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   STRAINS OF THE HOUSE FLY.

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FACTORS AFFECTING THE LATENT TOXICITY OF ALDRIN, DDT, AND

HEPTACHLOR TO RESISTANT AND SUSCEPTIBLE STRAINS OF

THE HOUSE FLY

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF THE

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THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN ENTOMOLOGY

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By

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ACKNOWLEDGMENT

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ABSTRACT

Latent toxicity is that phenomenon in which the full lethal effect of an insecticide applied to a larva is not apparent until it has differentiated into an adult. It was found that appreciable latent toxicity occurred when aldrin, heptachlor, and DDT were applied to larvae of a susceptible strain of the house fly, Musca domestica L., while little or no latent toxicity occurred when these insecticides were applied to a resistant strain. This study was undertaken to determine the effect of a number of factors on the latent toxicity of aldrin, DDT, and heptachlor to susceptible and resistant strains of the house fly. The factors investigated were: (1) rate of cuticular penetration of the insecticide; (2) rate of degradation of the insecticide in the insect body; and (3) amount of carry-over of the insecticide and/or its metabolites from the larval to the adult stage. The insecticides were topically applied to larvae of the resistant (Hawaiian) strain and the susceptible (SCR-60) strain of the house fly and their metabolism followed by the use of electron capture gas chromatography.

It was found that aldrin and heptachlor were rapidly converted to their epoxides, dieldrin and heptachlor epoxide, respectively, while DDT was converted to the non-toxic metabolite dichlorodiphenyldichloroethylene (DDE).

The rate of cuticular penetration of the three insecticides was somewhat faster with the susceptible than with the resistant strain.

Resistance of the larvae of the Hawaiian strain to DDT is attributable

to its rapid conversion to the non-toxic derivative DDE. No internal DDT

was recovered from the Hawaiian strain at any time, indicating complete

dehydrochlorination of all the DDT that penetrated. Measurable amounts

of DDT could be recovered from the susceptible SCR-60 larvae one hour

after topical application. The larvae of the SCR-60 strain were limited

in their ability to detoxify DDT to DDE.

The latent toxicity of DDT to the susceptible SCR-60 strain was due to

the carry-over of sufficient amounts of unchanged DDT from the larval to

the adult stage to cause mortality in the flies shortly after emergence. The

living flies contained very little DDT. There was no carry-over of DDT in

the restant Hawaiian strain. Only DDE was found internally, indicating com-

plete detoxification of the absorbed DDT.

There was sufficient carry-over of dieldrin and heptachlor epoxide from

the larva to the adult to account for the latent toxicity in the susceptible

SCR-60 flies. The failure to elicit the phenomenon of latent toxicity by aldrin

and heptachlor to the resistant strain is difficult to explain. The amount

carried over to the adult stage was practically the same in both strains.

Thus, it appears that the ability to convert aldrin and heptachlor to their

corresponding epoxides cannot explain resistance or the latent toxicity of

these two compounds. Latent mortality occurred only in the susceptible

SCR-60 flies despite the comparable pattern of metabolism in the two strains.

Also, the levels of dieldrin and heptachlor epoxide found in SCR-60

flies was practically the same in the living as in those that died of latent

toxicity.

FACTORS AFFECTING THE LATENT TOXICITY OF ALDRIN, DDT, AND HEPTACHLOR TO RESISTANT AND SUSCEPTIBLE STRAINS OF THE HOUSE FLY

INTRODUCTION

The term latent toxicity was first proposed to describe the phenome-non in which the full lethal effect of an insecticide applied to a larva is not apparent until it has differentiated into an adult (Tamashiro and Sherman, 1955). This phenomenon has been recorded in at least four dipterous species; the Oriental fruit fly, Dacus dorsalis Hendel; the melon fly, D. curcurbitae Coquillete; the Mediterranean fruit fly, Ceratitis capitata (Wiedemann); and the house fly, Musca domestica Linnaeus, in both the susceptible and resistant strains (Tamashiro and Sherman, 1955; Sherman, 1958; Sherman and Sanchez, In press). It was established in these studies that chemical structure, species, and degree of resistance are interrelated with latent toxicity. The chlorinated polycyclic hydrocarbon insecticides in particular showed a high order of latent toxicity.

Sherman and Sanchez (In press) found that the chlorinated polycyclic hydrocarbon insecticides manifested latent toxicity to both a DDT-susceptible and a DDT-resistant strain of the house fly. It was found that considerable latent toxicity was produced by aldrin, chlordane, DDT, dieldrin, and heptachlor to the susceptible strain. The resistant strain showed less latent

mortality in comparison with the susceptible strain. Only aldrin and heptachlor exhibited latent toxicity in the resistant strain, but only to a very limited extent and at very high dosages.

This study was undertaken to determine the role played by the following factors in the latent toxicity of aldrin, DDT, and heptachlor to a resistant and a susceptible strain of the house fly: (1) rate of cuticular penetration, (2) rate of degradation of the insecticide in the insect body, and (3) the amount of carry-over of the insecticide and/or its toxic metabolite from the larval to the adult stage.

MATERIALS AND METHODS

Insecticides and Metabolites. - The following purified compounds were used in this study: DDT   2, 2-bis(p-chlorophenyl)-1, 1, 1-trichloroethane   , p, p'-isomer, 99.3 per cent; DDE   2, 2-bis(p-chlorophenyl)-1, 1-dichloroethylene   , m.p 87-88°C; DDA   bis-(p-chlorophenyl)-acetic acid   , m.p. 163.4°C; aldrin   1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo, -exo-5, 8-dimethanonaphthalene   , recrystallized, 99+ per cent; dieldrin   1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo, exo-5, 8-dimethanonaphthalene   , recrystallized, 99+ per cent; heptachlor   1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-endomethanoindene   , analytical reference grade, 98.2 per cent; and heptachlor epoxide, analytical reference grade, 99.4 per cent. Stock solutions of these materials in redistilled acetone were prepared and used throughout the study. Where necessary, further dilutions of the solutions were made in redistilled acetone.

Insects.- The insects used in this study were a susceptible and a resistant strain of the house fly: the SCR-60 strain, a DDT-susceptible strain obtained from the University of California at Berkeley; a local resistant strain designated as the Hawaiian strain, originally obtained from a field collection in a poultry yard at Waimano Home, Pearl City, Oahu, during July, 1958, and subsequently reared continuously without insecticidal pressure.

Both strains were reared on the standard CSMA larval medium in a constant room temperature maintained at $21^{\circ}C \pm 2^{\circ}C$.

Treatments.- Mature third instar larvae (prepupae) of both strains were used to study the factors involved in latent toxicity. A representative number of larvae were removed from the rearing medium, washed in water and dried on filter paper. The test insects were inactivated with CO_2 before treatment. Each larva was held with a pair of forceps specially modified so that they could be firmly held without being injured by excessive pressure. A measured drop (0.001 ml in volume) insecticide in acetone was placed on the midsection of the larva. Aldrin, DDT, and heptachlor were applied at dosages of 0.250 µg, 0.125 µg, and 0.500 µg/larva, respectively. These dosages were selected because in a previous study (Sherman and Sanchez, In press) they were found to cause a high degree of latent toxicity in the SCR-60 strain of the house fly. These dosages were non-toxic to the resistant strain, but were applied so that comparisons could be made on a quantitative basis between the two strains. The acetone was allowed to evaporate and the treated larvae were placed in 50 ml beakers in batches of 20 for the larval and pupal metabolism studies.

The rate of cuticular penetration and metabolism of the insecticides were determined at the following time intervals: 1, 3, 6, 12, 24, 48, 96, and 192 hours after topical treatment. Two replications were made for each determination. The treated larvae of both strains transformed into what appeared

to be morphologically normal pupae within 48 hours after treatment.

Those treated larvae to be held for the emergence of the adult to

determine the effect of insecticidal carry-over from the larva to the adult

were placed in 50 ml beakers in groups of about 100 to 150 individuals.

Prior to emergence of the adults, the puparia were transferred to clean

100 ml beakers and covered with approximately 2 inches of washed beach

sand. This was done to prevent contact between the emerged adult flies

and any insecticide that might still have been present on the puparia. The

adult flies were allowed to emerge into wire-screen holding cages measuring

approximately 10 x 10 x 10 inches. They were given free access to Carna-

tion instant nonfat dry milk and water throughout the 14-day holding period.

No sugar was given to the adult flies as it was found early in the study that

sugar interfered with subsequent analysis.

Latent toxicity was observed over a three-day period after emergence

of the adults. Each day during this period, the dead flies were removed from

the holding cage, sexed, and placed in the freezer for preservation until

analysis. Moribund insects were treated as dead insects. One day and three

days after emergence representative samples of the living flies showing no

symptoms of intoxication were anaesthetized, sexed, and subjected to the

analytical procedure described below. If time did not permit immediate

analysis, these living adult flies were placed in the freezer until they could

be analyzed.

The adult flies were assayed for the parent compounds and metabolites 1, 3, 6, 10, and 14 days after emergence. They were anaesthetized with CO_2 before each sampling. Separate determinations were made for the male and female flies.

A study was also conducted to determine the metabolism of the three insecticides after application to the adult fly. The susceptible SCR-60 strain was used.

Two- to three-day old adult females were anaesthetized with CO_2. They were held by the wing with forceps and 0.001 ml of the required concentration of insecticide was applied on the notum by means of a micropippete. Aldrin, DDT, and heptachlor were applied at 0.014, 0.035, and 0.042 µg/female, respectively. These values had been established as the median-lethal dosages of these materials earlier (Sherman and Sanchez, In press). Forty females were treated with each insecticide. The acetone was allowed to evaporate and the treated adults were placed in a holding cage made of a 250 ml beaker covered with perforated aluminum foil. They were given free access to a nonfat milk solution during the first day after treatment. The milk solution was withdrawn from the second day because the flies became very much engorged and it was feared that this excessive body water would interfere with subsequent analysis.

Mortality was taken 48 hours after treatment. The dead and living flies were separated for analysis.

Extraction Procedure. - The amounts of the insecticides and their

metabolites contained by the larvae and pupae both externally and internally

were determined. To obtain the external residue, two replications of 20

insects each were rinsed twice in 5-ml portions of redistilled acetone

which were combined for analysis. This method was found to be highly

efficient in removing the external deposit for analysis. Rinsing three times

did not improve the efficiency of extraction and so only these two rinses

were utilized in the study.

