

MODE OF ACTION OF DDT ON THE HOUSEFLY, *MUSCA NEBULO* LINN.—I.

(The absorption, distribution, metabolism and excretion of DDT
when topically applied on the insect)

by

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ABSTRACT

The penetration of topically applied DDT through the cuticle of the housefly, *Musca nebulo* Linn. and its transference and distribution in various tissues of the insect have been studied. The detoxification and excretion of the insecticide from the housefly has also been investigated. The mechanism of intoxication and detoxification of DDT in nerve ganglion has been found to be similar to that in the whole fly. The nature of the solvent used in the application of the insecticide influences the penetration and detoxification of the toxicant.

Introduction

The penetration of a toxicant through the cuticle of the housefly and its deposition or decomposition within the insect have interested many workers. Added importance has been given to the subject on account of the spectacular development of resistance of the insect to several insecticides. The subject has been reviewed by Chadwick¹, Metcalf² and Brown³. Sternburg *et al*⁴ and Sternburg and Kearns⁵ have shown that normal flies are able to metabolize DDT to unknown non-toxic compounds. Lindquist *et al*⁶ reported on the basis of bioassay of the extracts of normal flies that approximately 31 to 71 per cent. of the DDT that penetrated the cuticle had been converted to non-toxic products. The primary degradation product of DDT in the resistant flies has been shown to be DDE [bis (*p*-chlorophenyl)—dichloroethylene] an observation first made by Sternburg *et al*⁴ and later by Perry and Hoskins^{7,8} and by Tierriere and Schonbrod⁹. According to Sternburg *et al*⁴ and Sternburg and Kearns⁵ resistant flies are able to metabolize DDT to DDA [bis (*p*-chlorophenyl)—acetic acid] as well.

Regarding the mode of action of DDT on the housefly, it can be assumed that there are a number of essential life processes or mechanisms which if interfered with by the insecticide can result in the eventual death of the insect. It is probable that one or more of the various enzyme systems important in digestive, respiratory and nerve functions are affected. There is, however, little information on the exact distribution of topically applied DDT in the various tissues of the housefly as also on the influence of various solvents used

in the application of the insecticide on the insect. Current knowledge regarding where and how DDT is metabolized within the insect body is also very limited. The present paper describes results of investigations on the penetration, distribution, metabolism and excretion of DDT in a normal laboratory strain of the housefly, *Musca nebulosa* Linn.

Experimental Procedure

Application of DDT on the housefly—The method of rearing flies in the laboratory was as described by Basden¹⁰ for *Musca domestica* Linn. Adult females being less susceptible to insecticides than the males were used in the experiments. The age of the insects used was 4 to 5 days. The insects were lightly immobilized with anaesthetic ether prior to the application of the insecticide. The method of application of the insecticide was essentially the same as described by March and Metcalf¹¹. Measured quantities of solutions of known concentrations of *pp'*-DDT (mp. 108°C) were applied on the dorsal thoracic region of each immobilized fly with a 1.0 ml tuberculine syringe attached to a micrometer head. Generally, batches of 25–50 treated flies were placed in dishes lined with filter paper where they were allowed to remain before proceeding to the next stage in the investigation.

Extraction of DDT from insects—At different intervals after the topical application of DDT the flies were rinsed successively with four 10-ml lots of acetone. The acetone rinses were combined and evaporated on a steam bath and the residue analyzed for DDT content. The DDT estimated was recorded as being 'outside' the insects. The washed flies or various organs taken out from flies were finely ground in a pestle and mortar in the presence of anhydrous sodium sulphate and extracted four times with 10-ml lots of ether. The united extracts were filtered through cotton wool. The solvent was evaporated and the residue analyzed for DDT. The insecticide recovered in this way was described as being 'inside' the flies or as being present in the various organs of the flies as the case may be.

Recovery of DDT from excreta of flies—After the topical application of DDT the flies were placed upside down with their wings on the side of a microscopic slide smeared with a thin film of an adhesive in such a manner that the abdomen of the insects rested on a second slide placed along the first. The wings were kept in position by covering with a microscopic cover glass. From the excreta deposited on the cover glass DDT was recovered by rinsing with acetone.

Estimation of DDT—The method followed was that of Schechter *et al*¹². The amount of DDT corresponding to the 'spekker' reading was read off from a calibration curve (chart 1). To check the sensitivity of the method known amounts of *pp'*-DDT were subjected to the colorimetric estimation with the results shown in Table 1. The amounts of DDT 'outside' and 'inside' the flies and from different organs and excreta were estimated at hourly intervals upto 6 hours and also 24 hours after the application of the insecticide. In each set of experiments flies treated with the solvent alone were subjected to the same process of estimation of DDT in order to obtain the 'blank'. These

extracts were invariably of a light yellow colour which, however, did not interfere with the optical density of colour produced by DDT.

