EFFECTS OF GAMMA AND X-IRRADIATION ON THE TOXICITY OF MALATHION TO HOUSE FLIES

by

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A Thesis Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ACKNOWLEDGMENTS

I have not only gained much experience and knowledge during my tenure as a graduate student in the Department of Entomology, but have also thoroughly enjoyed knowing its friendly people.

Dr. George W. Ware has provided me with professional and personal guidance and advice. For this and his eternal patience, I am sincerely grateful. In addition, Dr. Ware has provided me with funds through the National Institute of Health, without which this research would not have been possible.

I lovingly thank my husband who patiently taught me testing procedures, and more.

I would also like to geve thanks to Dr. Morton Wacks who answered so many of my questions concerning radiation. Wilson Cooper I thank, too, for his cooperative work in irradiating the insects.

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ABSTRACT

Dosage-mortality studies on house flies, *Musca domestica* L., exposed as pupae to gamma irradiation, ranging between 2.0 KR and 25.0 KR on varying days before emergence, indicated that emergence increases as age at time of radiation increases. Studies of controls and pupae irradiated with 12,000 R or 15,000 R at 2, 3, 4, 5, and 6 days before emergence indicated that greatest emergence occurred in pupae irradiated at 2 days before emergence.

Dosage-mortality studies on the combined effects of 5,000 R gamma irradiation and malathion applied topically to adults, showed that 1) newly pupated males, irradiated at 4 days before emergence, are significantly more tolerant than when irradiated later, 2 days before emergence; 2) female pupae irradiated 2 days before emergence are more tolerant to malathion than when irradiated earlier. It is suggested that the increased resistance in irradiated pupae may be attributed to increased carboxyesterase levels.

In comparing gamma with X-irradiated pupae, and their response to malathion toxicity, gamma irradiation increased malathion tolerance more than X-irradiation.

INTRODUCTION

There are virtually unlimited opportunities for the application of radiation to research. In entomology the applications of radiation are basically of two types. The first is concerned with the control of economic pests (O'Brien and Wolfe, 1964) and the second with the use of radiation as a physiological research tool.

In particular, irradiated house flies, Musca domestica L., manifested unexpected physiological responses after the application of certain insecticides (Guenthner and Ware, 1967). It follows then that an understanding of this aberrant response and the processes affecting such responses will lead to a better understanding of toxic action.

The aims of this project were to test further the effects of irradiation on the toxicity of the organophosphorous insecticide, malathion, O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate, to house flies, work originally reported by Guenthner and Ware (1967), and second, to initiate new radiation studies in the hope of establishing radiation response data on house flies in other areas. Although much research has been conducted in general radiation studies, relatively little has been done paralleling the major portions of this project.

The effects of increasing dosages of X-radiation on house fly emergence were studied by Rockstein, Dauer, and Bhatnagar (1965). They found that percentage of emergence decreased as the dosage increased and incomplete emergence increased as dosage increased.

The failure of house flies to emerge due to irradiation was investigated by Nair, Bhaskaran and Sivasubramanian (1967). From whole and partial X-irradiated house fly pupae, they found that damage to the target area resulted in failure to emerge. The target area in this case appeared to lie in the anterior seven segments. They suggested that failure to emerge was due to a direct effect of radiation on the differentiating myoblasts leading to the dystrophy of muscles. This dystrophy was associated with the emergence mechanism.

Male survival of X-irradiated house fly pupae was investigated by Dauer, Bhatnagar, and Rockstein (1965). Thirty-six to 48-hour pupae were exposed to dosages ranging from 10,000 through 30,000 rads. Males emerging from pupae irradiated with 10,000 and 20,000 rads lived an average of 16 days. Males from pupae receiving 30,000 rads lived 10 days while the controls lived 13.2 days.

A similar experiment by Rockstein, Dauer, and Bhatnagar (1966) exposed 36- to 48-hour pupae of the house fly to single doses of 2,000 to 8,000 rads. All levels of radiation produced a slight but significant decline in female life span. These low radiation levels had only a slight effect on male life span, with a decrease for 2,000 rads, no effect at 4,000 rads and a slight increase at 6,000 and 8,000 rads.

Very little work has been conducted on the combined effects of radiation and insecticides on insects. Varzandeh and Moos (1963) conducted studies dealing with the effects of X-radiation on the longevity, emergence and DDT-susceptibility of the house fly. Their findings showed that flies from pupae irradiated with the higher dosages exhibited less tolerance to DDT than those from lower dosages. Males exposed to higher dosages of radiation also had a shorter life span. Of interest was the fact that normal females mingling with irradiated males had a longer life span.

Hough (1963) worked with the irradiation of codling moth eggs to determine if this might influence response of the adult to insecticides. Sensitivity to gamma rays declined with aging of the eggs. Susceptibility of the adult to DDT and lead arsenate was not altered by irradiating eggs four days old; however, the resistance to DDT increased following irradiation of eggs less than one day old. Susceptibility to weak concentrations of azinphosmethyl or carbaryl was not affected.

Vashkov and Poleshchuk (1966) found that sensitivity of immature female house flies to DDT, benzene hexachloride and trichlorphon increased after exposure to X-radiation in proportion to the dose of the insecticide absorbed. For trichlorphon the sensitivity increased 1.2 to 6 times, for benzene hexachloride 1.2 to 9 times, and for DDT, 1.2 to 7 times.

The toxicity of malathion, heptachlor, and Temik® to X-irradiated house flies was also altered as shown in studies by Guenthner and Ware (1967). Generally, irradiation of pupae: 1) increased slightly the toxicity of heptachlor to males and females, 2) reduced significantly the toxicity of malathion to females with no effect on males, and 3) reduced significantly the toxicity of the carbamate, Temik, to males with no effect on females. It was suggested that the phenomenal response observed in 2) may be attributed to altered ratios of increased levels of esterases involved in detoxication of malathion.

The toxicities of DDT, azinphosmethyl and carbaryl to gamma irradiated pupae of the pink bollworm, Pectinophora gossypiella Saunders, were investigated by Rush and Ware (1969). Azinphosmethyl was 4 times as toxic to the irradiated moths as to the controls. Susceptibility to carbaryl was slightly increased in irradiated moths but DDT toxicity was not altered. They theorized that the increase in azinphosmethyl susceptibility may have been due to alterations in the enzyme systems which detoxify this insecticide in the pink bollworm.

The research reported here is a continuation of Guenthner and Ware's work (1967), in particular that portion involving irradiation of pupae at 2 days before peak emergence followed by topical application of malathion to the adult. Since they found no susceptibility change in males from pupae irradiated at 2 days before emergence the objective of this research was to duplicate their methods, but using gamma radiation at 2, 3, and 4 days before peak emergence, followed by topical treatment with malathion to the adults. Another objective was a study to determine the level of gamma radiation required to significantly decrease emergence in all stages of pupal development. From these studies it is hoped that the modes of insecticidal action can be explored more intricately.

MATERIALS AND METHODS

Rearing Methods

A Chemical Specialties Manufacturer's Association (CSMA) susceptible strain of house fly was used throughout this project. Larvae were reared on standard CSMA medium in one-gallon plastic containers which were covered with nylon netting to prevent unwanted oviposition in the larval medium.

The rearing room was illuminated with fluorescent light and the temperature was kept at 25 ± 2° C. The relative humidity ranged from 30% to 50% and was not controlled.

Eggs were collected in the rearing cages on milk-soaked cotton mats placed in petri dishes. The evaporated milk was diluted 1:2, milk : tap water.

The containers were each seeded with 0.6 ml of fly eggs and placed in the rearing room. After pupation the upper, drier layer of the medium containing the pupae was shaken onto a tray for drying. The pupae were separated from the dry medium using a screen, after which the medium chaff was fanned away.

The pupae were placed in one cubic foot, screened cages to await emergence. Food and water for the emerging adults were supplied in milk-filled 100 ml beakers on cotton mats placed in petri dishes. These also served as oviposition sites. In addition, sugar cubes were furnished as a dry food source.

When needed the flies were vacuumed from the sleeve-cages. A hole was cut in the bottom of a quart ice cream carton, into which a vacuum cleaner nozzle was firmly fitted. A metal screen was fitted and taped inside the carton above the nozzle. Flies were vacuumed into the carton which was capped with a screen-topped lid made from the ring portion of the original top.

Radiation Methods

Gamma radiation was supplied by a 530-curie $cobalt^{60}$ source. The X-radiation was carried out on a 1 MEV Dynamitron Electron Accelerator[1] utilizing a gold target.

Uniform radiation was achieved by containing the pupae in round, 1 3/4-inch diameter pill boxes, placed 2 1/2 inches from the center of a simple 1-rpm vertical turntable, 11 inches in diameter. For gamma radiation the turntable was placed in the 3 x 1 x 1 foot radiation chamber 12 inches from the $cobalt^{60}$ source. At this distance, the integrated dose was 83 R/minute.

Pupae shielded with lead were subjected to the same conditions in the radiation pit, and served as controls.

For X-radiation the turntable was placed several inches in front of the gold target so that a uniform dose rate averaging 150 R/minute was delivered. During the comparative irradiation study, the turntable was positioned so that the dose rate from the $cobalt^{60}$ source was the same as that from the X-ray machine.

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1. Manufactured by Radiation Dynamics, Inc., Westbury Industrial Park, Westbury Park, Long Island, New York.

In the gamma radiation chamber, ozone buildup was avoided by a small air compressor which continuously exchanged the chamber air at a rate of one cubic foot per minute. The X-radiation chamber was ventilated by a built-in blower of similar capacity.