To obtain the internal residue, the previously rinsed insects were

placed in a Thomas hand homogenizer (10 ml capacity). Using a teflon

pestle, the insects were macerated with two 5-ml portions of redistilled

acetone. The two acetone extracts were combined for analysis. This pro-

cedure was also found to be highly efficient in extracting the internal residues

since a third extraction did not yield significant amounts of insecticides and/

or metabolites. The combined extracts were centrifuged at 6,500 rpm for

three minutes before analysis.

Gas chromatography. - The cuticular penetration and metabolism

of the insecticides were followed by means of gas chromatography. An

Aerograph Hy-Fi gas chromatograph equipped with an electron capture

detector (cell voltage set at 90) and a Leeds and Northrup recorder (1 mv.)

was used. The mobile phase was nitrogen. The stationary phases were

either 5 per cent SE-30 silicone gum on Chromosorb W 60/80 mesh or

5 per cent Dow-11 silicone grease on Chromosorb W 60/80 mesh depending

upon the insecticide to be chromatographed. They were packed in pyrex

glass columns with the dimensions 5 ft. x 1/8 in. The SE-30 preparation

was the stationary phase for aldrin, heptachlor, and their metabolites. But

since DDT decomposed on this SE-30 column, it was necessary to use the

Dow-11 stationary phase for this material and its metabolite. A #705 Hamilton

syringe provided with a Chaney adapter and set to deliver 10 ul was used

for injecting the samples.

Aldrin, heptachlor, and their metabolites were chromatographed

under the following conditions: Column temperature, 185°C; injector

temperature, 250°C; N_2 flow rate, 45 ml/min; detector cell voltage, 90;

electrometer gain, 1x, 10^7; sample size, 10 ul. DDT and DDE were chromato-

graphed under the same conditions except that the injector temperature was

190°C and the nitrogen flow rate was 75 ml/min. Electrometer attenuation

was varied depending on the sensitivity of the detector.

When it was necessary to prepare new columns, they were conditioned

overnight at about 40°C above operating temperature, with a nitrogen flow

of 45 ml/min.

Standard solutions of the materials to be chromatographed were prepared

in redistilled acetone. Frequent injections of these standards were made to

take care of any change in sensitivity of the instrument.

Fortified samples of the fly homogenate were made to see if background

due to the tissue extracts would affect the recovery of the insecticidal

materials or their metabolites. These were made by adding aliquots of

the standard solutions to tissue extracts from untreated insects. Also,

it was necessary to inject these fortified samples prior to injecting the

experimental tissue samples to stabilize the detector response and to

obtain maximum reproducibility.

The quantity of insecticide and metabolite were recorded as peaks

and the peak areas were triangulated and measured with a Gelman model

39231 planimeter (compensating polar planimeter). The areas obtained in

square inches were converted to square millimeters for ease of computation

and correlated with concentration.

EXPERIMENTAL RESULTS

Chromatograms. - The effect of feeding non-fat dry milk or sugar

to the adult flies are shown in Figure 1. Sugar in the diet resulted in the

extraction of substances that gave high backgrounds and interfered with the

recovery of the insecticides or their metabolites. The feeding of non-fat

milk solids, on the other hand, resulted in no interfering background. This

points out the importance of selecting a diet which would be nutritious to the

fly and yet not require a complex clean-up procedure when conducting metabolism

studies.

Figure 2 compares the results obtained after injecting an acetone

solution of dieldrin and the acetone extractives from dieldrin-fortified

adult tissues. The tissue extractives did not interfere with recovery.

Figures 3, 4, and 5 show the typical electron capture gas chromato-

grams of the insecticides and their respective metabolites, and their separa-

tions as reflected by their retention times. The retention times of the

materials in minutes were determined by the time it took for each material

to pass through the column, using the beginning of the solvent peak as the

starting point. The retention times for aldrin and dieldrin, DDT and DDE,

and heptachlor and heptachlor epoxide, under the conditions prescribed for

each previously, were 3 and 6.25, 8.75 and 5, and 2.25 and 3.75 minutes,

respectively.
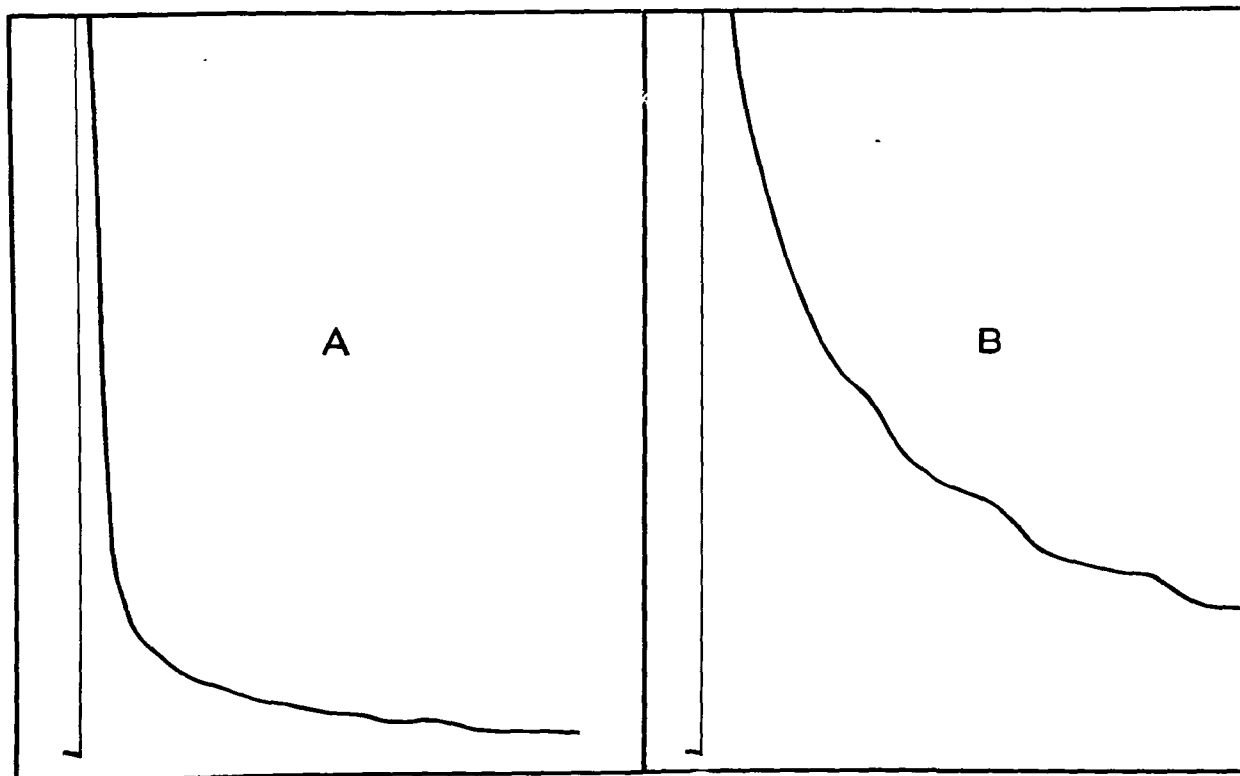
FIGURE 1. ELECTRON CAPTURE GAS CHROMATOGRAMS OF ONE-DAY OLD ADULT FLIES SHOWING THE EFFECT OF DIET ON THE AMOUNT OF BACKGROUND; A - ACETONE EXTRACTS (10 ML) OF 20 FLIES FED NON-FAT DRY MILK, AND B - ACETONE EXTRACTS (10 ML) OF 20 FLIES FED SUGAR.