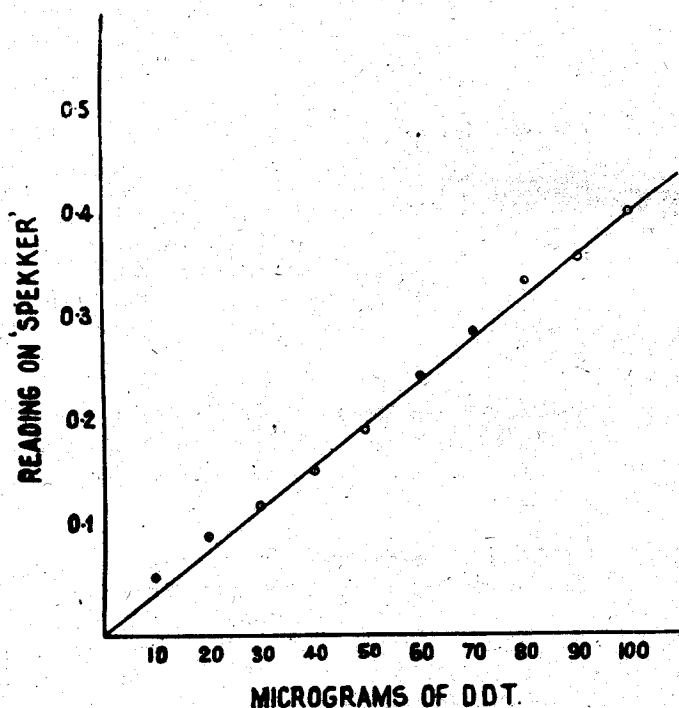
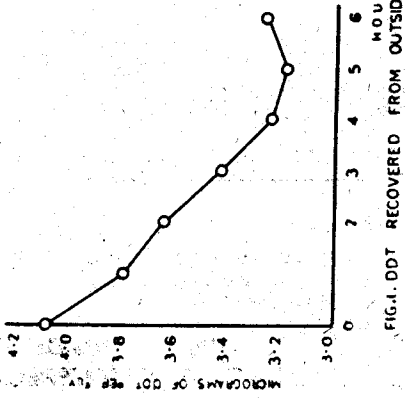


CHART 1. CALIBRATION CURVE FOR PP'-DDT.

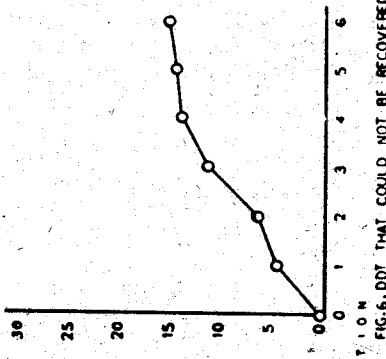
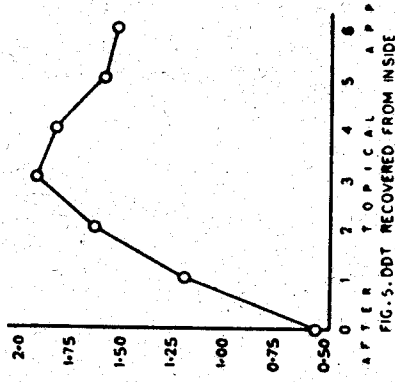
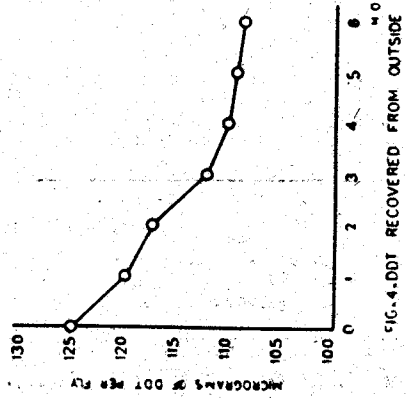
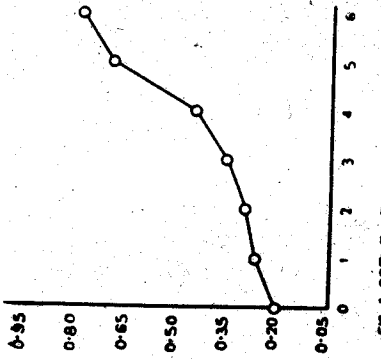
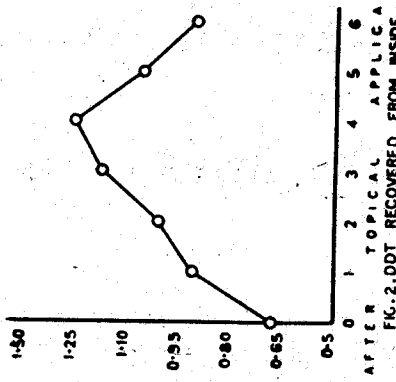
TABLE 1

Results of micro-estimation of pp'-DDT using colorimetric method (Schechter et al¹²)