Radiation Emergence Studies

To test the effects of gamma irradiation on house fly emergence, pupae were irradiated with gamma rays, ranging from 2.0 KR to 25.0 KR, on varying days before adult emergence.

One hundred pupae were placed in 1 1/2-inch diameter pill boxes, 200 pupae per dosage, and radiated at 83 R/minute. The same number were used for control groups plus an overall control not subjected to ozone. After irradiation, the pupae were transferred to one-pint Mason jars covered with screen-fitted lids. Diluted evaporated milk, in 1-ounce soufflé cups containing cotton, provided food and moisture for the emerging adults. These observation jars were placed in a climate chamber at 26 ± 1° C. with a 14-hour daylight photoperiod, for 24-, 48-, and 72-hour emergence counts.

To determine which pupal ages are most affected by a single dose of 15,000 R, one large population of pupae was separated into 5 groups. The first group was irradiated 24 hours after pupation (6 days before emergence), the second group after 48 hours, and in succeeding groups at 24-hour intervals up to 120 hours. The radiation and handling methods were the same as described previously. Emergence counts were taken at 24, 48, and 72 hours. This test was repeated with 12,000 R.

Topical Application of Malathion

Analytical grade malathion, 99.5%, was provided by the American Cyanamid Company and served as the test chemical. A 10 µg/µl stock solution was prepared periodically and refrigerated. From this stock solution, standard dilutions were prepared immediately prior to any test treatment.

After irradiation, the pupae were placed in the adult cages. These cages were then placed in the climate chamber until flies were withdrawn for topical application.

Four-day-old house flies of both sexes were used throughout this study. A one-microliter droplet of acetone containing a known amount of malathion was applied to the mesonotum of each fly with an ISCO Model M foot-operated microapplicator which drove a 1/4 cc tuberculin syringe fitted with a 26 gauge needle. To facilitate handling, the flies were lightly anesthetized with carbon dioxide and were kept under anesthesia no longer than 25 minutes. The anesthesia chamber was a flat-bottomed Büchner funnel connected with plastic tubing through which carbon dioxide was introduced. Control flies received only acetone without the toxicant. The flies were held as described under radiation emergence studies.

The temperature was recorded at the time of treatment. House-fly weights were determined for each test by weighing a 30-fly sample of each sex to the nearest 0.1 mg. Irradiated males and females were also weighed, since adults emerging from irradiated pupae tended to be smaller. Adult age was determined by counting the day of peak emergence as the first day of adult life. These would include flies emerging the day before and the day after, thus making their actual age 1 ± 1 day.

Combined Effects of Gamma Irradiation and Malathion

Range finding studies were conducted to establish an appropriate dosage range for male and female, irradiated and control flies. The dosage ranges are given in Table 1. To compare the relationship of malathion susceptibility to pupal age at the time of irradiation, pupae were irradiated at 2, 3, 4, and 5 days before peak emergence.

In the first series of tests, the gamma irradiated pupae received 5,000 R two days before peak emergence. The emerging adults were then topically treated, as were the controls, on the fourth day after peak adult emergence. The radiation and topical application procedures are identical to those previously described. Nine tests, consisting of 30 flies of each sex per dosage and per test were conducted for both the control and X-irradiated groups. However, three tests were incomplete due to the number of nonsignificant regressions. The next test was identical to the first but consisted of irradiating pupae 3 days before peak emergence. Six tests, of 30 flies of each sex per test, were conducted for both irradiated and control groups, with one incomplete test due to nonsignificant regression.

The next series of tests involved gamma irradiating pupae at four days before peak emergence. This work consisted of four tests of 30 flies of each sex, per test, per dosage for both radiated and control groups.

Table 1

Dosage ranges of malathion, expressed as μg per fly, applied topically to male and female house flies.

CONTROL		IRRADIATED	
Male	Female	Male	Female
0.175	0.225	0.175	0.25
0.20	0.275	0.20	0.30
0.25	0.350	0.25	0.40
0.30	0.425	0.30	0.50
0.33	0.50	0.33	0.60

The number of flies which emerged from pupae irradiated at 5 days before emergence was too small to run a complete test, although several unsuccessful attempts were made to rear a greater number of pupae.

Comparison of the Gamma and X-Irradiation Effects

Since Guenthner and Ware (1967) used only X-irradiated fly pupae before malathion treatment, the last series of tests involved a comparison of malathion-treated X-irradiated flies with malathion-treated gamma irradiated flies.

One population of house fly pupae was randomly divided into three groups. One was X-irradiated, a second gamma irradiated and a third left as a control group. No more than a 10-R-per-minute discrepancy between the gamma and X-ray dosage rate existed in each test. In the first three tests the dose rate for each source was around 150 ± 6 R, and in the last two tests the dose rate was around 110 R per minute ± 6. The length of the X-irradiation time varied up to one hour because of overheating and shutdown of the machine. The controls were subjected to the same ozone environment as the test pupae during the gamma irradiation.

The 4-day-old gamma and X-irradiated flies were treated simultaneously since the dosage ranges were identical. The controls were treated separately. The treated flies were placed in the 80° F. climate chamber to await 24-hour mortality counts.

The collected data were analyzed by log probit analysis to give LC_{30}, 50, 70, and 90's with the upper and lower 95% confidence limits for all points. The dosage-mortality curves were plotted on log probit paper. LD_{50}'s with corresponding upper and lower 95% confidence limits were calculated. The data for these studies are presented in the form of graphs and tables.

Variations within each study may be due to the following: age differences of the flies, temperature at time of irradiation exposure, rearing room temperature fluctuation, humidity, barometric pressure, evaporation of the malathion stock solution.

RESULTS AND DISCUSSION

Pupal Emergence Studies

Results from the preliminary studies of irradiating pupae at 1, 2, 3, and 4 days before peak emergence are shown in Table 2. The results show that emergence increases as pupal age at the time of irradiation increases. When irradiated at one and two days before peak emergence, there was no reduction in emergence at 20,000 R as compared with the controls. When irradiated 3 days before, emergence was reduced by approximately 50% at 25,000 R as compared with the controls. Only 1% emergence occurred when pupae were irradiated 4 days before emergence. Figures 1 and 2 show adult emergence from control pupae and pupae irradiated with 12,000 or 15,000 R at 2, 3, 4, 5, and 6 days before emergence. The greatest emergence for the irradiated flies occurred in pupae irradiated at 2 days before emergence, with no emergence from those irradiated 5 and 6 days before emergence.

Combined Effects of Gamma Irradiation and Malathion

The combined effects of pupae irradiated (5,000 R) 2 days before peak emergence followed by topical treatment of the adults with malathion, are shown in Table 3, which gives the LC_{30}'s, 50's, 70's and 90's with the upper and lower 95% confidence limits. The LD_{50}'s with upper and lower confidence limits are presented in Table 4. Figure 3 illustrates the dosage-mortality curves taken from the data of Table 3. The results show that in six tests, females irradiated

Table 2

Per cent mortality of pupae from different populations receiving gamma irradiation at 1, 2, 3, and 4 days before peak emergence.

Dosage in Kiloroentgens

Days before emergence	2.0	2.5	5.0	7.5	10.0	15.0	20.0	25.0	Control
1	9.5	-	-	-	7.5	8.5	16.5	-	11.0
2	-	5.0	13.5	-	6.0	12.5	9.5	-	7.5
3		10.5	10.3	13.5	14.3	12.3	12.0	39.0	8.0
4		11.0	13.6	-	30.0	73.0	95.6	99.0	10.5