RETENTION TIME (MINUTES)
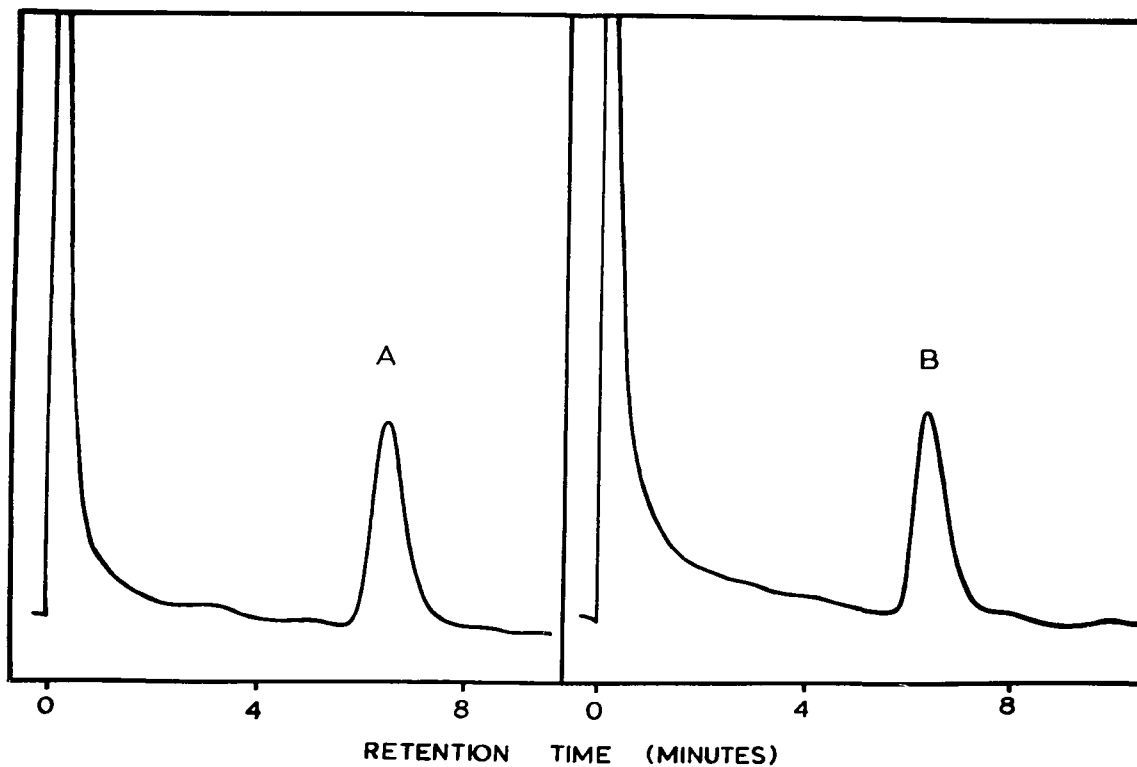
FIGURE 2. ELECTRON CAPTURE GAS CHROMATOGRAMS OF AN ACETONE SOLUTION OF DIELDRIN (A) AND ACETONE EXTRACTS OF INSECT TISSUES PLUS DIELDRIN (B). NOTE THE VERY LITTLE AMOUNT OF BACKGROUND IN CHROMATOGRAM B AS INDICATED BY THE SLIGHTLY HIGHER BASE LINE AFTER SOLVENT PEAK.
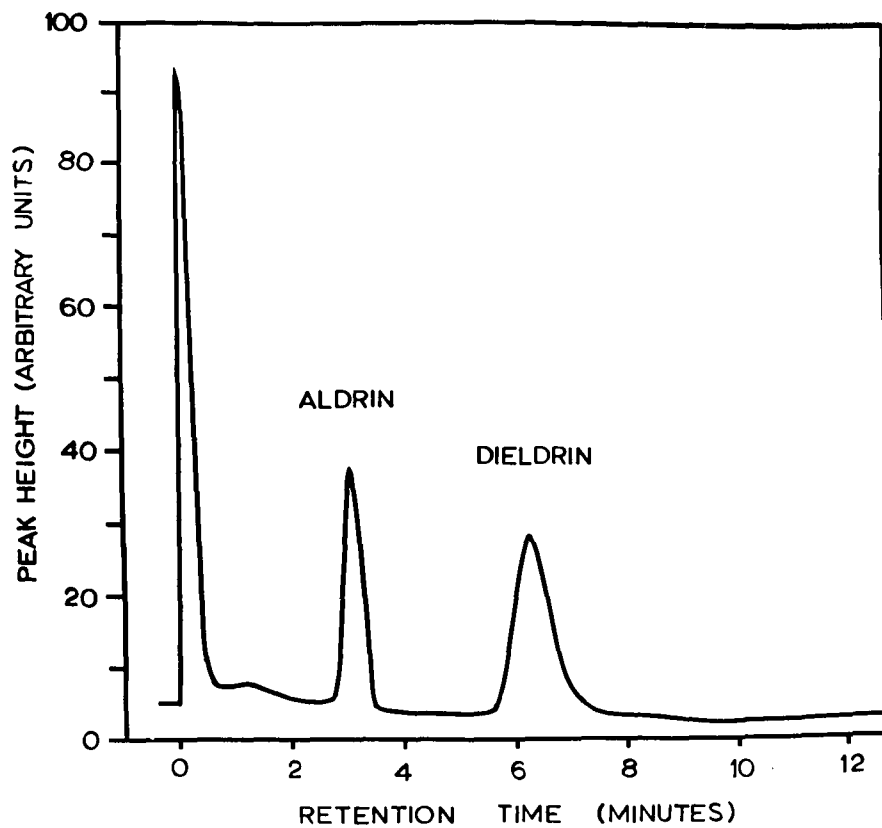
FIGURE 3. ELECTRON CAPTURE GAS CHROMATOGRAM OF ALDRIN
(0.002 UG) AND DIELDRIN (0.004 UG) SHOWING RELATIVE SENSI-
TIVITIES AND RETENTION TIMES. REFER TO TEXT FOR
CHROMATOGRAPHY CONDITIONS.
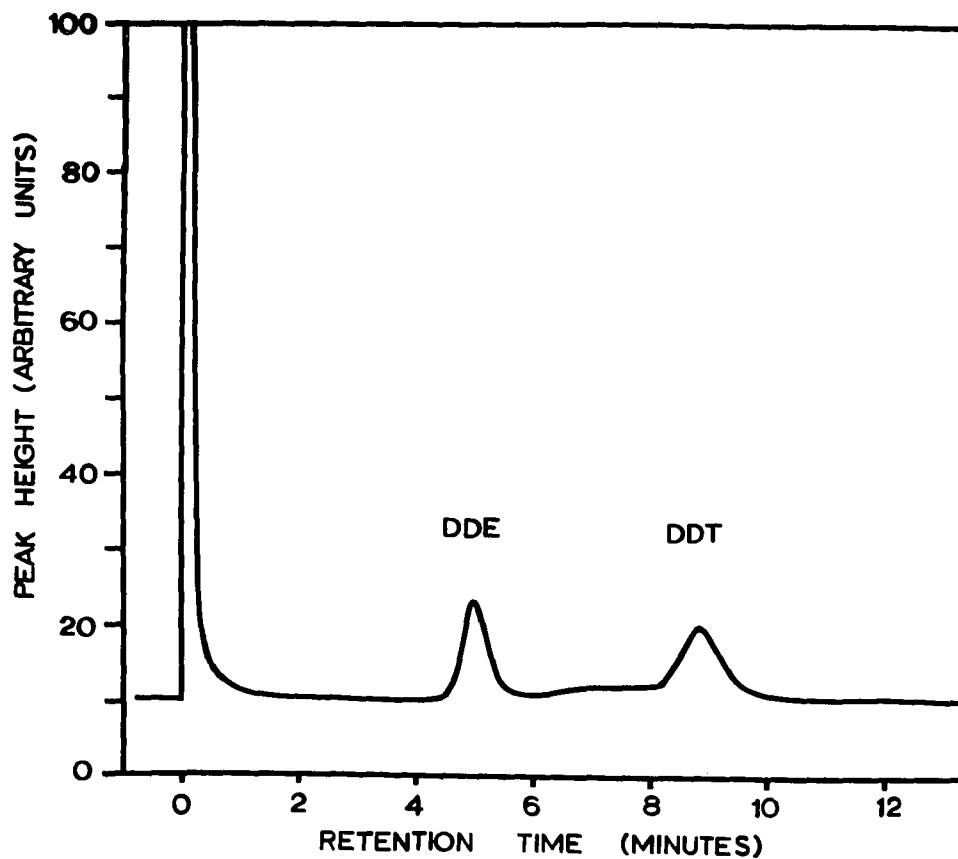
FIGURE 4. ELECTRON CAPTURE GAS CHROMATOGRAMS OF DDT
(0.0025 UG) AND DDE (0.00048 UG) SHOWING RELATIVE SENSITIVITIES
AND RETENTION TIMES. REFER TO TEXT FOR CHROMATO-
GRAPHY CONDITIONS.
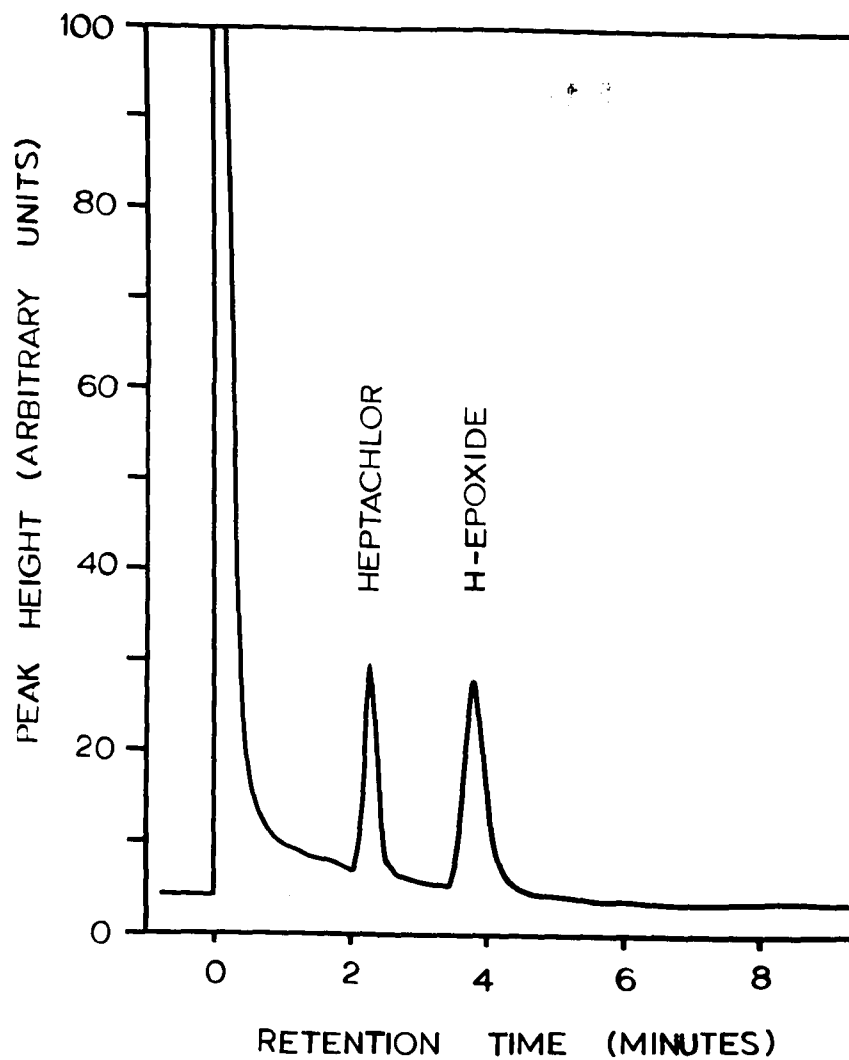
FIGURE 5. ELECTRON CAPTURE GAS CHROMATOGRAMS OF
HEPTACHLOR (0.002 µG) AND HEPTACHLOR EPOXIDE
(0.001 µG) SHOWING RELATIVE SENSITIVITIES AND
RETENTION TIMES. REFER TO TEXT FOR
CHROMATOGRAPHY CONDITIONS.

Calibration curves. - The calibration curves of aldrin and dieldrin,

DDT and DDE, and heptachlor and heptachlor epoxide are shown in Figures

6, 7, and 8. They were obtained by plotting sample size of each material

against detector response as measured by peak area in square millimeters.

The relative sensitivity of the electron capture detector to the insecti-

cides and their metabolites found in this study are presented in Table I.

These values are the relative electron affinities of the detector under the

conditions described for each material in this study. They do not represent

maximum sensitivity of the detector, but rather practical detectability of

the compounds. Levels of each material lower than those in the table could

be detected, but could not be quantified with any degree of reliability.

DDT

For the penetration and metabolism studies, the susceptible and re-

sistant flies were assayed for DDT and its metabolite DDE, 1, 3, 6, 12, 24,

48, 96, and 192 hours after topical treatment with DDT at a dosage of 0.125

μg/larva. Figure 9 and Table II summarize the amounts of DDT and DDE

detected externally and internally during these periods.