Actual amount of pp'-DDT taken (μ gms)	Amount of pp'-DDT estimated			
	Experiment I		Experiment II	
	μ /gms	%	μ /gms	%
1.0	1.00	100.0	1.12	112.0
2.0	2.13	106.5	2.00	100.0
5.0	5.14	102.8	5.00	100.0
10.0	10.00	100.0	10.15	101.5
20.0	21.16	105.8	21.00	105.0
50.0	50.00	100.0	48.50	97.0
100.0	97.90	97.9	100.00	100.0
M \pm S.E.	..	101.9 \pm 3.27	..	102.2 \pm 4.93



FIGS. 1, 2 & 3. DDT APPLIED AT THE RATE OF 5 MICROGRAMS PER FLY



FIGS. 4, 5 & 6. DDT APPLIED AT THE RATE OF 126 MICROGRAMS PER FLY

Results and Discussion

Absorption, metabolism and excretion of DDT—For studies on the absorption, metabolism and excretion of topically applied DDT the insects were treated with the insecticide in acetone solution in 2 batches of 25 flies each at the rate of 5 and also 126 micrograms of *pp'*-DDT per insect. The object of investigating the two toxicant dosages was to ascertain how the absorption, metabolism and excretion of the insecticide was influenced by two such widely different dosages.

In Table 2 and figures 1 to 6 are shown the quantities of DDT applied and recovered from the 'outside' and 'inside' of the treated flies. The amounts of the insecticide that could not be recovered have been calculated by difference and have also been shown. The results have been presented on per fly basis. Figures 1 to 6 are the composite of two experiments the results of which have been separately described in Table 2.

The results in Table 2 and figures 1 to 6 show that fairly large quantities of DDT are not recovered from flies after topical application of the insecticide showing thereby that the insecticide is converted into compounds that do not respond to the colorimetric test of Schechter *et al*¹² for DDT. It is now well recognised that the primary degradation product of DDT is DDE. Tahori and Hoskins^{13, 14} and Babers and Pratt¹⁵ suggest the formation of other unknown metabolites in both susceptible and resistant flies. This aspect is being investigated in these laboratories and will be reported later.

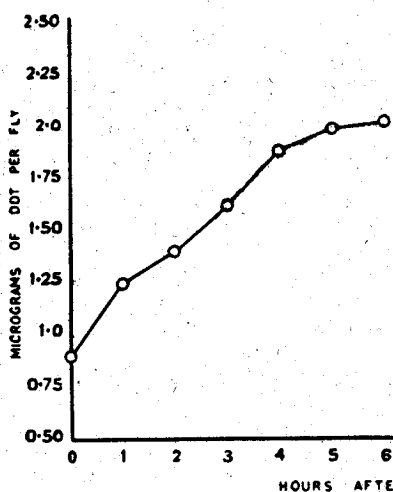


FIG. 7. DDT ABSORBED AFTER TOPICAL APPLICATION AT THE RATE OF 5 MICROGRAMS PER FLY.

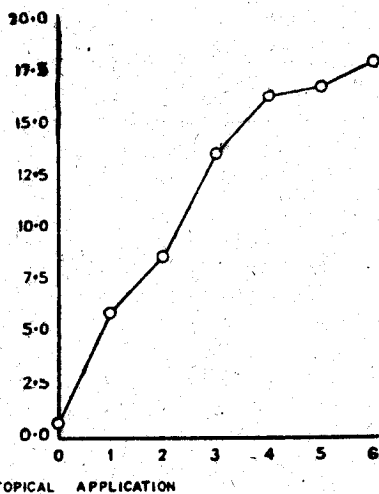


FIG. 8. DDT ABSORBED AFTER TOPICAL APPLICATION AT THE RATE OF 126 MICROGRAMS PER FLY.

FIGS 7 & 8. REPRESENT AMOUNT OF DDT RETAINED INSIDE FLIES+AMOUNT EXCRETED+AMOUNT THAT COULD NOT BE RECOVERED.

The results in figures 7 and 8 show that there is progressive increase with time in the quantities of DDT absorbed by the insects.