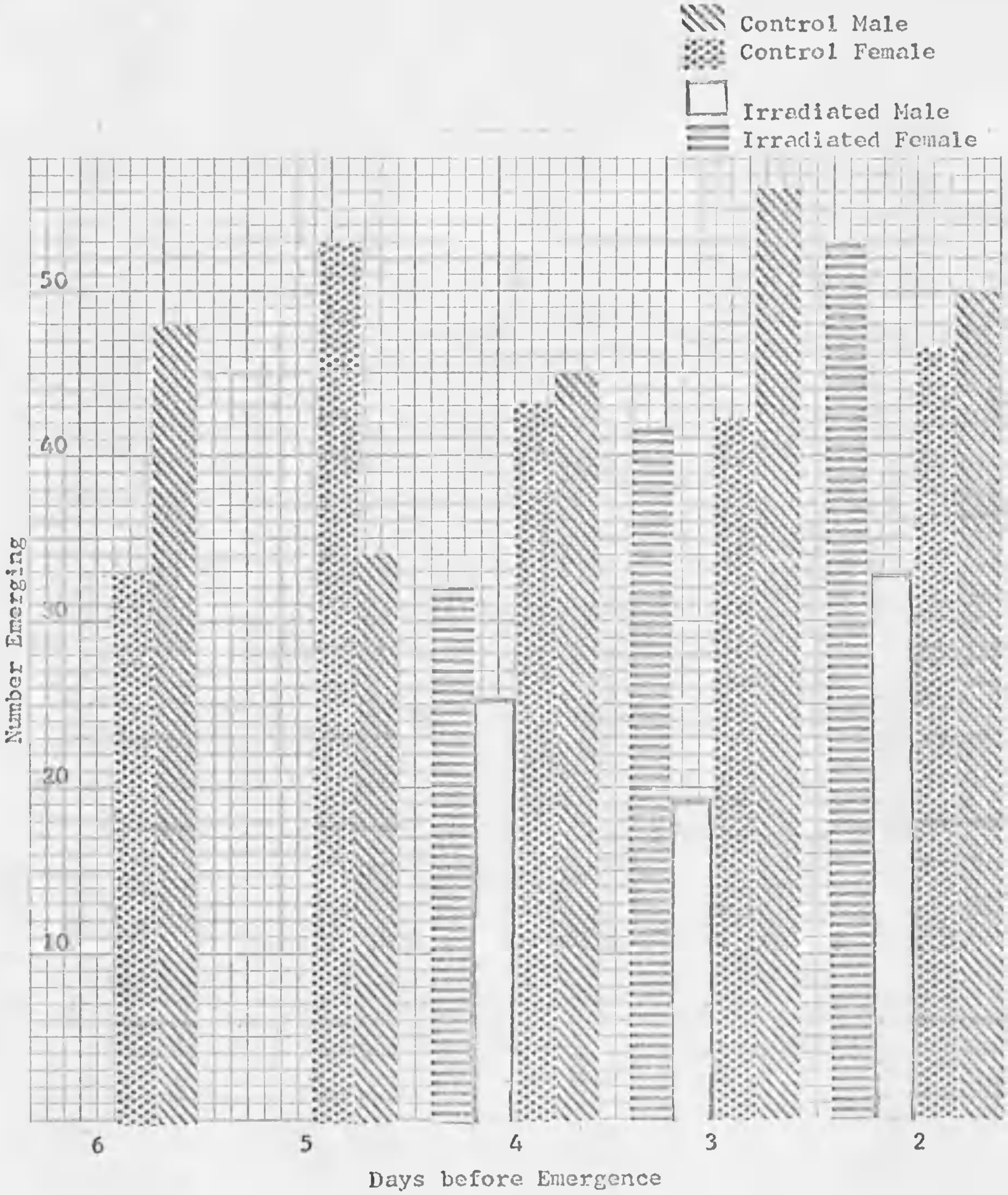


Figure 1. Adult emergence from control pupae and pupae receiving 12,000 R gamma radiation. Each age group included 100 control and 100 irradiated pupae.

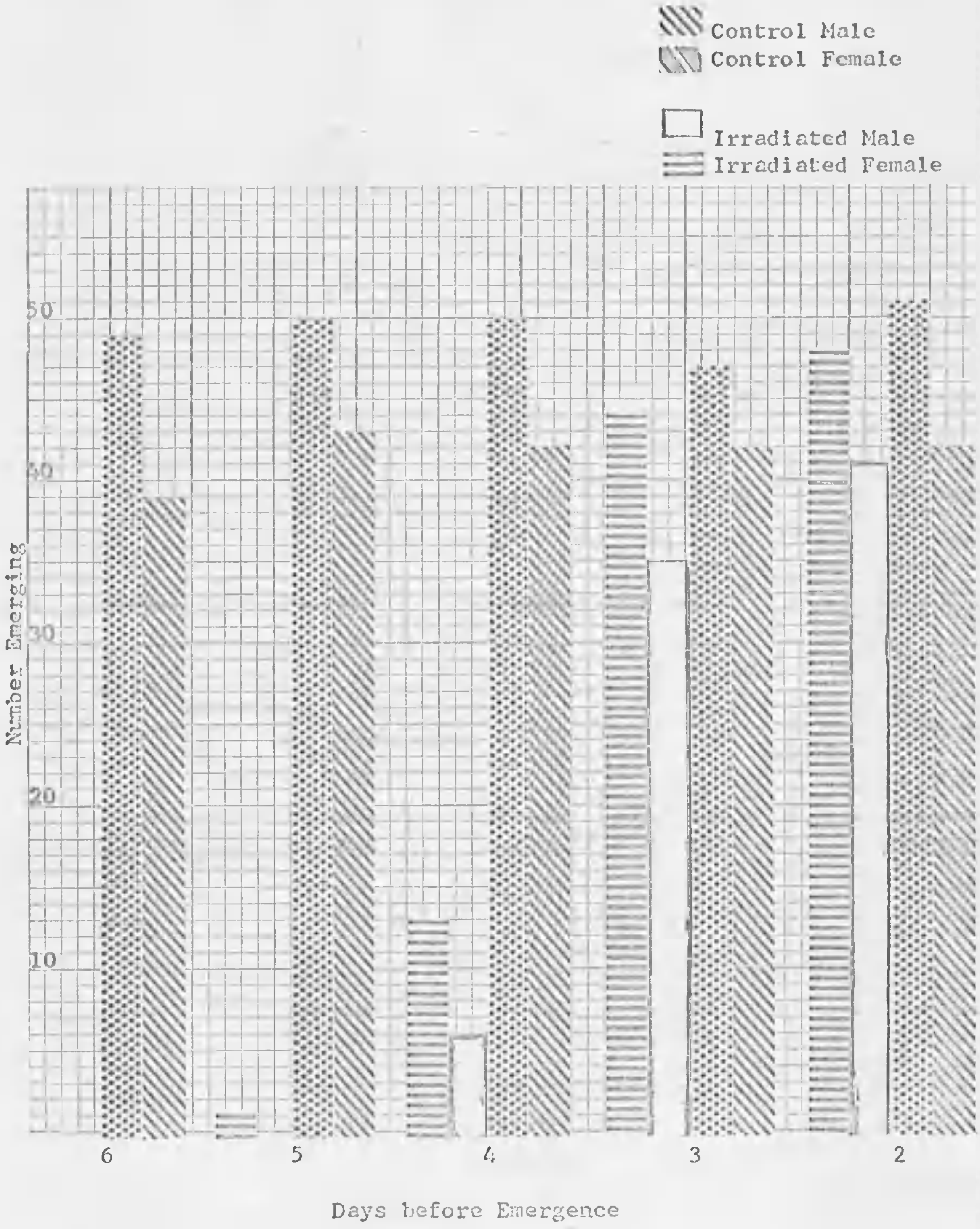


Figure 2. Adult emergence from control pupae and pupae receiving 15,000 R gamma radiation. Each age group included 100 control and 100 irradiated pupae.

Table 3

LC 30-50-70-90 values for house flies (μg malathion per fly) with upper and lower 95% confidence limits, for controls and those receiving 5,000 R gamma irradiation as pupae 2 days prior to peak emergence.

TEST	1		2		3		4		5		6	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
					CONTROL							
Upper Limit	.2695	.3337	.2779	.5107	.2505	.3788	.2678	.3481	--[a]	.3302	.1725	.1930
LC_{30}	.2216	.3053	.2620	.4408	.2344	.3487	.2538	.3199	--	.2764	.1626	.1566
Lower Limit	.0918	.2696	.2440	.3989	.2151	.3154	.2371	.2858	--	.1478	.1345	.0838
Upper Limit	.3718	.3920	.3202	.7058	.2942	.4701	.3013	.4298	--	.3972	.1815	.2200
LC_{50}	.2574	.3615	.2967	.5379	.2724	.4202	.2836	.3886	--	.3059	.1735	.1884
Lower Limit	.1840	.3302	.2796	.4747	.2551	.3865	.2688	.3574	--	.2206	.1555	.1225
Upper Limit	.7672	.4719	.3780	.9988	.3563	.6065	.3458	.5549	--	.5635	.1954	.2543
LC_{70}	.2989	.4280	.3360	.6562	.3165	.5063	.3169	.4721	--	.3385	.1852	.2265
Lower Limit	.2464	.3946	.3127	.5515	.2933	.4556	.2986	.4273	--	.2792	.1756	.1764
Upper Limit	2.7746	.6398	.4880	.6669	.4808	.8961	.4292	.8268	--	1.1156	.2353	.3542
LC_{90}	.3709	.5462	.4021	.8744	.3932	.6628	.3719	.6253	--	.3919	.2034	.2957
Lower Limit	.2957	.4920	.3617	.6777	.3507	.5648	.3418	.5368	--	.3284	.1934	.2645
					GAMMA IRRADIATED							
Upper Limit	.2447	.3795	.2726	.6220	.2560	.4959	.2713	.3885	.1777	.2693	--[a]	.1995
LC_{30}	.2291	.3459	.2601	.5224	.2345	.4181	.2564	.3586	.1631	.2377	--	.1469
Lower Limit	.2092	.3043	.2441	.4651	.2084	.3526	.2390	.3233	.1399	.1916	--	.0589
Upper Limit	.2813	.4623	.2981	.9088	.3306	.8789	.3089	.4641	.1980	.3216	--	.2451
LC_{50}	.2630	.4208	.2840	.6585	.2893	.5939	.2887	.4265	.1851	.2919	--	.1969
Lower Limit	.2464	.3839	.2709	.5686	.2647	.4998	.2729	.3940	.1674	.2543	--	.1070
Upper Limit	.3324	.5857	.3314	1.3637	.4541	1.6989	.3597	.5713	.2253	.3981	--	.3081
LC_{70}	.3020	.5119	.3101	.8301	.3569	.8436	.3252	.5073	.2101	.3584	--	.2640
Lower Limit	.2823	.4656	.2957	.6767	.3162	.6495	.3046	.4659	.1962	.3256	--	.1900
Upper Limit	.4345	.8565	.3930	2.4796	.7376	4.5114	.4558	.7932	.2863	.5885	--	.5382
LC_{90}	.3687	.6793	.3520	1.1597	.4832	1.4003	.3861	.6516	.2523	.4820	--	.4030
Lower Limit	.3345	.5922	.3297	.8599	.3980	.9247	.3511	.5773	.2340	.4283	--	.3469

a) nonsignificant regression

Table 4

LD_{50}'s (μg malathion per gram of fly) for flies from control and pupae receiving 5,000 R gamma irradiation 2 days before emergence.