Penetration of DDT. - The rate of penetration of DDT was based on

the disappearance of the external DDT residue and the appearance of internal

DDT and/or DDE. There was a more rapid rate of disappearance of external

DDT from the susceptible SCR-60 strain than from the resistant Hawaiian

strain. At the end of 12 hours, the level of external DDT were 32 per cent
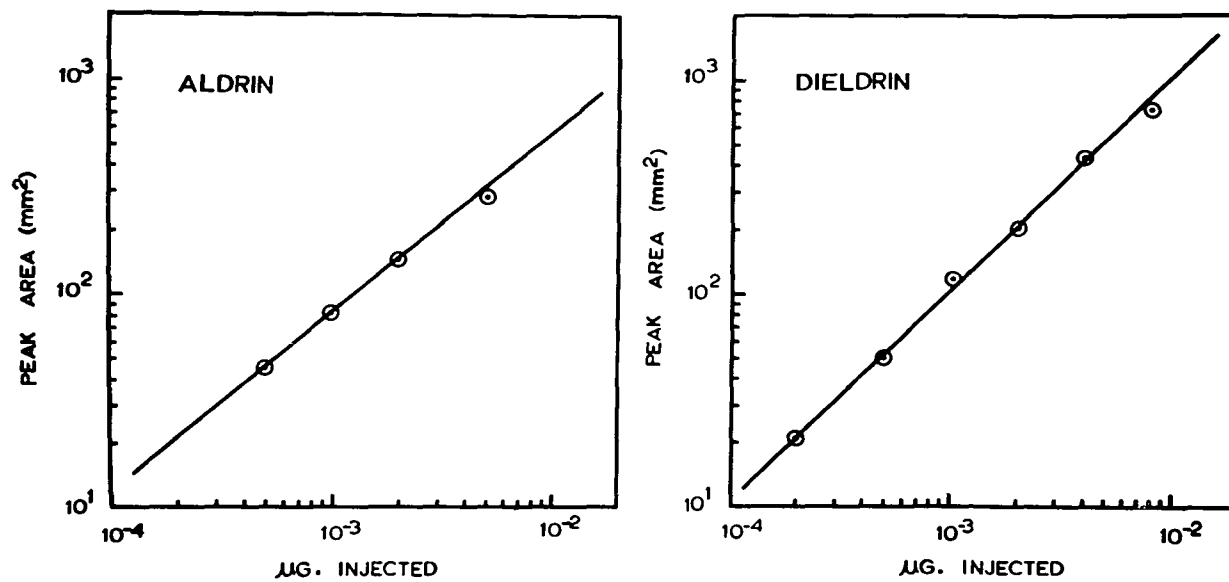
FIGURE 6. CALIBRATION CURVES OF ALDRIN AND DIELDRIN OBTAINED BY PLOTTING SAMPLE SIZE AGAINST DETECTOR RESPONSE AS MEASURED BY PEAK AREA IN MM^2.
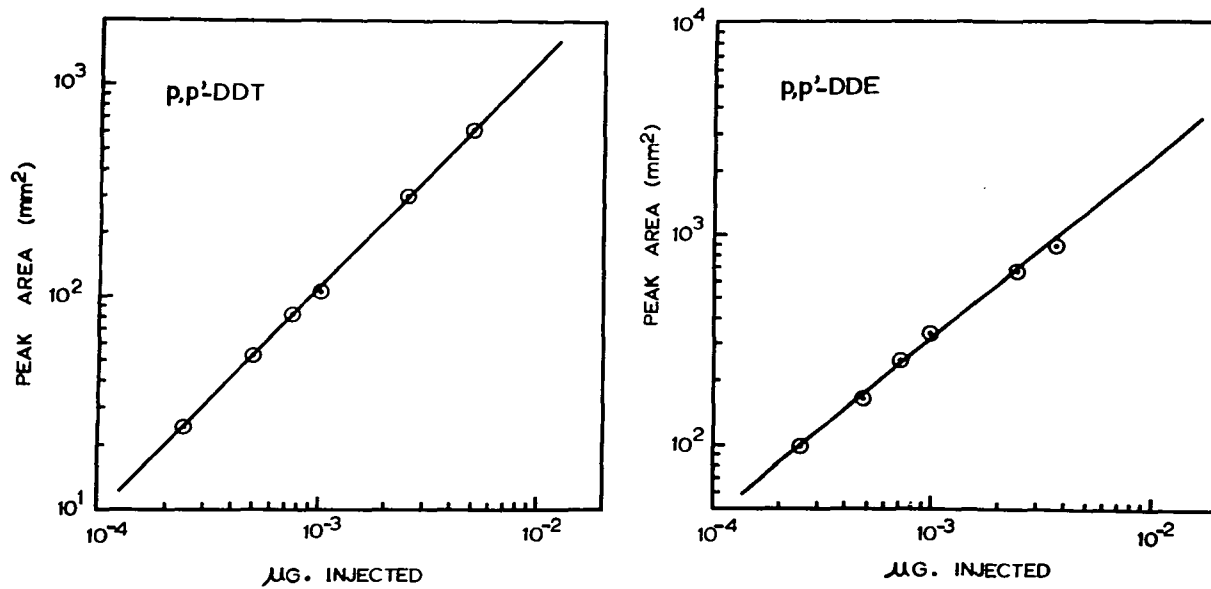
FIGURE 7. CALIBRATION CURVES OF DDT AND DDE OBTAINED BY PLOTTING SAMPLE SIZE
AGAINST DETECTOR RESPONSE AS MEASURED BY PEAK AREA IN MM^2.
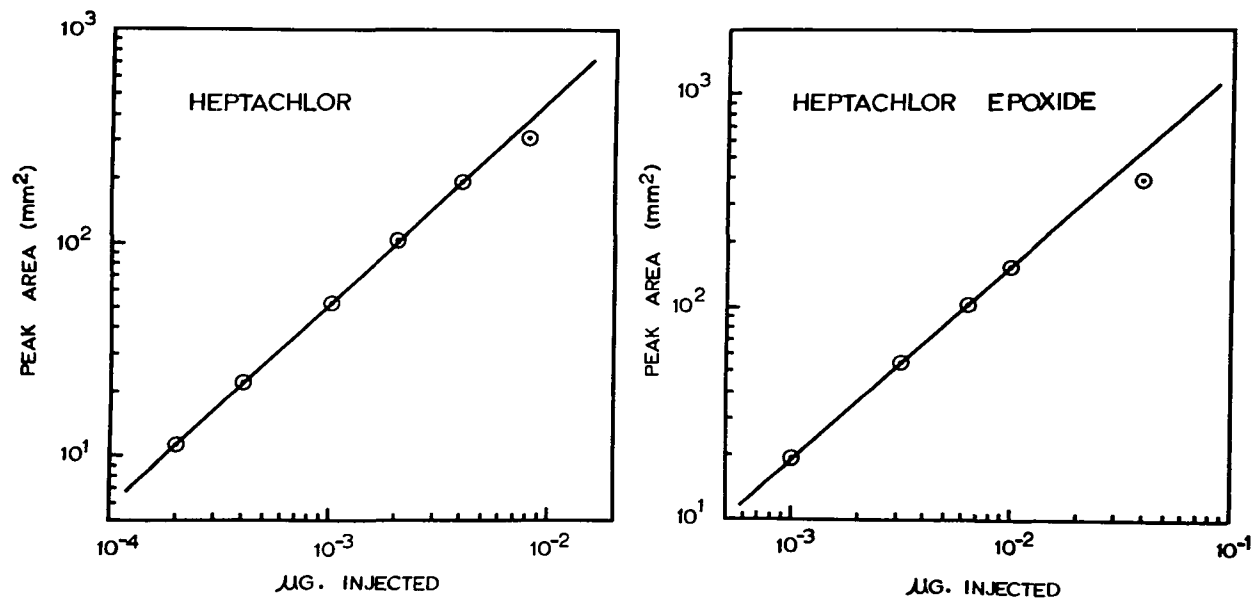
FIGURE 8. CALIBRATION CURVES OF HEPTACHLOR AND HEPTACHLOR-EPOXIDE OBTAINED
BY PLOTTING SAMPLE SIZE AGAINST DETECTOR RESPONSE AS MEASURED BY
PEAK AREA IN MM^2.

TABLE I. APPROXIMATE LIMIT OF SENSITIVITY OF
EXPERIMENTAL CHEMICALS

Chemicals	Sensitivity μg/larva
Aldrin	0.001
Dieldrin	0.004
Heptachlor	0.005
Heptachlor epoxide	0.002
DDT	0.010
DDE	0.002
DDA	12.0
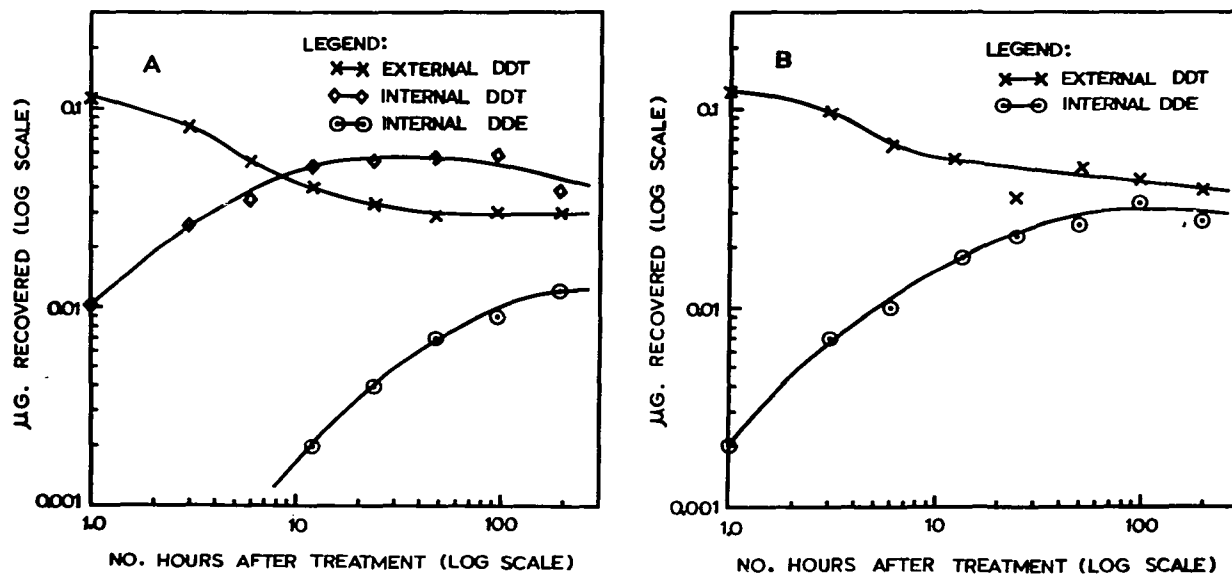
FIGURE 9. THE PENETRATION AND METABOLISM OF TOPICALLY APPLIED DDT TO LARVAE OF
SUSCEPTIBLE SCR-60 (A) AND RESISTANT HAWAIIAN (B) STRAINS OF THE HOUSE FLY AT
1, 3, 6, 12, 24, 48, 96, AND 192 HOURS AFTER TREATMENT.