TABLE 2
DDT Estimated from Flies after Topical Application

DDT applied in acetone (μ gms/fly)	Hours after topical application	Experiment I*						Experiment II*					
		DDT Recovered				DDT not recovered		DDT Recovered				DDT not recovered	
		Outside†		Inside		μ gms/ fly	% ‡	Outside†		Inside		μ gms/ fly	% ‡
		μ gms/ fly	% ‡	μ gms/ fly	% ‡			μ gms/ fly	% ‡	μ gms/ fly	% ‡		
5	§ 0	4.40	88.0	0.63	12.0	0.0	0.0	3.80	76.0	0.75	15.0	0.45	9.0
	1	3.70	74.0	0.87	17.6	0.42	8.4	3.92	78.4	0.96	19.2	0.12	2.4
	2	3.52	70.4	1.05	21.0	0.43	8.6	3.80	76.0	1.01	20.2	0.19	3.8
	3	3.28	65.6	1.14	22.8	0.58	11.6	3.62	72.4	1.24	24.8	0.14	2.8
	4	3.08	61.6	1.26	25.2	0.66	13.2	3.44	68.8	1.28	25.6	0.28	5.6
	5	3.32	66.4	1.10	22.0	0.58	11.6	3.08	61.6	1.05	21.0	0.87	17.4
	6††	3.24	64.8	0.88	17.6	0.88	17.6	3.32	66.4	0.96	19.2	0.72	14.4
126	0	125.5	99.6	0.50	0.4	0.0	0.0	125.0	99.2	0.64	0.5	0.36	0.3
	1	120.5	95.6	1.26	1.0	4.24	3.4	120.0	95.2	1.16	0.9	4.84	3.8
	2	119.0	94.4	1.62	1.3	5.38	4.3	116.5	92.5	1.70	1.3	7.80	6.2
	3	111.5	88.5	1.90	1.5	12.60	10.0	114.0	90.5	2.00	1.6	10.00	7.9
	4	111.5	88.5	1.70	1.4	12.80	10.2	109.0	86.5	2.00	1.6	15.00	11.9
	5	109.0	86.5	1.62	1.3	15.38	12.2	110.5	87.7	1.62	1.3	13.88	11.0
	6††	109.0	86.5	1.52	1.2	15.48	12.3	109.0	86.5	1.52	1.2	15.48	12.3

* The temperature and relative humidity during the experiments lay between 28.4°C—30.6°C & 60—80% respectively.

† Includes the amount present in the excreta of flies as in the technique adopted quantity of the insecticide excreted by the insect could not be separated from the amount actually present 'outside' of the treated flies. However, the amount of DDT in the excreta is in no case more than about 12.4 per cent. (Tables 4 and 6B) of the amount estimated from the 'outside' of the treated flies. For details please see the sections on 'Excretion of DDT'.

†† There was no mortality of the insects upto 6 hours after the application of the insecticide.

‡ Expressed as percentages of the amount of toxicant applied.

§ The experiments at '0' hour were carried out by treating the individual flies with the toxicant and transferring them immediately into acetone.

A batch of 25 flies could be treated in 2-3 minutes.

|| The values are the difference of total amounts of DDT applied and recovered.

The pattern of results obtained with the two toxicant dosages investigated shows that the larger dosage (126 micro grams per fly) gives increased absorption of DDT and so also greater detoxification of the insecticide than that obtained when the insecticide is applied at the rate of 5 micrograms per fly although the increases are not proportional to the dosages applied. Barker¹⁶ has also shown that increased dosages give increased absorption and greater metabolization of DDT in resistant flies although the increases are not proportional to the dosages applied.

Extent of absorption and detoxification of DDT in poisoned flies when death supervened—In order to ascertain whether the extent of absorption and detoxification of the insecticide was different depending on the treated insect being alive or dead after the topical application, the above experiments were repeated, the period of contact with the topically applied insecticide being progressively increased to 24 hours. The insects were treated at the rate of 127 micrograms of *pp'*-DDT per fly. At each interval, the dead flies were segregated from the surviving flies and the two sets were separately examined. The results are shown in Table 3 and figures 9 to 11.