TEST	CONTROL			GAMMA IRRADIATED		
	Upper Limit[a]	LD_{50}	Lower Limit[a]	Upper Limit	LD_{50}	Lower Limit
MALES						
1	40.50	28.04	20.04	33.20	31.04	29.08
2	29.67	27.49	25.91	29.03	27.65	26.38
3	26.27	24.32	22.78	30.61	26.79	24.51
4	31.06	29.24	27.71	32.52	30.39	28.73
5	--[b]	--	--	23.86	22.30	20.17
6	21.29	20.35	18.24	--	--	--
FEMALES						
1	31.95	29.46	26.91	38.14	34.72	31.67
2	42.06	32.06	28.29	60.99	44.16	38.16
3	27.33	24.43	22.47	60.61	40.96	34.47
4	34.38	31.09	28.59	36.26	33.32	30.78
5	29.42	22.66	16.34	29.50	26.78	23.33
6	20.27	17.36	11.29	26.56	21.34	11.60

a) 95% upper and lower confidence limits

b) nonsignificant regression

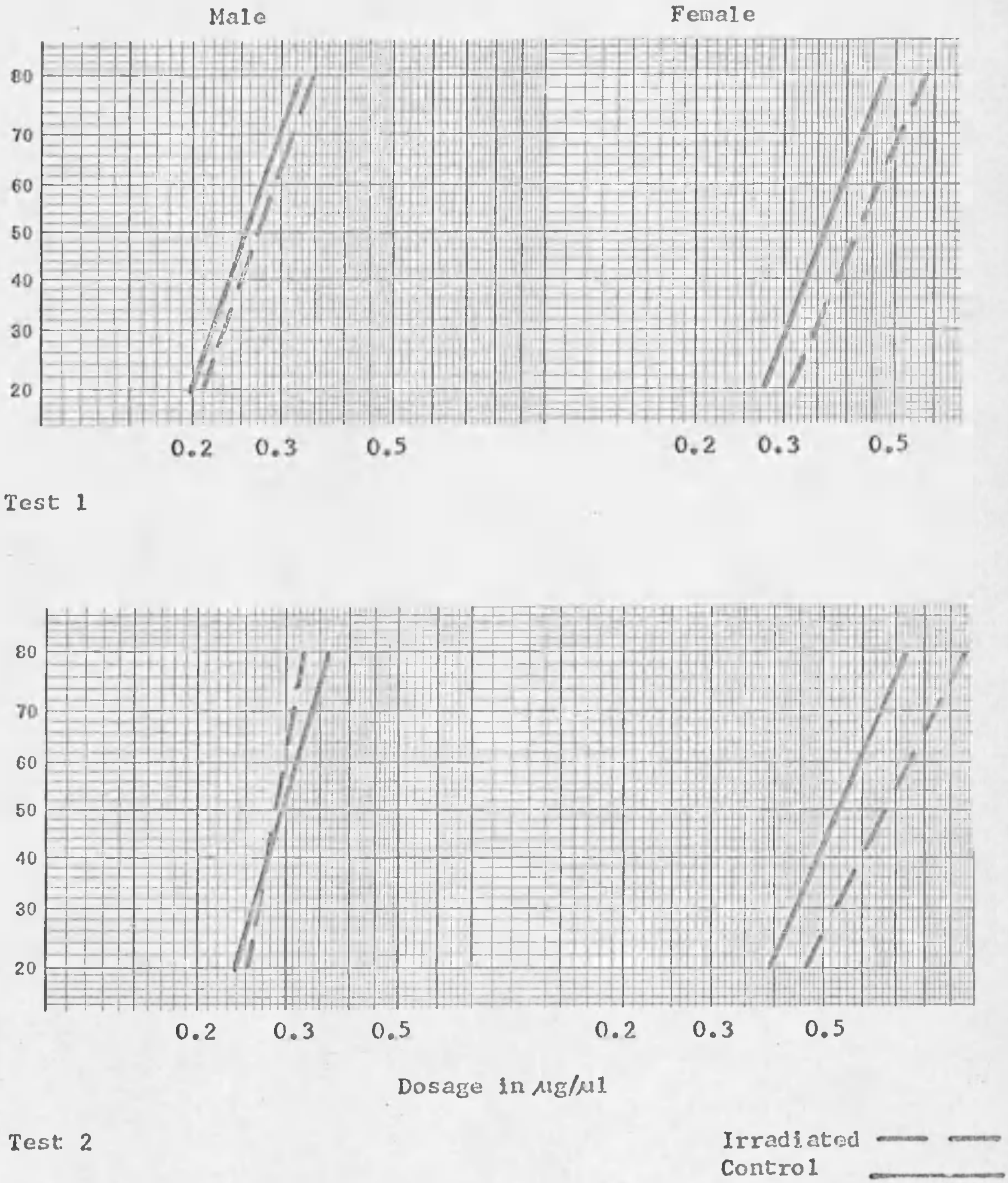
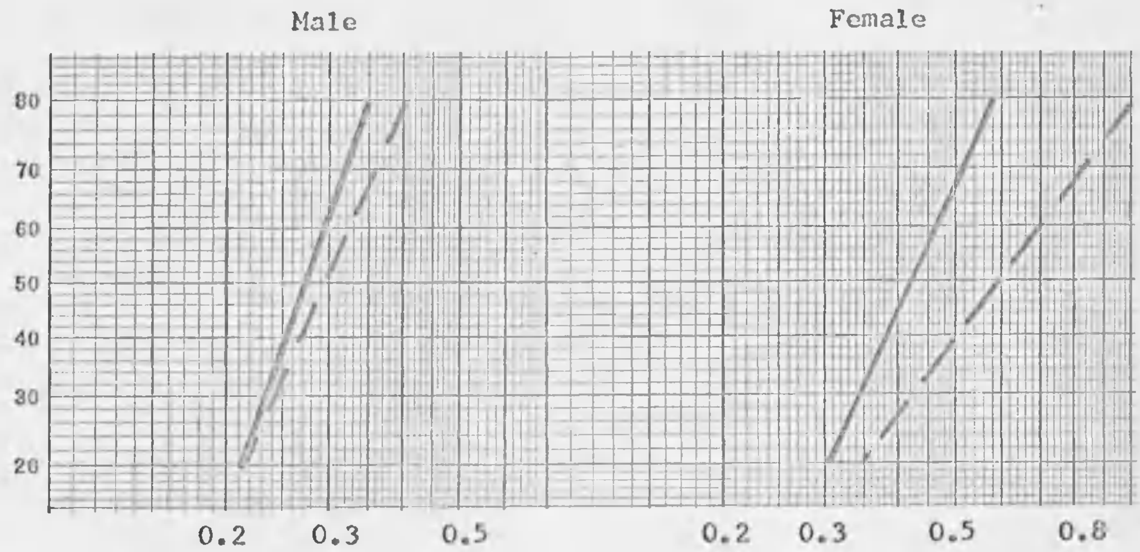
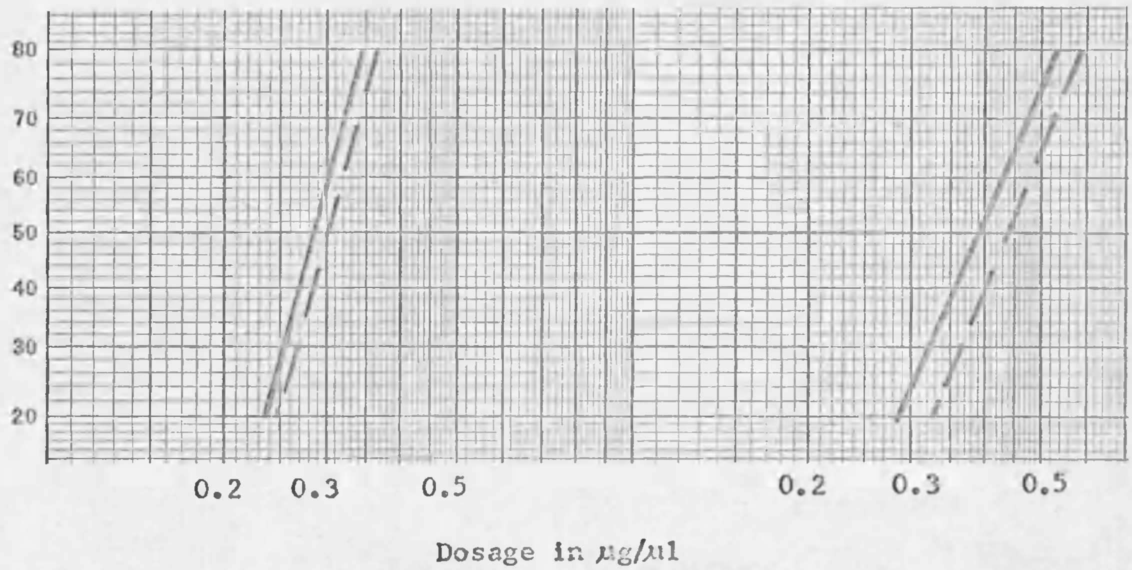


Figure 3. Dosage-mortality curves for pupae receiving gamma radiation two days prior to peak emergence.

Tests 1 and 2 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R two days prior to peak emergence.