TABLE II. THE PENETRATION AND THE METABOLISM OF TOPICALLY
APPLIED DDT TO LARVAE OF SUSCEPTIBLE SCR-60 AND RESIS-
TANT HAWAIIAN STRAINS OF THE HOUSE FLY AT DIFFERENT
TIME INTERVALS AFTER TREATMENT

Number Hours After Treat- ment[a]	SCR - 60 STRAIN				
	External DDT Re- covered[b] μg/larva	Internal DDT Re- covered μg/larva	Internal DDE Re- covered μg/larva	%Applied Toxicant Recovered[c] Internally	%Applied Toxicant Re- covered[d]
1	0.111	0.010	0	8.0	96.8
3	0.081	0.026	0	20.8	85.6
6	0.054	0.035	<0.002	28.0	71.2
12	0.040	0.052	0.002	43.2	75.2
24	0.033	0.054	0.004	46.4	72.8
48	0.029	0.056	0.006	49.6	72.8
96	0.031	0.058	0.008	52.8	77.6
192	0.030	0.038	0.012	40.0	64.0
	HAWAIIAN STRAIN				
1	0.120	0	0.002	1.6	97.6
3	0.096	0	0.007	5.6	82.4
6	0.064	0	0.010	8.0	59.2
12	0.056	0	0.018	14.4	59.2
24	0.036	0	0.023	18.4	47.2
48	0.050	0	0.026	20.8	60.8
96	0.044	0	0.034	27.2	62.4
192	0.039	0	0.027	21.6	52.8

[a] Pupation of mature third instar larvae occurred in about 24-48 hours after
treatment.

[b] The external DDT comprised of the beaker rinse and insect rinse.

[c] Combined amount of internal DDT and internal DDE expressed as percentage
in terms of amount of DDT originally applied.

[d] Determined by combining together the amount of external DDT, internal DDT
and DDE and expressed as percentage in terms of the amount of DDT originally
applied.

and 44 per cent of the dose applied on the SCR-60 and Hawaiian strain,
respectively. At the end of 48 hours, the SCR-60 strain contained 23 per
cent of the initial deposit while the Hawaiian strain still contained 40 per
cent of the deposit. At the same time, the presence of internal DDT in
the SCR-60 strain was rapidly detected.

Metabolism of DDT. - Resistance of the larvae of the Hawaiian
strain to DDT is attributable to its rapid conversion to the nontoxic deriva-
tive DDE. No internal DDT was recovered from the Hawaiian strain at any
time indicating complete dehydrochlorination of all the DDT that penetrated.
Measurable amounts of p, p'-DDT could be recovered from the susceptible
larvae one hour after topical application, building up to a maximum of 0.058
µg/pupa 96 hours after treatment. The larvae of the SCR-60 strain have a
very limited ability to detoxify DDT to DDE. No DDE could be detected in
the SCR-60 larvae prior to 12 hours after treatment.

Latent Toxicity of DDT. - To elucidate the phenomenon of latent
toxicity, a study of insecticidal storage and carry-over to the adult stage
was undertaken. Table III summarizes the latent toxicity and carry-over
of DDT to the adult stage in the susceptible and resistant strains of the house
fly. There were no differences in detectable DDT and DDE due to sex. The
adult females of the SCR-60 strain that died between 1 and 3 days after emer-
gence due to latent toxicity contained 0.042 µg DDT and 0.010 µg DDE. On
the other hand, surviving adults of this strain contained insignificant amounts

TABLE III. THE LATENT TOXICITY AND CARRY-OVER OF DDT AND
EXCRETION OF DDE IN THE SUSCEPTIBLE AND RESISTANT
STRAIN OF THE HOUSE FLY TOPICALLY TREATED IN
THE LARVAL STAGE

Days After Emergence	SCR - 60 STRAIN							
	Dead Adults				Live Adults			
	DDT μg/fly		DDE μg/fly		DDT μg/fly		DDE μg/fly	
	M	F	M	F	M	F	M	F
1	-	-	-	-	0.010	0.010	0.0025	0.0025
(1–3 bracket)	0.037	0.042	0.010	0.010				
3	-	-	-	-	0	0	0.0020	0.0020
6	-	-	-	-	0	0	0	0
10	-	-	-	-	0	0	0	0
14	-	-	-	-	0	0	0	0

	HAWAIIAN STRAIN							
1	No dead adults	No dead adults	No dead adults	No dead adults	0	0	0.016	0.025
3					0	0	0.014	0.016
6					0	0	0.0025	0.0065
10					0	0	<0.002	0.0060
14					0	0	0	0.0025

of DDT 1 day after emergence, but no DDT was found 3 days after emergence.

DDE was present at levels of 0.025 and 0.020 µg 1- and 3- days after emer-

gence, respectively. DDT had no latent toxicity to the Hawaiian strain nor

was DDT detected in this strain.

Excretion of DDT. - Excretion was determined by the loss of internal

DDE. Surviving flies from the latent toxicity study were observed for 14

days after emergence and the amounts of DDT and DDE present were deter-

mined. The results are summarized in Table III. Lower amounts of DDE

were found in the survivors of the susceptible strain than in the resistant

strain. There was no difference between the sexes of the SCR-60 strain in

the rate of excretion of DDE. However, there appeared to be a more rapid

loss of DDE from the males of the resistant strain than from the females.

Adult Toxicity and Metabolism. - Sherman and Sanchez (In press)

determined the LD_{50} of DDT to the susceptible strain of the housefly to be

0.035 µg/female. The metabolism of this dosage of DDT by the adult females

was studied. Forty-eight hours after application, 40 per cent mortality

occurred. Table IV presents the amounts of DDT and DDE recovered from

these flies. The DDT topically applied to the adult susceptible female fly

was rapidly metabolized. The results were comparable to those found in the

latent toxicity studies since in both cases, the dead flies contained more DDT

and DDE than did the survivors.

ALDRIN

Table V and Figure 10 summarizes the amounts of aldrin and its

TABLE IV. METABOLISM OF DDT APPLIED AT THE RATE OF 0.035
UG/FLY TO 2-3 DAY OLD FEMALES OF THE SCR-60 STRAIN
48 HOURS AFTER TREATMENT[1]/

Insecticide and Metabolite	Beaker Rinse	TOXICANT RECOVERED		
		External μg/female	Internal μg/female	% of Applied
DDT (Living flies)		0	< 0.002	--
DDT (Dead flies)	} 0	0	0.006	17.14
DDE (Living flies)		0	0.001	2.85
DDE (Dead flies)	} 0	0	0.002	5.71

[1]/ Output sensitivity setting of 10X used rather than the usual 1X. This
was necessitated by the very low amounts of DDT to be analyzed.

TABLE V. THE PENETRATION AND METABOLISM OF TOPICALLY
APPLIED ALDRIN TO LARVAE OF SUSCEPTIBLE SCR-60
AND RESISTANT HAWAIIAN STRAINS OF THE
HOUSE FLY AT DIFFERENT TIME INTERVALS
AFTER TREATMENT

Number Hours After Treatment[a]	SCR-60 STRAIN				
	External Aldrin Recovered [b] μg/larva	Internal Aldrin Recovered μg/larva	Internal Dieldrin Recovered μg/larva	%Applied Toxicant Recovered Internally[c]	%Applied Toxicant Re-covered[d]
1	0.150	0.008	< 0.004	3.2	63.2
3	0.119	0.014	0.008	8.8	56.4
6	0.073	0.020	0.028	19.2	48.4
12	0.025	0.007	0.054	24.4	34.4
24	0.012	0.004	0.058	24.8	29.6
48	0.005	0	0.077	30.8	32.8
96	< 0.001	0	0.068	27.2	27.2
192	< 0.001	0	0.042	16.8	16.8

	HAWAIIAN STRAIN				
1	0.168	0.008	0	3.2	70.4
3	0.123	0.014	0.008	8.8	58.0
6	0.072	0.014	0.012	10.4	39.2
12	0.056	0.016	0.032	19.2	41.6
24	0.032	0.012	0.048	24.0	36.8
48	0.016	0.006	0.047	21.2	27.6
96	0.010	0.004	0.043	18.8	22.8
192	< 0.001	0.004	0.068	28.8	28.8

[a] Pupation of mature third instar larvae occurred in about 24-48 hours after treatment.

[b] The external aldrin comprised of the beaker rinse and insect rinse.

[c] Combined amount of internal aldrin and internal dieldrin expressed as percentage in terms of amount of aldrin originally applied.

