that mosquito metabolism is not responsible for DDD in these studies. However, recent studies have reported that axenic rat liver is responsible for such a conversion (Ottoboni *et al.*, 1968). A third experiment conducted at the same time (February) in different years similarly yielded appreciable amounts of DDD as contrasted to its absence in Experiment I, conducted in May. Infrequently in these and other studies, cloudiness strongly associated with excess food has been observed and assumed to be indicative of bacterial growth. This seems to support the suggestion that an agent other than the mosquito is responsible for DDD production.

Some variability was also encountered between individual samples within an experiment, particularly in larval samples. Generally this variation (2–3 fold) was similar to that found by other workers dealing with similar research in mosquitoes (Khan, 1964) and fish (Allison *et al.*, 1963). This variation does not appear to be related to chemical analyses but instead to biological variation. However, it does not appear to be related to the slight variation in sample size or mortality. It should be noted, however, that the percent of DDT absorbed by the organism is low. For example, in Experiment I, Larval Sample #1, 122 picograms per individual per sample of 750 individuals indicates that 4.6 percent of the DDT introduced into the system is located in the larvae after 24 hours. Therefore, an expression of the experimental data not as the weight of DDT absorbed but as percent absorbed or percent not absorbed, would lessen any apparent importance of this variability. It should be noted that factors other than the biological organism

TABLE 1.—Mortality of mosquitoes and recoveries of DDT, DDE, and DDD after exposure to 0.001 ppm in the early larval instars.

Sample	Individuals per sample	% mortality above control	picograms/individual *		
			DDE	DDD	DDT
		(Experiment I)			
Larval, control	810	....	0	0	0
Larval 1	750	7.4	11.1	0	122.0
Larval 2	730	9.8	3.9	0	34.9
Larval 3	732	9.6	11.7	0	119.0
Pupal, control	770	....	0	0	0
Pupal 1	720	6.5	29.6	0	81.5
Pupal 2	712	7.6	32.6	0	102.7
Pupal 3	732	5.2	23.8	0	94.9
Adult, control	720	0	0	0	0
Adult 1	672	6.6	36.4	0	110.0
Adult 2	694	3.6	38.3	0	113.2
Adult 3	621	13.6	34.1	0	108.5
		(Experiment II)			
Larval, control	810	....	0	0	0
Larval 1	720	11.1	12.2	114.5	178.1
Larval 2	698	13.8	0	188.8	142.7
Larval 3	732	9.6	51.1	56.6	119.6
Pupal, control	820	....	0	0	0
Pupal 1	711	13.0	58.8	33.0	195.2
Pupal 2	782	4.6	36.6	29.5	118.3
Pupal 3	766	6.6	41.1	35.4	75.6
Adult, control	710	....	0	0	0
Adult 1	688	3.0	27.8	0	45.1
Adult 2	650	8.4	51.1	0	96.4
Adult 3	702	1.1	15.4	0	65.9

* Each value based on 700–900 individuals/sample.

are probably contributing to the variation. For example, as shown in Table 2, DDT disappears rapidly (approximately 90 percent in 48 hours) from a similar but non-biological system with only small quantities of DDE being formed. Such disappearance has already been shown to be at least partly due to the codistillation of DDT from aqueous systems (Bowman *et al.*, 1964). In studies reported here where sealing of the systems is assumed to prevent loss due to codistillation, rapid loss of DDT is attributable to adsorption on glass surfaces of the container.

Irrespective of the variation in and between experiments, the data indicate that metabolism of DDT, adsorbed during the early larval stages, proceeds slowly during the pupal and early adult stage. The slight changes in the concentration of DDT and DDE during these periods were not consistent between the experiments, and such differences could be due in part to differences in binding to and extractability from larval, pupal, and adult tissues. DDD, however, was so rapidly metabolized by either the mosquito and/or microorganisms that negligible amounts of it were found in the adult tissues. A metabolite of DDD, MDE, was not detected by the analytical methods employed, although an enzyme system responsible for such metabolism has been demonstrated in the mosquito (Kimura and Brown, 1964).

These studies on DDT metabolism do not further elucidate the possible mechanism by which the stress of sublethal amounts of insecticide induce increased reproduction. It has been previously suggested (Sutherland, Beam and Gupta, 1967) that such induction might be triggered before the fourth larval instar, by which time basal follicle development has been initiated. Since the response is not specific for the insecticide, it has been subsequently postulated that the external

TABLE 2.—Amounts of DDT and DDE recovered from aqueous systems treated with 1.25 μg DDT.

Sample	Hours after treatment	μg DDT	μg DDE
1	24	0.9	0.004
2	24	0.72	0.004
3	24	0.88	0.000
4	48	0.076	0.070
5	48	0.078	0.070
6	48	0.103	0.080
7	72	0.000	0.000
8	72	0.000	0.000
9	72	0.000	0.000

stress agent (the insecticide) causes the release of a common internal stress agent which in turn influences reproduction levels. However, because of the high levels of DDT and DDE existing in pupal and adult tissues after larval treatment, one can not assume that these compounds do not directly affect the reproductive processes.

Assuming 300 larvae/gram wet tissue, the average amount of DDT found in this stage was 36 ppb. While this level may seem low, the relatively slow metabolism of DDT under these conditions indicates that mosquitoes as they survive sublethal quantities of insecticide and emerge as adults, may serve as an efficient dispersal agent of insecticides and their metabolites from aqueous to terrestrial environments.

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