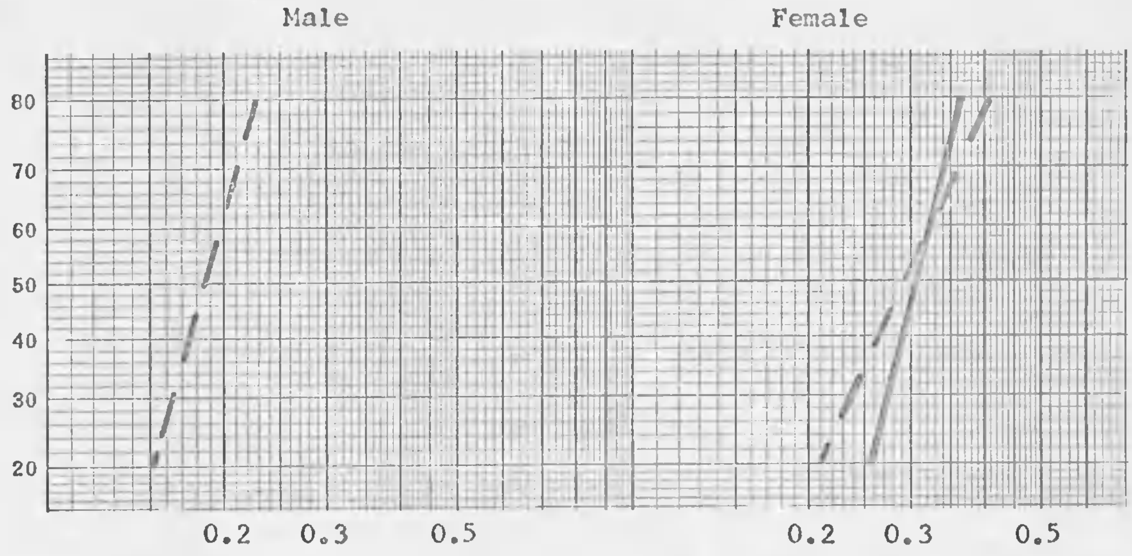
Test 3



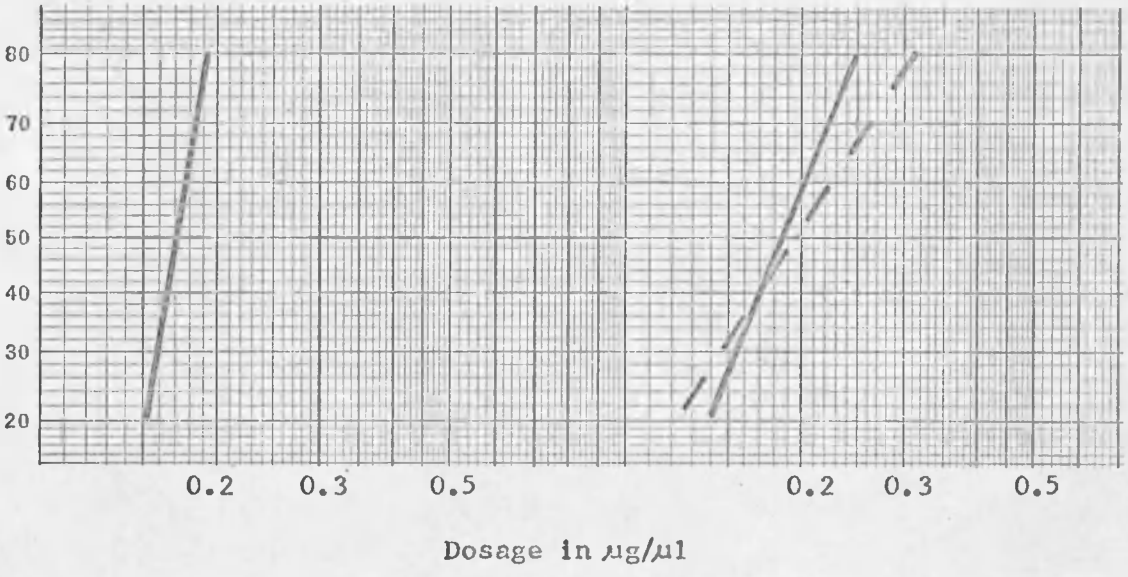
Test 4

Figure 3, Continued

Tests 3 and 4 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R two days prior to peak emergence.



Test 5



Test 6

Figure 3, <u>Continued</u>

Tests 5 and 6 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R two days prior to peak emergence.

2 days before peak emergence were more tolerant to malathion than non-irradiated females. In only two tests was the tolerance significant. However, in the comparative radiation study, three of the four tests showed that irradiated females were significantly more tolerant to malathion than the controls. Irradiated males showed only a slight tolerance to malathion.

These data support the work of Guenthner and Ware (1967) who found that female flies irradiated 2 days before peak emergence were significantly more tolerant to malathion than the controls.

In Table 5, the LC values with the upper and lower 95% confidence limits are given, and in Table 6 the LD_{50} values with the 95% confidence limits are presented. These data along with the dosage-mortality curves (Figure 4) show that in three of five tests, males from pupae irradiated 3 days before emergence have a significantly increased tolerance to malathion. In only two of the five tests were the data on the females complete, due to the number of nonsignificant regressions. In these two cases, the females were slightly more tolerant to malathion than the controls.

Data on pupae irradiated 4 days before emergence are presented as LC values in Table 7, LD_{50} values in Table 8 and as dosage-mortality curves in Figure 5. The males were significantly more tolerant than controls in three of four tests. Females were only slightly more tolerant.

These data show that male pupae, irradiated 4 days before emergence are significantly more tolerant than when irradiated as older pupae. With females the reverse is true. Female pupae irradiated at

Table 5

LC 30-50-70-90 values for house flies (μg malathion per fly) with upper and lower 95% confidence limits, for controls and those receiving 5,000 R gamma irradiation as pupae 3 days prior to peak emergence.

TEST	1		2		3		4		5	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
CONTROL										
Upper Limit	.2522	--[a]	.2194	--[a]	.1883	.3567	.2144	.5142	.2012	.3267
LC_{30}	.2375	--	.2064	--	.1781	.2998	.2007	.3659	.1913	.3016
Lower Limit	.2199	--	.1901	--	.1634	.1963	.1828	.1108	.1785	.2697
Upper Limit	.2893	--	.2472	--	.2053	.4271	.2438	2.3745	.2167	.3740
LC_{50}	.2708	--	.2335	--	.1952	.3398	.2295	.4280	.2047	.3451
Lower Limit	.2551	--	.2197	--	.1840	.2650	.2148	.3285	.1942	.3177
Upper Limit	.3397	--	.2838	--	.2278	.5673	.2833	26.4971	.2369	.4384
LC_{70}	.3087	--	.2641	--	.2139	.3851	.2623	.5007	.2191	.3948
Lower Limit	.2889	--	.2493	--	.2035	.3225	.2468	.4030	.2081	.3649
Upper Limit	.4373	--	.3547	--	.2720	.9567	.3625	98.7708	.2740	.5714
LC_{90}	.3729	--	.3155	--	.2441	.4614	.3183	.6279	.2416	.4795
Lower Limit	.3390	--	.2922	--	.2290	.3825	.2930	.4729	.2263	.4323
GAMMA IRRADIATED										
Upper Limit	.2527	.3686	.2313	.4621	.2160	.3258	.2537	--[a]	.1947	--[a]
LC_{30}	.2390	.3402	.2133	.4036	.1994	.3006	.2363	--	.1839	--
Lower Limit	.2226	.3061	.1889	.3463	.1764	.2691	.2157	--	.1680	--
Upper Limit	.2848	.4373	.2800	.7075	.2539	.3800	.2910	--	.2111	--
LC_{50}	.2686	.4035	.2568	.5467	.2361	.3526	.2699	--	.1991	--
Lower Limit	.2541	.3727	.2373	.4755	.2183	.3252	.2513	--	.1870	--
Upper Limit	.3271	.5337	.3573	1.1691	.3108	.4540	.3413	--	.2336	--
LC_{70}	.3017	.4786	.3091	.7406	.2796	.4136	.3083	--	.2155	--
Lower Limit	.2846	.4412	.2830	.6048	.2595	.3836	.2863	--	.2039	--
Upper Limit	.4070	.7329	.5273	2.4803	.4343	.6063	.4396	--	.2774	--
LC_{90}	.3570	.6122	.4041	1.1480	.3569	.5208	.3735	--	.2417	--
Lower Limit	.3289	.5465	.3514	.8333	.3191	.4715	.3380	--	.2252	--

a) nonsignificant regression

Table 6

LD_{50}'s (µg malathion per gram of fly) for flies from control and pupae receiving 5,000 R gamma irradiation 3 days before emergence.