[d] Determined by combining together the amount of external aldrin, internal aldrin, and internal dieldrin and expressed as percentage in terms of the amount of aldrin originally applied.
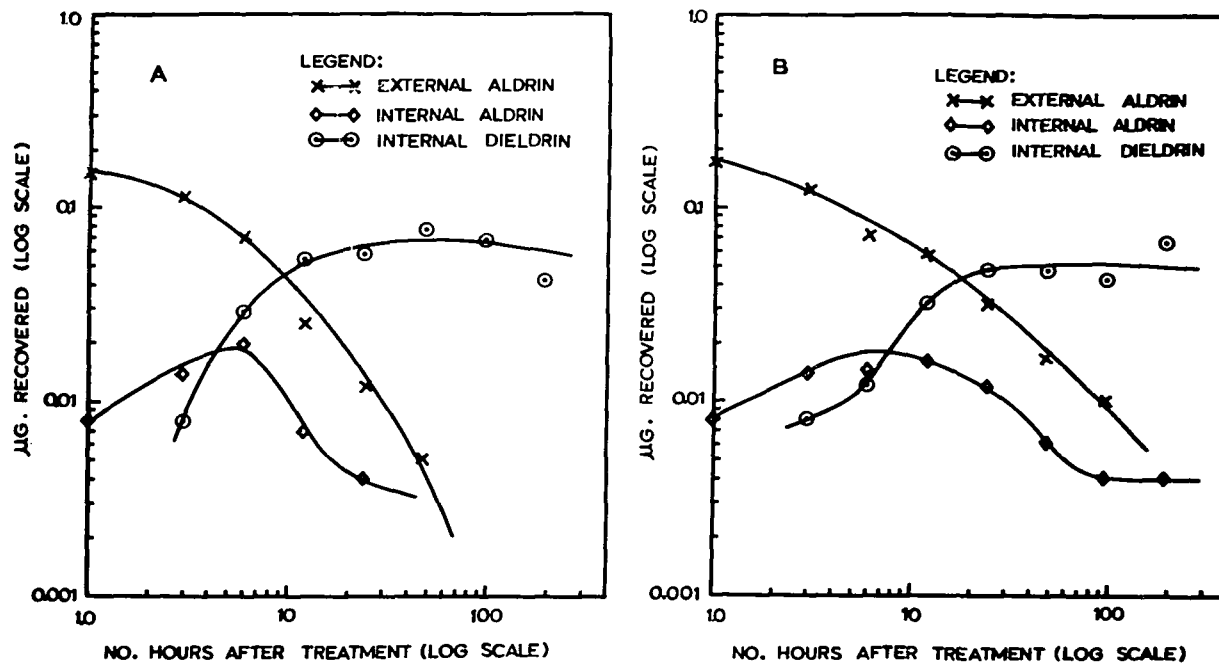
FIGURE 10. THE PENETRATION AND METABOLISM OF TOPICALLY APPLIED ALDRIN TO LARVAE OF SUSCEPTIBLE SCR-60 (A) AND RESISTANT HAWAIIAN (B) STRAINS OF THE HOUSE FLY AT 1, 3, 6, 12, 24, 48, 96, AND 192 HOURS AFTER TREATMENT.

epoxide dieldrin detected externally and internally during the 192 hours

following topical application of 0.250 μg/larva.

Penetration of aldrin. - The rate of disappearance of external deposits of·

topically applied aldrin was significantly faster in the susceptible than in the

resistant strain. At the end of 12 hours, the amount of external aldrin re-

maining on the SCR-60 strain was about 10 per cent of the applied dosage

compared to 23 per cent for the Hawaiian strain; decreasing to 2 and 7 per

cent for each, respectively, at the end of 48 hours. There was also a more

rapid increase of aldrin and dieldrin detected internally in the susceptible

strain than in the resistant strain.

Metabolism of aldrin. - The amounts of internal aldrin during the

first 6 hours after treatment in both SCR-60 and Hawaiian strains were about

the same. Thereafter the amount of internal aldrin was considerably less

in the SCR-60 strain than in the Hawaiian strain. Forty-eight hours after

treatment no internal aldrin could be detected in the susceptible strain.

Complete epoxidation of aldrin to dieldrin apparently occurred during this

time. Internal aldrin was still present in the Hawaiian strain at the end of

192 hours (8 days). The rapid rate of disappearance of internal aldrin corre-

lates very well with the more rapid build-up of its epoxide, dieldrin in the

SCR-60 strain. Dieldrin could be detected in the SCR-60 strain but not in

the Hawaiian strain 1 hour after treatment. The concentration of this metabolite

reached a maximum after 48 hours (0.077 μg/insect) in the SCR-60 strain

compared to 192 hours in the Hawaiian strain (0.068 μg/insect).

Latent toxicity of aldrin. - Table VI summarizes the latent toxicity

of aldrin and the carry-over of dieldrin to the adult stage in the susceptible

and resistant strains of the house fly. No aldrin was found in the adults

of either strain of house fly. Complete epoxidation had occurred by this

time. During the first three days after emergence of the adult flies there

appeared to be no significant differences in the amounts of detectable dieldrin

due to sex or strain of house fly. In the susceptible strain, the dead and living

flies had approximately the same content of dieldrin.

Sherman and Sanchez (In press) found the topical LD_{50} for aldrin and

dieldrin to be 0.014 μg and 0.107 μg/fly, respectively for the susceptible

adult female and 4.74 μg and 15.8 μg/fly, respectively for the resistant

adult female. The amounts of dieldrin found in the dead and surviving adult

flies were more than enough to cause latent toxicity to the susceptible adult.

Excretion of dieldrin. - Excretion was determined by the loss of in-

ternal dieldrin. The results are summarized in Table VI. The males of

both strains of house fly excreted dieldrin at a more rapid rate than their

respective females. The resistant strain excreted dieldrin at a significantly

more rapid rate than did the susceptible strain.

Adult toxicity and metabolism. - The previously determined LD_{50} of

aldrin for the female susceptible fly (0.014 μg) was applied to susceptible

females and the metabolism studied. Forty-eight hours after application

25 per cent mortality occurred. Table VII presents the amounts of aldrin

TABLE VI. THE LATENT TOXICITY, CARRY-OVER AND EXCRETION OF DIELDRIN IN THE SUSCEPTIBLE AND RESISTANT HOUSE FLY TOPICALLY TREATED WITH ALDRIN IN THE LARVAL STAGE

Days After Emergence	SCR-60 STRAIN							
	Dead Adults				Live Adults			
	Aldrin $\mu g/fly$		Dieldrin $\mu g/fly$		Aldrin $\mu g/fly$		Dieldrin $\mu g/fly$	
	M	F	M	F	M	F	M	F
1	-	-	-	-	0	0	0.114	0.125
	0	0	0.096	0.129				
3	-	-	-	-	0	0	0.078	0.090
6	-	-	-	-	0	0	0.060	0.081
10	-	-	-	-	0	0	0.021	0.052
14	-	-	-	-	0	0	0.025	0.057
	HAWAIIAN STRAIN							
1	No dead adults	No dead adults	No dead adults	No dead adults	0	0	0.126	0.137
3					0	0	0.088	0.089
6					0	0	0.032	0.065
10					0	0	0.014	0.036
14					0	0	0.010	0.020

TABLE VII. METABOLISM OF ALDRIN APPLIED AT THE RATE OF 0.014 JIG/FLY TO 2-3 DAY OLD FEMALES OF THE SCR-60 STRAIN 48 HOURS AFTER TREATMENT

Insecticide and Metabolite	Beaker Rinse	TOXICANT RECOVERED		
		External	Internal	
		μg/ female	μg/ female	% of Applied

Aldrin (Living flies)		0	0	
	0			
Aldrin (Dead flies)		0	0	----
Dieldrin (Living flies)		0	0.007	50.00
	0			
Dieldrin (Dead flies)		0	0.008	57.14

and dieldrin recovered from these flies.

HEPTACHLOR

Table VIII and Figure 11 summarize the amounts of heptachlor and its epoxide detected externally and internally during the 192 hours following topical application of 0.500 µg/larva.

Penetration of heptachlor. - There was a very marked difference in disappearance of external deposits between the susceptible and resistant strains of house fly. Six hours from the time of treatment only 4 per cent of the applied dose of heptachlor was detectable on the SCR-60 strain compared to 54 per cent on the Hawaiian strain. No external heptachlor was detectable on the SCR-60 strain at the end of 24 hours but 16 per cent of the applied dose was still present externally on the Hawaiian strain during the same period. There was also a more rapid increase of internal heptachlor and its epoxide in the susceptible strain than in the resistant larvae in the first few hours after treatment.

Metabolism of heptachlor. - The rate of detection of internal heptachlor in the susceptible strain was more rapid during the first 12 hours after treatment than in the resistant strain. However, after this period the amounts of heptachlor detected were comparable for both strains. The rate of epoxidation of heptachlor progressed at similar rates in both strain of flies. The amount of heptachlor epoxide detected in the SCR-60 strain at the different time intervals was similar to that found in the Hawaiian strain.

TABLE VIII. THE PENETRATION AND METABOLISM OF TOPICALLY
APPLIED HEPTACHLOR TO LARVAE OF SUSCEPTIBLE SCR-60
AND RESISTANT HAWAIIAN STRAINS OF THE HOUSE FLY AT
DIFFERENT TIME INTERVALS AFTER TREATMENT

Number Hours After Treatment[a]	SCR -60 STRAIN				
	External Heptachlor Recovered[b] µg/larva	Internal Heptachlor Recovered µg/larva	Internal H-epoxide Recovered µg/larva	% Applied Toxicant Recovered internally[c]	% Applied Toxicant Recovered[d]
1	0.405	0.012	<0.002	2.4	83.4
3	0.110	0.051	0.008	13.0	33.8
6	0.022	0.058	0.014	14.5	18.80
12	0.006	0.055	0.025	16.0	17.2
24	0	0.029	0.026	11.0	11.0
48	0	0.016	0.046	12.4	12.4
96	0	0.012	0.042	10.8	10.8
192	0	0.008	0.043	10.2	10.2
	HAWAIIAN STRAIN				
1	0.445	0.010	< 0.002	2.0	91.0
3	0.330	0.028	0.003	6.2	72.2
6	0.268	0.052	0.018	14.0	67.6
12	0.095	0.041	0.028	13.8	32.8
24	0.080	0.033	0.068	20.2	36.2
48	0.020	0.016	0.058	14.8	18.8
96	--	0.011	0.044	11.0	11.0
192	0	0.006	0.075	16.2	16.2

[a] Pupation of mature third instar larvae occurred in about 24-48 hours after treatment.

[b] The external heptachlor comprised of the beaker rinse and insect rinse.

[c] Combined amount of internal heptachlor and internal heptachlor epoxide expressed as percentage in terms of amount of heptachlor originally applied.