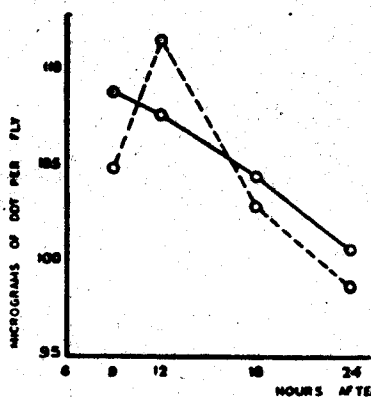


FIG. 9. DDT RECOVERED FROM OUTSIDE

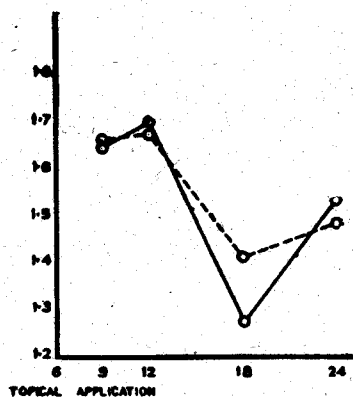


FIG. 10. DDT RECOVERED FROM INSIDE

TABLE 3

DDT estimated from 'Surviving' and 'Dead' flies

DDT estimated from	Hours after topical application	Survival of flies after application %	Experiment I*				DDT not recovered*	Experiment II*						
			DDT Recovered		%††	DDT Recovered		%††						
			Outsidet	Inside		Outsidet			Inside					
μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly	μ gm/ fly					
'Surviving' flies	9	80	111.54	87.9	2.00	1.5	13.46	10.6	106.54	83.9	1.30	1.0	19.16	15.1
	12	75	113.70	89.5	1.80	1.4	11.53	9.1	102.20	80.4	1.62	1.3	23.18	18.3
	18	55	102.14	80.4	1.12	0.9	23.74	18.7	107.14	84.4	1.44	1.1	18.42	14.5
	24	15	99.88	78.7	1.44	1.1	25.68	20.3	101.88	80.1	1.64	1.3	23.48	18.6
'Dead' flies	9	80	103.04	81.2	2.0	1.6	21.96	17.2	107.04	84.2	1.34	1.1	18.62	14.7
	12	75	111.70	87.9	1.68	1.3	14.60	10.8	111.70	87.9	1.68	1.3	14.60	10.8
	18	55	104.14	82.0	1.12	0.9	21.76	17.1	102.14	80.4	1.72	1.4	23.14	18.2
	24	15	99.88	78.7	1.64	1.3	25.42	20.0	97.88	77.1	1.34	1.1	27.80	21.8

* The experiments were carried out at $26.5 \pm 1^\circ \text{C}$ and $55 \pm 5\% \text{R.H.}$

† Includes the amount present in the excreta of flies. Please see footnote to Table 2.

†† Expressed as percentages of the amount of DDT applied.

‡ The values are the difference of total amounts of DDT applied and recovered.

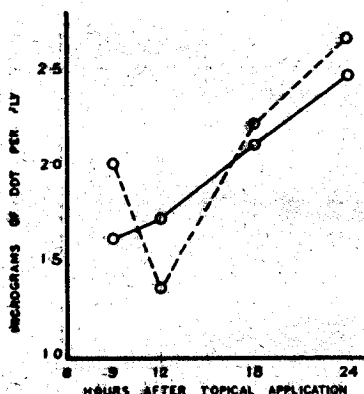


FIG. 11. DDT THAT COULD NOT BE RECOVERED

FIGS 9, 10 & 11. DDT APPLIED AT THE RATE OF 127 MICROGRAMS PER FLY.

○—○ SURVIVING FLIES
○- - -○ DEAD FLIES

It will be noted from the results in Table 3 and figures 9 to 11 that there are no appreciable differences in the amount of the insecticide found 'outside' or 'inside' or 'not recovered' from both 'surviving' and 'dead' flies. There is no reference in the literature on this aspect.

Excretion of DDT—The results in Table 4 and figures 12 and 13 show that DDT when applied topically gets excreted by flies in varying amounts depending on the period of intoxicification. It will be noted from the results that the larger dosage gives increased excretion although the increase is not proportional to the dosages applied and that the maximum amount of the insecticide excreted is in no case more than about 11.3 per cent. of that estimated from the 'outside' of the treated flies.

TABLE 4
DDT estimated from excreta of flies

DDT applied in acetone (μ gm/fly)	Hours after topical application	Survival of flies after application (%)	DDT Excreted					
			Experiment I ††			Experiment II ††		
			μ gm/fly	%*	%**	μ gm/fly	%*	%**
5	1	100	0.050	1.0	1.4	0.062	1.2	1.6
	2	100	0.073	1.5	2.0	0.050	1.0	1.3
	3	100	0.092	1.8	2.8	0.086	1.7	2.4
	4	100	0.155	3.1	5.0	0.193	3.9	5.6
	5	100	0.241	4.8	7.2	0.169	3.4	5.5
	6	100	0.370	7.4	11.3	0.241	4.8	7.3
	24	50	0.420§	8.4	†	0.370§	7.4	†
126	1	100	0.147	0.1	0.1	0.100	0.1	0.1
	2	100	0.244	0.2	0.2	0.290	0.2	0.2
	3	100	0.388	0.3	0.3	0.338	0.3	0.3
	4	100	0.388	0.3	0.3	0.434	0.3	0.4
	5	100	0.454	0.4	0.4	0.473	0.4	0.4
	6	100	0.483	0.4	0.4	0.530	0.4	0.5
	24	15	0.530§	0.4	†	0.770§	0.6	†

*Expressed as percentages of the amount of DDT applied.

**Expressed as percentages of the amount of DDT estimated from the 'outside' of treated flies (Table 2).

†† The experiments were carried out at $27.2 \pm 3.0^\circ\text{C}$ and $71 \pm 8\%$ R.H.

† Not done.

§ Includes the amount which had been excreted by dead flies also.

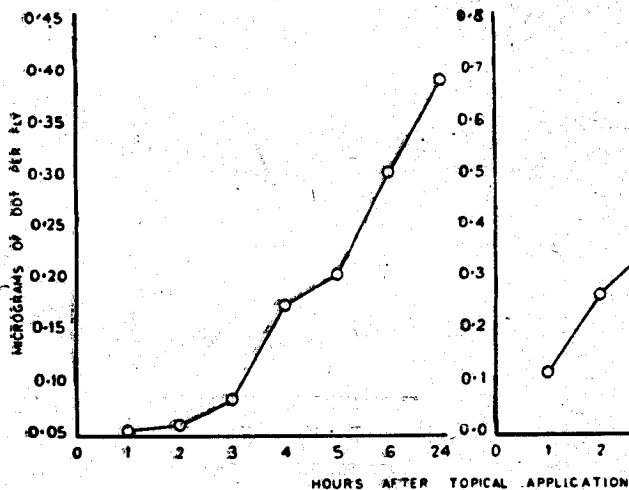


FIG.12.—DDT EXCRETED AFTER TOPICAL APPLICATION AT THE RATE OF 5 MICROGRAMS PER FLY.