TEST	CONTROL			GAMMA IRRADIATED		
	Upper Limit[a]	LD_{50}	Lower Limit[a]	Upper Limit	LD_{50}	Lower Limit
			MALES			
1	30.66	28.70	27.03	23.83	22.47	21.26
2	25.02	23.63	22.24	27.01	25.73	22.89
3	18.33	17.43	16.43	25.39	23.61	21.83
4	19.35	18.21	17.05	25.75	23.89	22.24
5	17.20	16.25	15.41	18.68	17.62	16.55
			FEMALES			
1	--[b]	--	--	48.03	44.32	40.94
2	--	--	--	48.67	37.61	32.71
3	23.60	18.77	14.64	25.68	23.82	21.97
4	124.97	22.53	17.29	--	--	--
5	19.68	18.16	16.72	--	--	--

a) 95% upper and lower confidence limits

b) nonsignificant regression

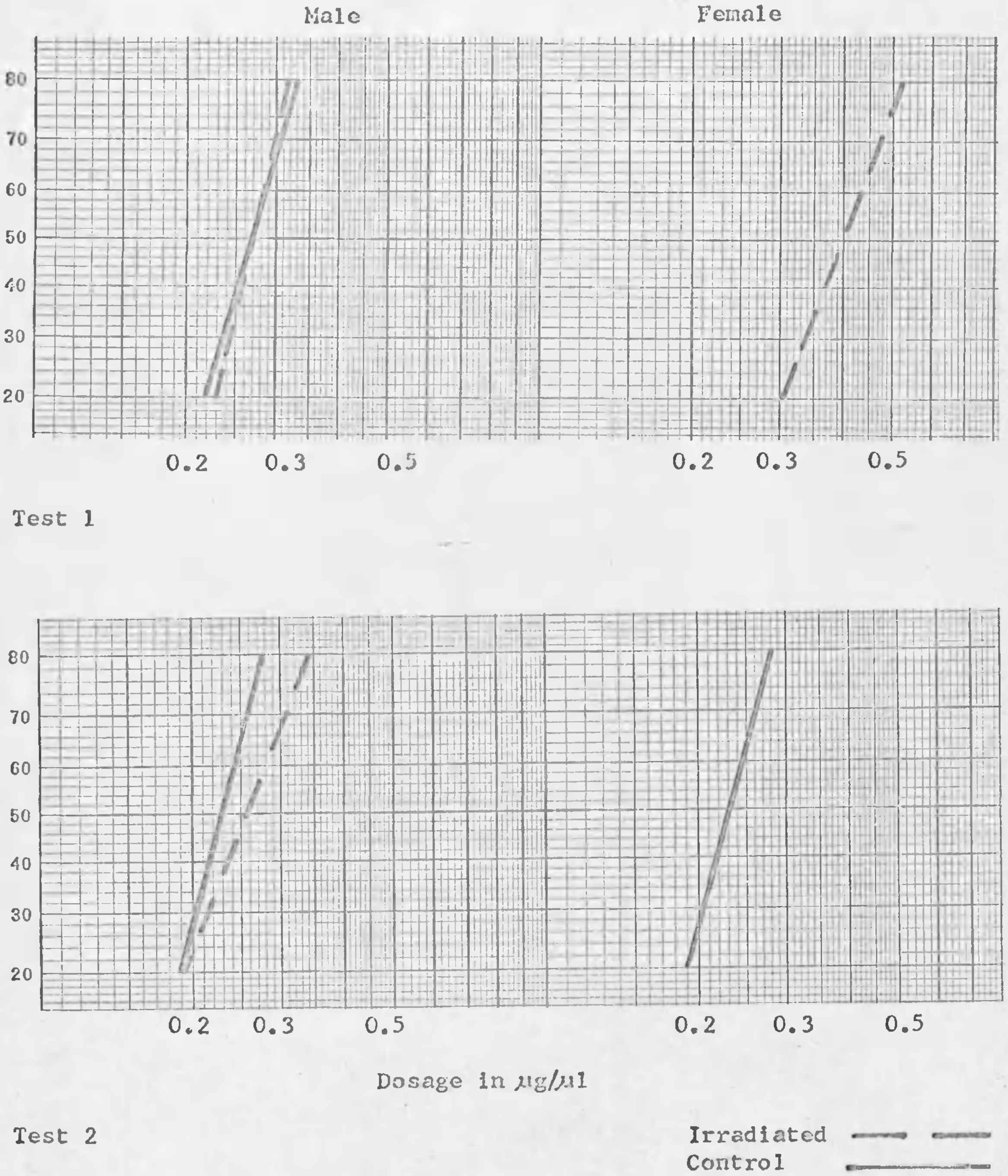
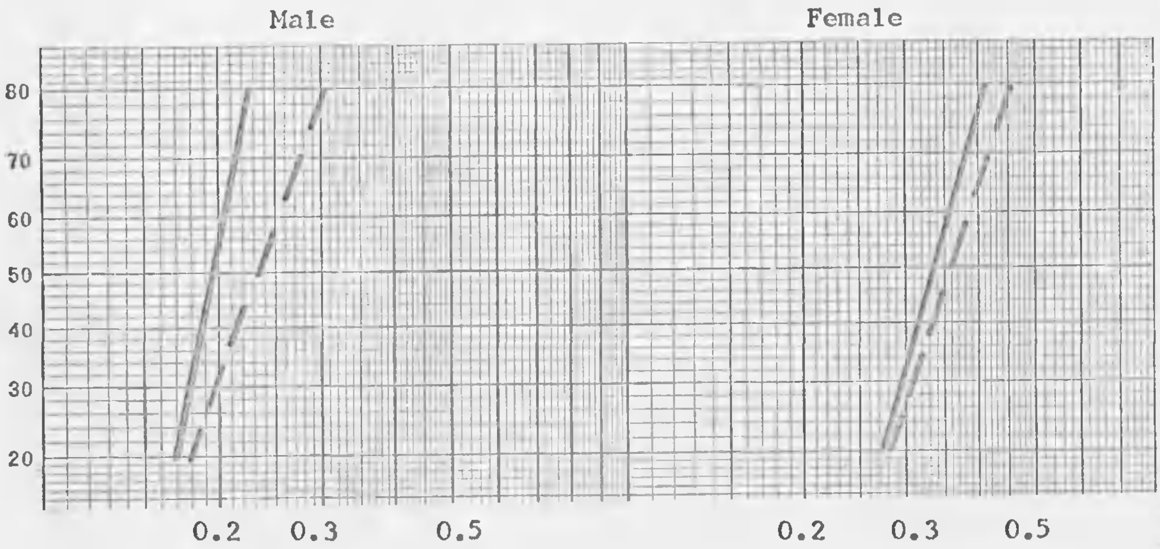
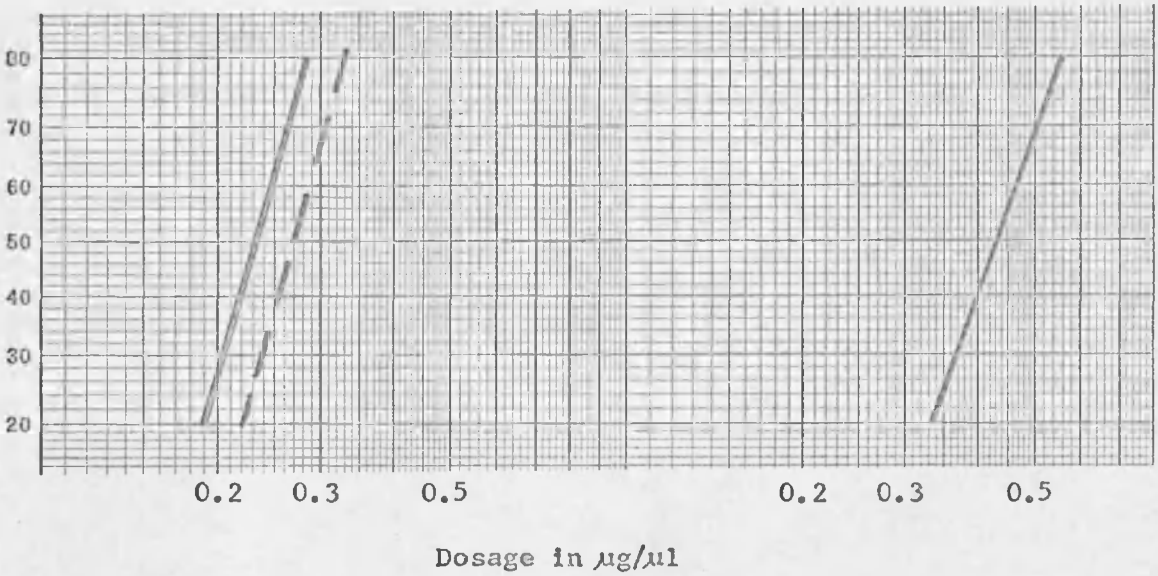


Figure 4. Dosage-mortality curves for pupae receiving gamma radiation three days prior to peak emergence.

Tests 1 and 2 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R two days prior to peak emergence.



Test 3



Test 4

Irradiated — —
Control ———

Figure 4, <u>Continued</u>

Tests 3 and 4 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R three days prior to peak emergence.