[d] Determined by combinging together the amount of external heptachlor, internal heptachlor, and internal heptachlor epoxide and expressed as percentage in terms of heptachlor originally applied.
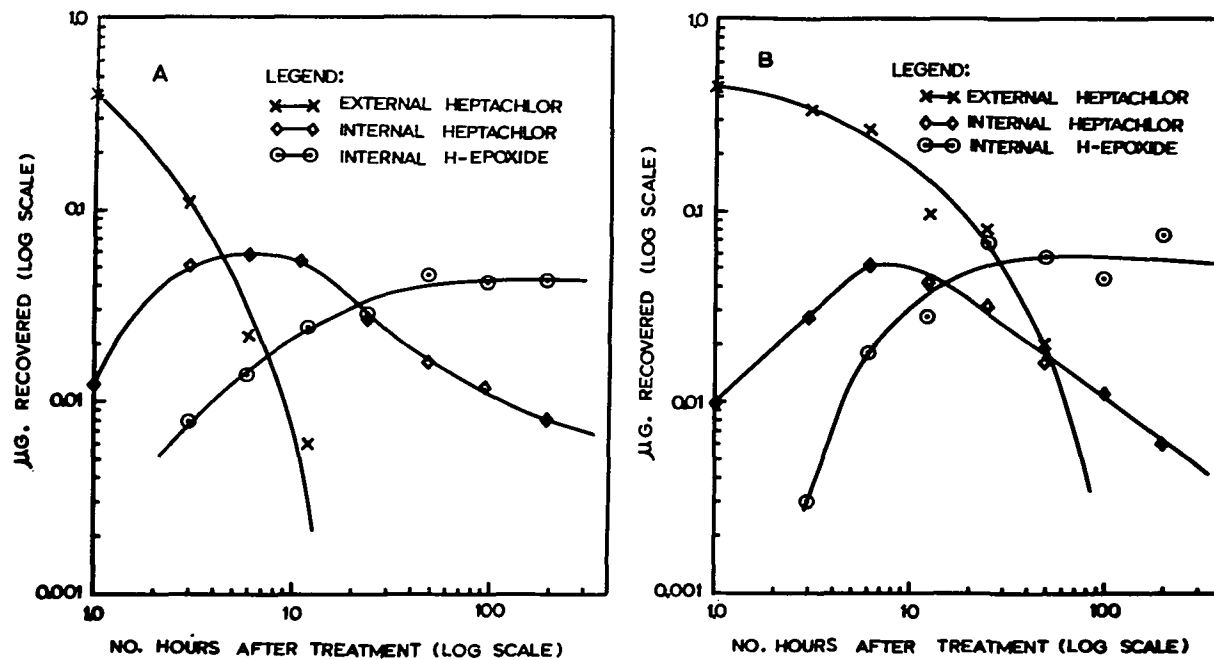
FIGURE 11. THE PENETRATION AND METABOLISM OF TOPICALLY APPLIED HEPTACHLOR TO LARVAE OF SUSCEPTIBLE SCR-60 (A) AND RESISTANT HAWAIIAN (B) STRAINS OF THE HOUSE FLY AT 1, 3, 6, 12, 24, 48, 96, AND 192 HOURS AFTER TREATMENT.

Latent toxicity of heptachlor. - Table IX summarizes the latent toxicity

of heptachlor and the carryover of its epoxide to the adult stage in the sus-

ceptible and resistant strains of the house fly. No heptachlor was found in

the adults of either strain of house fly. Complete epoxidation had occurred

by this time. During the first three days after emergence of the adult flies,

there appeared to be no significant differences in the amounts of detectable

heptachlor epoxide due to sex or strain of house fly. In the susceptible

strain, the dead and living flies had approximately the same content of

heptachlor epoxide.

The topical heptachlor LD_{50} for adult females of the susceptible strain

and the resistant strain was found to be 0.042 and 24.2 μg/fly, respectively

(Sherman and Sanchez, In Press). The amounts of heptachlor epoxide found

in the dead and surviving susceptible flies were more than enough to account

for latent toxicity to the adult.

Excretion of heptachlor epoxide. - Excretion was determined by the

loss of internal heptachlor epoxide. The results are summarized in Table IX.

The males of both strains excreted heptachlor epoxide at a more rapid rate

than their respective females. The resistant strain appeared to excrete

the epoxide at a slightly slower rate than did the susceptible strain.

Adult toxicity and metabolism. - The previously determined LD_{50} of

heptachlor for the female susceptible fly (0.042 μg) was applied to 40 suscep-

tible females and the metabolism studied. Forty-eight hours after application

TABLE IX. THE LATENT TOXOCITY, CARRY-OVER AND EXCRETION OF HEPTACHLOR EPOXIDE IN THE SUSCEPTIBLE AND RESISTANT STRAIN OF THE HOUSE FLY TOPICALLY TREATED WITH HEPTACHLOR IN THE LARVAL STAGE

Days After Emergence	SCR-60 STRAIN							
	Dead Adults				Live Adults			
	Heptachlor µg/fly		H-epoxide µg/fly		Heptachlor µg/fly		H-epoxide µg/fly	
	M	F	M	F	M	F	M	F
1	-	-	-	-	0	0	0.175	0.172
	0	0	0.180	0.185				
3					0	0	0.092	0.125
6	-	-	-	-	0	0	0.050	0.080
10	-	-	-	-	0	0	0.014	0.041
14	-	-	-	-	0	0	0.008	0.022

Days After Emergence	HAWAIIAN STRAIN							
	Heptachlor µg/fly		H-epoxide µg/fly		Heptachlor µg/fly		H-epoxide µg/fly	
	M	F	M	F	M	F	M	F
1	No dead adults	No dead adults	No dead adults	No dead adults	0	0	0.175	0.185
3					0	0	0.104	0.120
6					0	0	0.053	0.067
10					0	0	0.028	0.065
14					0	0	0.017	0.047

38 per cent mortality occurred. Table X presents the amounts of heptachlor and its epoxide recovered from these flies. No heptachlor was found in any of the flies 48 hours after treatment. The amount of heptachlor epoxide detected in the dead flies was somewhat higher than in the survivors.

TABLE X. THE METABOLISM OF HEPTACHLOR APPLIED AT THE
RATE OF 0.042 µG/FLY TO 2-3 DAY OLD SCR-60 STRAIN
FEMALES 48 HOURS AFTER TREATMENT

Insecticide and Metabolite	Beaker Rinse	TOXICANT RECOVERED		
		External µg/ female	Internal µg/ female	% of Applied
Heptachlor (Living flies)	0	0	0	----
Heptachlor (Dead flies)		0	0	----
Heptachlor epoxide (Living flies)	0	0	0.008	19.04
Heptachlor epoxide (Dead flies)		0	0.012	28.57

DISCUSSION

The slower rate of cuticular penetration of DDT observed in the larvae

of the resistant Hawaiian strain may greatly intensify its resistance to DDT.

The slower rate of cuticular penetration of DDT per se cannot explain the

resistance of the Hawaiian strain to this compound. It can only postpone

or retard the accumulation of toxicant till enough has penetrated to initiate

toxic action. However, this property, in association with the ability to

rapidly metabolize DDT to DDE, is definitely of significance. It is contribut-

ing to the detoxification mechanism by reducing the rate of penetration of the

toxicant, such that it can easily be handled or detoxified.

The pattern of aldrin and heptachlor was not as clear as that presented

by DDT. The somewhat slower penetration of aldrin and heptachlor into

larvae of the resistant Hawaiian strain as compared to the susceptible

SCR-60 strain appears not to be important in explaining the latent toxicity

of these compounds to the susceptible strain or resistance, since the amounts

of the metabolites dieldrin and heptachlor epoxide carried over from the larval

to the adult stage were practically the same for both strains.

Several workers have shown that aldrin undergoes epoxidation in insects

to form the compound dieldrin (Gianotti et al., 1956; Brooks, 1960; Cohen

and Smith, 1961; and Earle, 1963). It has also been shown (Perry et al., 1958)

that heptachlor was converted by both susceptible and resistant house flies

to a toxic derivative, heptachlor epoxide. Metabolism of heptachlor by house

flies was not considered to be an important factor in resistance to said com-
pound (Perry, 1960). Perry considered the probable cause of resistance to
be the removal of the toxic metabolite heptachlor epoxide from the site of
action and its storage in non-sensitive tissues.

In the present study, the mature third instar larvae of both the susceptible
SCR-60 and resistant Hawaiian strains rapidly converted absorbed aldrin
and heptachlor to their corresponding epoxides, dieldrin and heptachlor
epoxide. The metabolism patterns of aldrin and heptachlor in both strains
did not shed any light as to the cause of either resistance or latent toxicity.
The conversion of aldrin to dieldrin was faster in the susceptible than in
the resistant strain, while the rate of conversion of heptachlor to the
corresponding more toxic epoxide was about equal in both strains after the
first 12 hours. However, this phenomenon does not appear to be involved in
the difference in resistance between the two strains, since eventually both
contained comparable levels of both epoxides.

In experiments employing radioactive materials, it was indicated that
resistance to dieldrin in house flies is due to factors other than cuticular
penetration, differences in metabolism, or excretion of the insecticide
(Brooks, 1960). Earle (1963) concluded that the metabolism of cyclodiene
insecticides is a relatively unimportant resistance mechanism in house flies.
He found that large quantities of dieldrin absorbed during the larval stage of
resistant flies remained unchanged throughout the pupal stage and were

excreted during the first few days of adult life. Winteringham and

Harrison (1959) using S^{35}-labeled analog of dieldrin showed that it was

partly metabolized by resistant and susceptible flies. Approximately 3 per

cent of the dosage applied was excreted unchanged and 1 to 3 per cent

was excreted as water-soluble and water-insoluble metabolites. They also

showed that the non-insecticidal bromine analog of dieldrin was excreted

unchanged in equal proportions of both resistant and susceptible house

flies. Again, small amounts of water-soluble metabolites were found.

Most of the dieldrin and heptachlor epoxide carried over from the

larval to the adult stage were excreted by the susceptible SCR-60 and

resistant Hawaiian flies within two weeks of adult life. The male flies

of both strains excreted the metabolites of aldrin and heptachlor at a faster

rate than the female flies. This may be due to the higher rate of metabolism

in the males as indicated by its higher oxygen consumption (Edwards, 1953).

In attempting to explain the differential latent toxicity of the chlorinated

hydrocarbon insecticides to the various species of fruit flies, Tamashiro

and Sherman (1955) in effect suggested the following:

The larvae, pupae, and adults of one species may detoxify the insecti-

cide more readily than the corresponding stages of the other. Or, the larvae

of one species may be much more resistant than the corresponding adult, but

there is little difference in resistance between the larva and adult of the

other species.