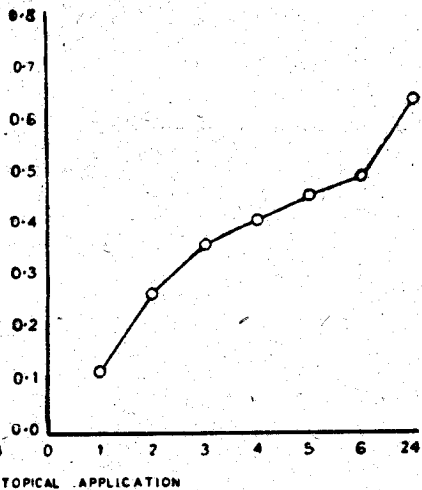


FIG.13.—DDT EXCRETED AFTER TOPICAL APPLICATION AT THE RATE OF 126 MICROGRAMS PER FLY.

Distribution of DDT in various organs—To ascertain the distribution of topically applied DDT the insects were treated in acetone solution in 2 batches of 50 flies each at the rate of 126 micrograms of *pp'*-DDT per fly. The quantities of DDT estimated from various organs of the insect at different intervals of time after the topical application of the insecticide are shown in Table 5 and figures 14 to 18.

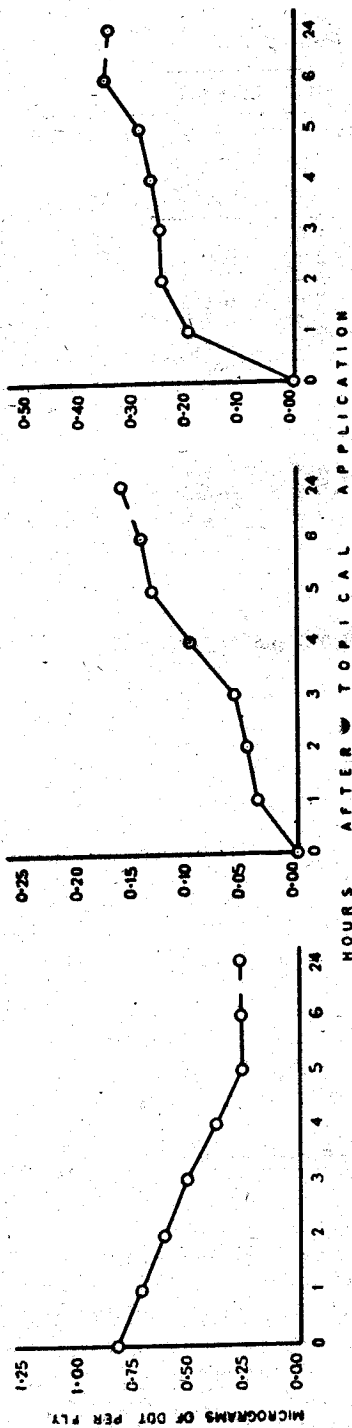


FIG. 16. HINDGUT

FIG. 15. MIDGUT

FIG. 14. DORSAL THORACIC REGION

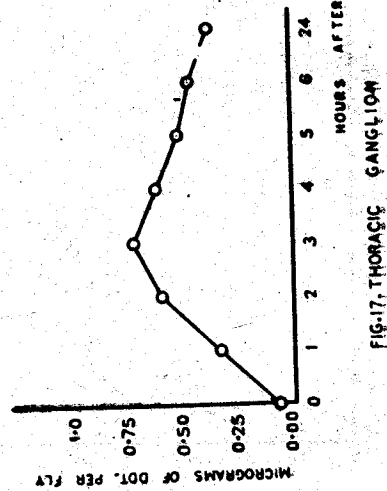


FIG. 17. THORACIC GANGLION

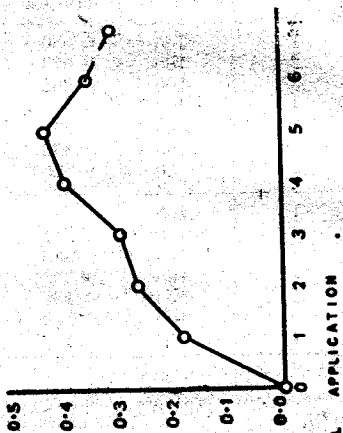


FIG. 18. MALPIGHIAN TUBULES

TABLE 5

DDT recovered from various organs of the housefly

Hours after topical application	Survival of flies after application (%)	Amount of DDT (μ gms/fly) recovered †									
		Experiment I *					Experiment II *				
		Dorsal thoracic region	Midgut	Hindgut	Thoracic nerve ganglion	Malpighian tubules	Dorsal thoracic region	Midgut	Hindgut	Thoracic nerve ganglion	Malpighian tubules
0§	100	0.85	0.0	0.0	0.098	0.0	0.82	0.0	0.0	0.054	0.0
1	100	0.72	0.047	0.192	0.385	0.169	0.74	0.024	0.212	0.301	0.198
2	100	0.52	0.047	0.260	0.630	0.291	0.72	0.047	0.240	0.580	0.260
3	100	0.52	0.071	0.265	0.670	0.313	0.50	0.047	0.240	0.810	0.300
4	100	0.43	0.096	0.265	0.550	0.401	0.34	0.105	0.284	0.720	0.420
5	100	0.27	0.130	0.307	0.530	0.430	0.25	0.144	0.289	0.530	0.480
6	100	0.27	0.130	0.384	0.510	0.380	0.27	0.162	0.337	0.460	0.363
24	15	0.29†	0.144†	0.313†	0.410†	0.337†	0.25†	0.182	0.380†	0.380†	0.325†

*The experiments were carried out at 28-29°C & 66±3% R.H.

†There was no trace of DDT in the foregut, wings and legs.

‡Amount present in both 'surviving' and 'dead' flies.

§The experiments at '0' hour were carried out as described in the footnote to Table 2.

The results presented in Table 5 and figures 14 to 18 show that DDT gets widely distributed in the housefly. The distribution however, does not appear to be uniform as in the early stages upto 2 hours after application, the major portion of the insecticide was recovered from the dorsal thoracic region and subsequently from the thoracic nerve