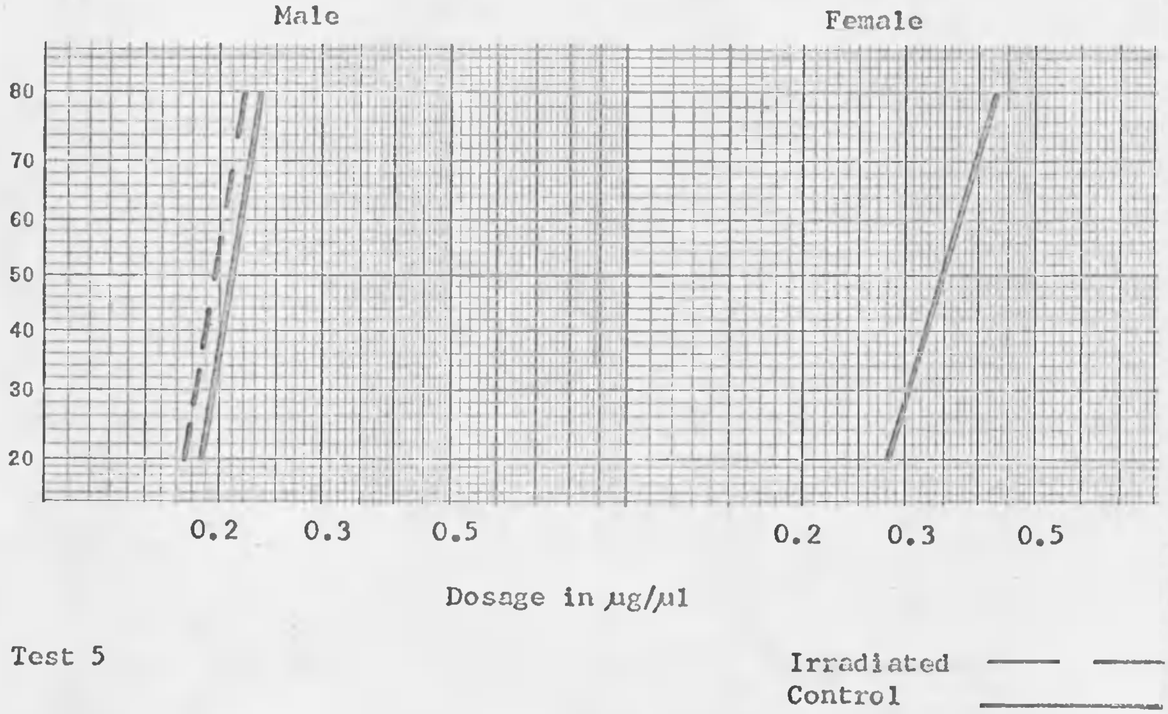


Figure 4, Continued

Test 5 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R three days prior to peak emergence.

Table 7

LC 30-50-70-90 values for house flies (µg malathion per fly) with upper and lower 95% confidence limits, for controls and those receiving 5,000 R gamma irradiation as pupae 4 days prior to peak emergence.

TEST	1		2		3		4	
	Male	Female	Male	Female	Male	Female	Male	Female
				CONTROL				
Upper Limit	.1876	.3531	.4590	.5713	.1667	.2916	.2333	.4242
LC_{30}	.1729	.2778	.3259	.4700	.1486	.2698	.2205	.3940
Lower Limit	.1514	.0488	.2857	.4226	.1166	.2418	.2050	.3606
Upper Limit	.2119	.5665	.8383	.8192	.1866	.3382	.2613	.5095
LC_{50}	.1984	.3430	.4225	.5725	.1713	.3151	.2473	.4557
Lower Limit	.1817	.2052	.3466	.4967	.1461	.2916	.2339	.4233
Upper Limit	.2448	2.3517	1.5623	1.1968	.2129	.4025	.2975	.6344
LC_{70}	.2276	.4236	.5478	.6974	.1974	.3681	.2774	.5270
Lower Limit	.2131	.3331	.4122	.5730	.1797	.3428	.2625	.4793
Upper Limit	.3159	29.0276	3.8690	2.0869	.2791	.5354	.3660	.8868
LC_{90}	.2775	.5745	.7970	.9272	.2424	.4605	.3273	.6501
Lower Limit	.2561	.4244	.5251	.6982	.2236	.4183	.3040	.5631
				GAMMA IRRADIATED				
Upper Limit	.2343	.4113	.4353	--[a]	.2258	.3443	.3115	.4106
LC_{30}	.2200	.3829	.3124	--	.2138	.3189	.2786	.3770
Lower Limit	.2022	.3468	.2730	--	.1991	.2882	.2539	.3385
Upper Limit	.2684	.4707	.8626	--	.2511	.4006	.4214	.5039
LC_{50}	.2520	.4386	.4165	--	.2382	.3723	.3397	.4562
Lower Limit	.2368	.4080	.3396	--	.2255	.3449	.3053	.4191
Upper Limit	.3151	.5520	1.7575	--	.2833	.4767	.5885	.6414
LC_{70}	.2887	.5025	.5552	--	.2655	.4345	.4141	.5519
Lower Limit	.2708	.4684	.4111	--	.2518	.4036	.3556	.5002
Upper Limit	.4069	.7149	4.9588	--	.3436	.6303	.9653	.9343
LC_{90}	.3512	.6114	.8410	--	.3105	.5432	.5512	.7266
Lower Limit	.3207	.5558	.5363	--	.2900	.4924	.4377	.6283

a) nonsignificant regression

Table 8

LD_{50}'s (µg malathion per gram of fly) for flies from control and pupae receiving 5,000 R gamma irradiation 4 days before emergence.

TEST	CONTROL			GAMMA IRRADIATED		
	Upper Limit[a]	LD_{50}	Lower Limit[a]	Upper Limit	LD_{50}	Lower Limit
			MALES			
1	19.02	17.81	16.31	20.97	19.69	18.50
2	75.53	38.17	31.23	77.02	37.19	30.32
3	15.79	14.49	12.36	24.26	23.01	21.79
4	23.46	22.20	20.99	38.24	36.02	27.70
			FEMALES			
1	31.13	18.85	11.28	24.80	23.11	21.50
2	49.05	34.28	29.74	--	--	--[b]
3	20.06	18.69	17.29	28.61	26.59	24.64
4	32.38	28.96	26.90	32.03	29.00	26.64

a) 95% upper and lower confidence limits

b) nonsignificant regression

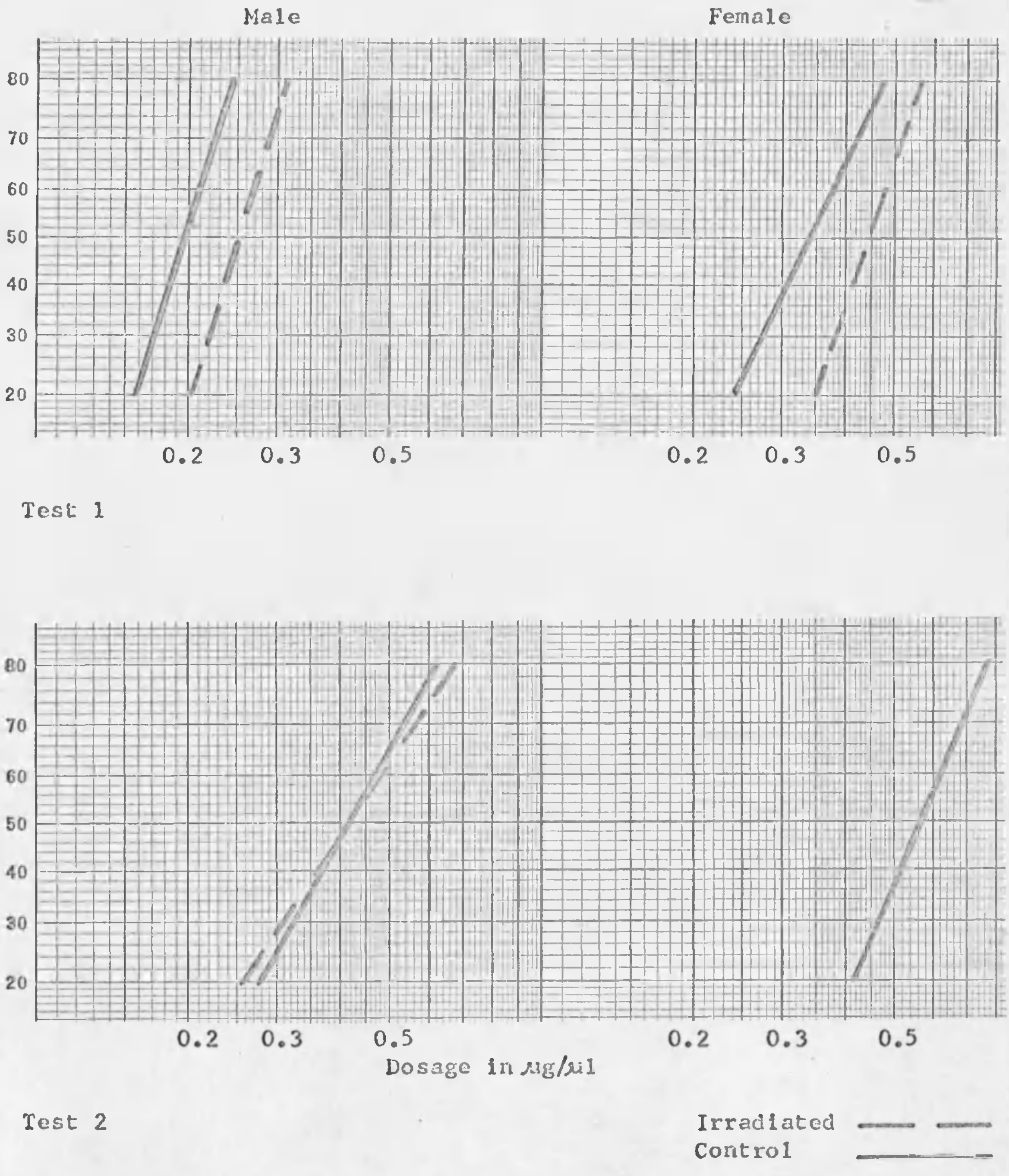
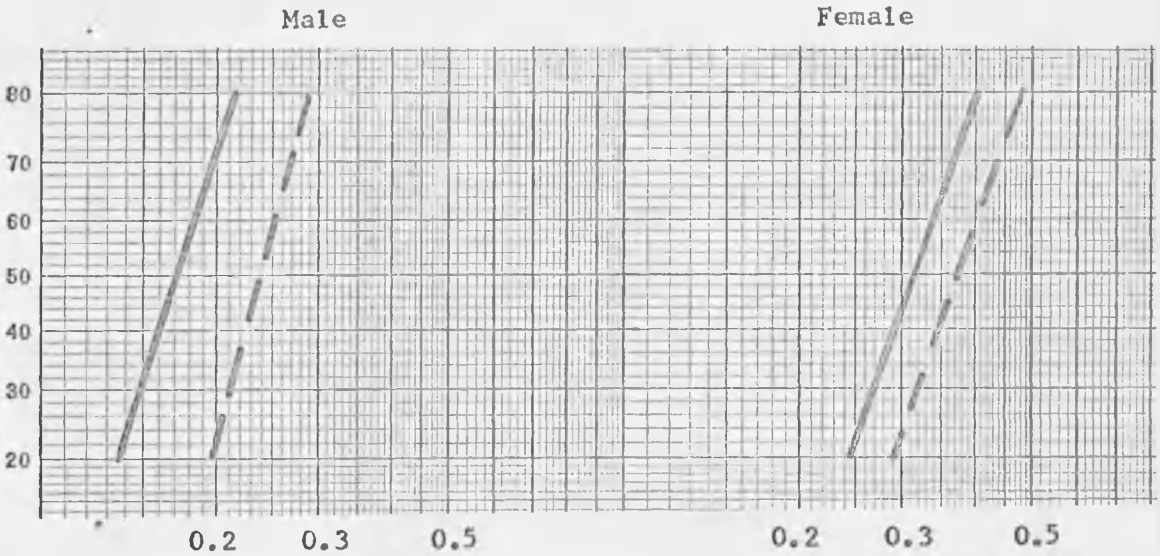
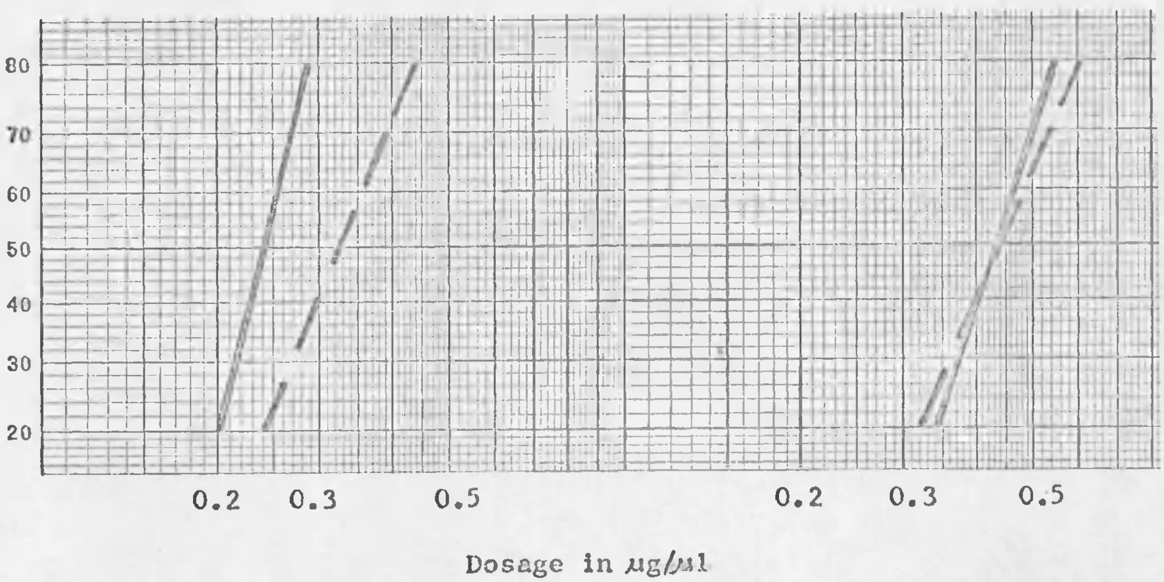