Where larvae are much more resistant than the corresponding adults,

the carry-over of the insecticide and/or its toxic metabolite(s) from the

larval to the adult stage may be sufficient to cause mortalities among the

susceptible flies shortly after emergence.

During transformation to the pupa, the larval tissues are largely or

completely destroyed and the disintegration products of the larval fat-body

provide the necessary material for histogenesis. In the light of the findings

of Gianotti et al. (1956) that dieldrin is accumulated in the fat-body of

Periplaneta americana (L.) and the observation in Calliphora erythrocephala

Meigen that the larval fat-body is entirely replaced by adult tissue which is

completed only after the emergence of the adult (Perez, 1910), it is very

tempting to speculate that at the time of transformation to an adult, insecti-

cide which is absorbed and stored in the larval tissues is completely released,

so that toxic symptoms start to appear after emergence of the adult. On

the other hand, Perry et al. (1958) suggested that the ability of a heptachlor-

resistant strain of house fly to withhold large quantities of the heptachlor

epoxide without apparent harmful effects may involve a mechanism of in-

activation of the toxicant by solubilization or storage in non-sensitive tissues,

such as the fat-body. They cited the observation that starting heptachlor-

treated resistant flies after the epoxide had been formed caused the onset

of strong symptoms of poisoning. This observation is not without merit.

Since the food reserves in the fat-body are mobilized and depleted during

starvation, it is conceivable that the toxicant tied-up in the fat-body is released when it is utilized by the insect.

Sherman and Sanchez (In Press) found the Hawaiian strain to be more resistant than the SCR-60 strain to aldrin, DDT, heptachlor, and a number of other chlorinated hydrocarbon insecticides in both adult and larval stages. The difference in resistance, however, was greatest in the larval stage. The larvae of the Hawaiian strain required a tremendous amount of insecticide applied to their bodies in order to cause even low levels of mortality. The three insecticides were more toxic to the larvae of the SCR-60 strain than to the local Hawaiian strain as follows: aldrin $>2,273$ times; DDT, $> 8,333$ times; and heptachlor, $> 2, 813$ times.

They found, also, the characteristic of latent toxicity to be exhibited to a considerable degree by aldrin, heptachlor, and DDT on the SCR-60 strain. Only the first two compounds caused some latent toxicity to the Hawaiian strain and only to a very limited extent and at very high dosages.

Resistance of the larvae of the Hawaiian strain to DDT is attributable to its rapid conversion to the non-toxic derivative DDE. No internal DDT was recovered from the Hawaiian strain at any time, indicating complete dehydrochlorination of all the DDT that penetrated. Measurable amounts of DDT could be recovered from the susceptible SCR-60 larvae one hour after topical application, increasing to a maximum 96 hours after treatment. The larvae of the SCR-60 strain were limited in their ability to detoxify DDT to DDE.

The role of speed of metabolism in DDT resistance was shown by the work of Menn et al. (1957) with susceptible and resistant adult house flies exposed to a sub-lethal dose of DDT at $35^{o}C$. The amount of internal unreacted DDT in susceptible flies was high within 2 hours and decreased very slowly with time. The level of unreacted DDT in the resistant flies decreased sharply after 2 hours, to a level much lower than in the susceptible flies.

The latent toxicity of DDT to the susceptible strain of the house fly may be explained by the discovery of Moorefield (1958) that in a susceptible strain, the larval stage contains considerable DDT-dehycrochlorinase but this enzyme cannot be found in the adults (amounts may be too low to be detected by available analytical methods). However, both the larvae and adults of the resistant strain of house fly contain a great amount of the enzyme (Moorefield and Kearns, 1957; and Moorefield, 1958). Although Kerr et al. (1957) reported that DDT-ase can be detected in susceptible adult house flies, the amounts detected were insignificant. Assuming these findings to be applicable to Hawaiian and SCR-60 strains, the presence of the enzyme in the larval stage of the susceptible strain may be one of the factors responsible for the greater resistance of the larvae to DDT compared to that of the adults. The larvae may reduce the level of active DDT but do not deactivate all of it, so that the remaining DDT may affect the emerging adults. This view is further strengthened by an earlier work in which a direct correlation has been established between the presence of DDT resistance

in house fly and the occurrence of the detoxification enzyme DDT-dehy-drochlorinase (Sternberg et al. 1954).

The latent toxicity of DDT to the susceptible SCR-60 strain was due to the carry-over of sufficient amounts of unchanged DDT from the larval to the adult stage to cause mortality in the flies shortly after emergence. The living flies contained very little DDT. A small amount of internal DDE was found in both dead and living flies. There was no carry-over of DDT in the resistant Hawaiian strain. Only the non-toxic derivative, DDE was found internally, indicating complete detoxification of the absorbed DDT. The metabolism of DDE to DDA could not be followed in this study since the electron capture detector was not sensitive to amounts less than 12 ug/insect. This is far in excess of any expected DDA.

Hoskins and Witt (1958) found both DDE and DDA in the excreta of a resistant strain of house fly. Considerable quantities of a water-soluble conjugate was obtained from the excreta of susceptible and resistant house flies treated with C^{14}-DDT (Terriere and Schonbrod, 1955). Perry et al. (1955) found that DDE was the major metabolic product of DDT in resistant strains of the house fly. Both DDT and DDE were found in the ether-soluble portion of the excreta. Small amounts of unidentified excretory products were also produced after extended time intervals.

In this study, the susceptible adult flies of both sexes that survived latent toxicity excreted the DDE during the first 6 days of adult life. The

very small amount of DDT present in one-day old flies could not be detected

after 3 days. The inability to detect any difference in rates of excretion

between the sexes in this strain may be due to the low levels of DDT and

DDE carried over from the larval stage.

There was sufficient carry-over of dieldrin and heptachlor epoxide from

the larval stage to the adult to account for the latent toxicity to the susceptible

SCR-60 flies. The failure to elicit the phenomenon of latent toxicity by aldrin

and heptachlor to the resistant strain is difficult to explain. The amount

carried over to the adult stage was practically the same in both strains of

the house fly. Thus, it appears that the ability to convert aldrin and heptachlor

to their corresponding epoxides cannot, alone, explain the resistance pheno-

menon or the latent toxicity of these two compounds. Latent mortality

occurred only in the susceptible SCR-60 flies despite the comparable pattern

of metabolism in the two strains. Also, the levels of dieldrin and heptachlor

epoxide found in SCR-60 flies was practically the same in the living as in

those that died of latent toxicity.

The high values for dieldrin and heptachlor epoxide obtained in this

carry-over study when compared to levels obtained in the larval-pupal

metabolism studies on the same compounds could be explained by the way

the larvae were handled. The grouping of the larvae to be held for adult

emergence in batches of 100-150 in 50 ml beakers after topical treatment

allowed for greater contact between larvae. Thus the insecticide was

was spread to a greater body surface area and penetration was increased.

This, plus the reduced rate of volatilization of the insecticides in such

large groupings would account for the higher values in the carry-over

studies when compared to the 192 hour values in the larval-pupal studies.

SUMMARY AND CONCLUSIONS

Latent toxicity is that phenomenon in which the full lethal effect of an insecticide applied to a larva is not apparent until it has differentiated into an adult. It was found that appreciable latent toxicity occurred when aldrin, heptachlor, and DDT were applied to larvae of a susceptible strain of the house fly, Musca domestica L., while little or no latent toxicity occurred when these insecticides were applied to a resistant strain. This study was undertaken to determine the effect of a number of factors on the latent toxicity of aldrin, DDT, and heptachlor to susceptible and resistant strains of the house fly. The factors investigated were: (1) rate of cuticular penetration of the insecticide; (2) rate of degradation of the insecticide in the insect body; and (3) amount of carry-over of the insecticide and/or its metabolites from the larval to the adult stage. The insecticides were topically applied to larvae of the resistant (Hawaiian) strain and the susceptible (SCR-60) strain of the house fly and their metabolism followed by the use of electron capture gas chromatography.

It was found that aldrin and heptachlor were rapidly converted to their epoxides, dieldrin and heptachlor epoxide, respectively, while DDT was converted to the non-toxic metabolite dichlorodiphenyldichloroethylene (DDE).

The rate of cuticular penetration of the three insecticides was some- what faster with the susceptible than with the resistant strain.

Resistance of the larvae of the Hawaiian strain to DDT is attributable

to its rapid conversion to the non-toxic derivative DDE. No internal DDT

was recovered from the Hawaiian strain at any time, indicating complete

dehydrochlorination of all the DDT that penetrated. Measurable amounts

of DDT could be recovered from the susceptible SCR-60 larvae one hour

after treatment, increasing to a maximum 96 hours after treatment. The

larvae of the SCR-60 strain were limited in their ability to detoxify DDT

to DDE.

The latent toxicity of DDT to the susceptible SCR-60 strain was due to

the carry-over of sufficient amounts of unchanged DDT from the larval to

the adult stage to cause mortality in the flies shortly after emergence.

The living flies contained very little DDT. A small amount of internal DDE

was found in both dead and living flies. There was no carry-over of DDT

in the resistant Hawaiian strain. Only DDE was found internally, indicating

complete detoxification of the absorbed DDT.

The picture with aldrin and heptachlor was not as clear as with DDT.

The conversion of aldrin to dieldrin was faster in the susceptible than in

the resistant strain, while the rate of conversion of heptachlor to the

corresponding more toxic epoxide was about equal in both strains.

There was sufficient carry-over of dieldrin and heptachlor epoxide

from the larval stage to the adult to account for the latent toxicity to the

susceptible SCR-60 flies. The failure to elicit the phenomenon of latent

toxicity by aldrin and heptachlor to the resistant strain is difficult to explain. The amount carried-over to the adult stage was practically the same in both strains. Thus, it appears that the ability to convert aldrin and heptachlor to their corresponding epoxides alone cannot explain resistance or the latent toxicity of these two compounds. Latent mortality occurred only in the susceptible SCR-60 flies despite the comparable pattern of metabolism in the two strains. Also, the levels of dieldrin and heptachlor epoxide found in SCR-60 flies were practically the same in the living as in those that died of latent toxicity.

The adult flies of the susceptible and resistant strains excreted the dieldrin and heptachlor epoxide carried over from the larval stage within two weeks of adult life. The male flies of both strains excreted these metabolites at a faster rate than the female flies.

There was no difference in the rate of excretion of DDE between the sexes of the SCR-60 strain. However, the males of the resistant strain excreted DDE at a faster rate than the females. --

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