ganglion. Sternburg and Kearns⁵ found that DDT occurred only in the cuticle hypoderm. Tahori and Hoskins^{13, 14} have disputed the findings and have reported that they found DDT in all tissues investigated (after the topical application of the insecticide). Their observation supported the work of Lindquist *et al*¹⁷ who found DDT in the body fluid, gut ganglia, reproductive organs and muscles as well as in the cuticle hypoderm. The results of present investigations given in Table 5 and figures 14 to 18 reveal that the amount of DDT recovered from the dorsal thoracic region at different intervals decreases progressively upto 5 hours, and thereafter becomes steady. The hind and midguts show a progressive increase in deposition of the insecticide with the passage of time. There was, however, no trace of the insecticide in the extracts of the foregut, wings and legs of the treated flies. The malpighian tubules show an increase in the amount of DDT deposited upto 5 hours after which there is a slight decrease thereby indicating that greater excretion of the insecticide is taking place. This is in agreement with the results presented in Table 3. The results of DDT estimation from the thoracic nerve ganglion (Table 5 and figure 17) and from 'inside' the fly (Table 2 and figure 5) show an increase in the amounts of DDT absorbed upto 3 hours after which there is a decrease in the amounts of the insecticide recovered. This shows that the mechanism of intoxication and detoxification of DDT in the nerve ganglion is similar to that in the whole fly.

Effect of solvents on the penetration, detoxification and excretion of DDT—For studies on the effect of different solvents on the penetration and detoxification of topically applied DDT the insects were treated in 2 batches of 25 flies each at the rate of approximately 5 micrograms of the insecticide per fly. In Table 6 are shown the actual quantities of DDT applied in respect of different solvents investigated. The results are presented in Tables 6A and 6B.

TABLE 6
Quantities of DDT applied in different solvents

Solvents investigated	Concentration of DDT in different solvents % (w/v)	Amount of DDT applied (μ gm/fly)
Acetone	1.0	5.00
Ether	1.0	7.26
Benzene	1.0	6.04
Ethyl alcohol	1.0	5.22
Kerosene	1.0	4.67
Liquid paraffin	3.0	4.88

TABLE 6A

Effect of Solvents of Penetration and Detoxification of DDT

		DDT estimated after topical application expressed as percentages of the amount applied in different solvents																		
		Acetone π			Ether			Benzene			Ethyl alcohol			Kerosene			Liquid paraffin			
		Out-side†	In-side	Not re-cov-ered‡	Out-side†	In-side	Not re-cov-ered‡	Out-side†	In-side	Not re-cov-ered‡	Out-side†	In-side	Not re-cov-ered‡	Out-side†	In-side	Not re-cov-ered‡	Out-side†	In-side	Not re-cov-ered‡	
Experi- ment I	Hours after topical applica- tion	0††	88.0	12.0	0.0	92.0	7.4	0.6	89.4	8.0	2.6	92.7	3.4	3.9	87.4	4.2	8.4	98.5	3.0	0.0
	1	74.0	17.6	8.4	86.5	13.3	0.2	84.1	13.5	2.4	83.4	6.7	9.9	88.2	7.5	4.3	92.6	8.8	0.0	
	2	70.4	21.0	8.6	81.0	15.2	3.8	78.2	16.8	5.0	87.4	9.3	3.3	83.1	11.1	6.8	87.7	11.8	0.5	
	3	65.6	22.8	11.6	73.3	17.9	8.8	73.6	21.2	5.2	72.8	12.0	15.2	73.7	17.2	9.1	81.9	18.5	0.0	
	4	61.6	25.2	13.2	61.7	18.2	20.1	72.5	20.0	7.5	65.2	12.0	22.8	71.1	17.3	11.6	76.2	18.6	5.2	
	5	66.4	22.0	11.6	55.1	14.6	30.3	67.0	18.2	14.8	67.4	13.0	19.6	65.1	14.8	20.1	76.2	15.6	8.2	
	6	64.8	17.6	17.6	57.9	12.5	27.6	67.9	15.9	16.2	65.1	11.6	23.3	61.0	12.4	26.6	74.5	15.7	9.8	
	24§	71.6*	14.6*	13.8*	57.9*	10.7*	31.4*	67.8*	15.5*	16.7*	58.2*	10.7*	31.1*	66.8*	13.1*	20.1*	72.7*	11.8*	15.5*	
Experi- ment II	Hours after topical applica- tion	0††	76.0	15.6	8.4	89.3	8.2	2.5	89.4	9.6	1.0	96.6	3.8	0.0	85.7	4.5	9.8	98.9	3.6	0.0
	1	78.4	19.2	2.4	83.8	11.2	5.0	84.8	13.5	2.7	98.1	8.3	0.0	83.1	8.2	8.7	91.8	8.8	0.0	
	2	76.0	20.2	3.8	79.9	16.0	4.1	78.8	17.8	3.4	87.4	10.2	2.4	81.4	11.4	7.2	86.1	12.8	1.1	
	3	72.4	24.8	2.8	70.8	18.4	10.8	75.9	20.9	3.2	76.7	13.8	9.5	77.1	17.4	5.5	80.3	16.7	3.0	
	4	68.8	25.6	5.6	55.1	18.4	26.5	67.9	20.8	1.3	69.0	12.5	18.5	67.6	16.2	16.2	77.9	18.6	3.5	
	5	61.6	21.1	17.3	52.4	15.3	32.3	68.9	18.7	12.4	65.2	11.1	23.6	69.4	16.3	14.3	75.4	16.7	7.9	
	6	66.4	19.2	14.4	58.4	13.4	28.2	66.2	16.8	17.0	58.2	10.4	31.4	65.1	13.3	21.6	74.5	14.7	10.8	
	24§	64.4*	16.5*	19.1*	57.8*	13.4*	28.8*	70.8*	15.9*	13.3*	63.6*	10.5*	25.9*	61.2*	11.1*	27.7*	73.9*	12.8*	13.3*	

π The data are from Table 2.

*This includes the amount present in the excreta of flies. Please see footnote on the aspect (Table 2).

†The values are the difference of total amounts of DDT applied and recovered.

‡Represents the amount present in both 'surviving' and 'dead' flies.

§The experiments were carried out at $27.2 \pm 0.6^\circ\text{C}$ & $60 \pm 10\%$ R.H.

||The experiments at 'O' hour were carried out as described in the footnote to Table 2.

¶The survival of insects 24 hours after application was 50 per cent. There was, however, no mortality of the insects upto 6 hours after application.

TABLE 6B

Effect of solvents on excretion of DDT

DDT applied (μ gm/fly)	Solvents used in the application of DDT	DDT excreted ††					
		Experiment I †			Experiment II †		
		μ gm/fly	%*	%†	μ gm/fly	%*	%†
5.00	Acetone ..	0.38	7.6	10.6	0.40	8.0	12.4
7.26	Ether ..	0.08	1.1	2.0	0.23	3.2	5.6
6.04	Benzene ...	0.23	3.8	5.6	0.18	3.0	4.2
5.22	Ethyl alcohol ..	0.18	3.5	5.9	0.20	3.8	6.0
4.67	Kerosene ..	0.23	4.9	7.4	0.20	4.3	7.0
4.88	Liquid paraffin	0.1	2.0	2.8	0.10	2.0	2.8

*Expressed as percentages of the amount of DDT applied.

†Expressed as percentages of the amount of DDT estimated from the 'outside' of treated flies (Table 6A).

††Estimated 24 hours after topical application. The survival of insects was 50 per cent.

‡The experiments were carried out at 28°C and 42±2% R. H.

The results presented in Table 6A show that with ether, acetone and benzene larger amounts of DDT are recovered from inside the flies than with kerosene, ethyl alcohol and liquid paraffin showing thereby that the penetration of the insecticide through the insect cuticle is influenced by the nature of solvent used for dissolving the insecticide. It will be noted that with ether, kerosene and ethyl alcohol greater detoxification of the insecticide is observed than with the other three solvents. The results in Table 6B show that the nature of solvent influences the quantity of DDT excreted by the treated flies, the maximum amount being excreted when acetone is used for dissolving the insecticide.

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