Figure 5. Dosage-mortality curves for pupae receiving gamma radiation four days prior to peak emergence.

Tests 1 and 2 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R four days prior to peak emergence.



Test 3



Test 4

Irradiated — —
Control ———

Figure 5, <u>Continued</u>

Tests 3 and 4 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma irradiated with 5,000 R four days prior to peak emergence.

4 days before emergence are less tolerant than when irradiated as older pupae.

In the comparative irradiation studies, it was found that in most cases gamma irradiation had a greater effect than X-irradiation as shown in the LC values in Table 9, the LD_{50} values in Table 10, and the dosage-mortality curves in Figure 6. Gamma irradiation seems to increase malathion tolerance more than X-irradiation. Difficulty was encountered in delivering a precise X-ray dosage. Also, the X-ray machine stopped several times during a treatment due to overheating. Therefore, although the dosage rate was nearly the same for both gamma and X-rays, the delivery time of the X-rays was 30 minutes longer than for the gamma rays. These margins of error may explain the unexpected differences between the radiations.

Although gamma and X-rays are both forms of ionizing radiation, their energy levels differ. In a comparison of gamma and X-rays based on the effects of Drosophila eggs and on wheat seedlings, Henshaw, Henshaw, and Francis (1933) found that roentgen rays of 165 KV and 700 KV were equally effective in producing egg mortality, but gamma rays were 32% more effective in producing certain effects on wheat seedlings. To interpret the results of increased tolerance of malathion due to some effect of irradiation, it is necessary to discuss the malathion detoxification mechanism.

According to O'Brien (1960) the anticholinesterase potency of the malathion molecule is increased about 10,000 times when it is converted to malaoxon by the microsomal system of insects. Krueger and O'Brien (1959) found that malathion degradation in the mouse is much

Table 9

LC 30-50-70-90 values for house flies (μg malathion per fly) with upper and lower 95% confidence limits, for controls and those receiving 5,000 R gamma or X-irradiation as pupae 2 days prior to peak emergence.

TEST	1		2		3		4		5	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
CONTROL										
Upper Limit	.1946	.3069	.1555	.2710	.1669	.2673	.2461	.3377	.2164	.3426
LC_{30}	.1826	.2828	.1331	.2481	.1513	.2483	.2038	.3211	.2055	.3209
Lower Limit	.1663	.2517	.0818	.2172	.1184	.2233	.0398	.3024	.1894	.2962
Upper Limit	.2160	.3640	.1716	.3157	.1816	.3026	.4021	.3787	.2358	.4075
LC_{50}	.2043	.3367	.1529	.2929	.1688	.2834	.2450	.3589	.2256	.3772
Lower Limit	.1913	.3105	.1099	.2677	.1443	.2627	.1683	.3414	.2137	.3533
Upper Limit	.2439	.4468	.1916	.3784	.2012	.3495	1.9143	.4308	.2614	.4981
LC_{70}	.2285	.4009	.1757	.3456	.1882	.3236	.2944	.4011	.2477	.4433
Lower Limit	.2161	.3703	.1458	.3206	.1726	.3031	.2440	.3710	.2370	.4100
Upper Limit	.2993	.6233	.2501	.5145	.2538	.4442	25.7017	.5261	.3119	.6789
LC_{90}	.2686	.5157	.2147	.4390	.2204	.3917	.3840	.4709	.2675	.4983
Lower Limit	.2504	.4599	.1972	.3975	.2055	.3608	.2956	.4373	.2675	.4983
GAMMA IRRADIATED										
Upper Limit	.2300	.4400	.1788	.3139	.3598	.6523	.2052	--[a]	.2260	.5699
LC_{30}	.2148	.4120	.1631	.2873	.2998	.5174	.1946	--	.2116	.4942
Lower Limit	.1951	.3786	.1384	.2529	.2696	.4506	.1807	--	.1926	.4462
Upper Limit	.2674	.5023	.2015	.3699	.5465	1.0954	.2278	--	.2667	.8232
LC_{50}	.2497	.4683	.1876	.3417	.3760	.6920	.2174	--	.2491	.6259
Lower Limit	.2334	.4384	.1686	.3125	.3254	.5747	.2063	--	.2338	.5485
Upper Limit	.3205	.5859	.2321	.4484	.8519	1.8910	.2573	--	.3272	1.2220
LC_{70}	.2903	.5323	.2158	.4064	.4716	.9254	.2427	--	.2933	.7926
Lower Limit	.2707	.4968	.2008	.3753	.3828	.7095	.2315	--	.2730	.6562
Upper Limit	.4287	.7476	.3019	.6167	1.6334	4.2626	.3142	--	.4526	2.1858
LC_{90}	.3608	.6404	.2641	.5220	.6540	1.4079	.2847	--	.3712	1.1149
Lower Limit	.3256	.5825	.2437	.4694	.4791	.9497	.2669	--	.3316	.8407
X-IRRADIATED										
Upper Limit	.1941	--[a]	.1714	.2633	.2186	.4272	.2369	.4713	.1833	.5558
LC_{30}	.1812	--	.1530	.2324	.2062	.3950	.2251	.4427	.1502	.5086
Lower Limit	.1635	--	.1226	.1866	.1907	.3585	.2110	.4134	.0764	.4715
Upper Limit	.2169	--	.1946	.3119	.2448	.5123	.2688	.5457	.2427	.6755
LC_{50}	.2046	--	.1788	.2832	.2317	.4677	.2545	.5042	.2138	.5876
Lower Limit	.1905	--	.1549	.2459	.2185	.4325	.2420	.4735	.1682	.5404
Upper Limit	.2471	--	.2259	.3820	.2787	.6329	.3110	.6433	.4499	.8358
LC_{70}	.2309	--	.2089	.3451	.2603	.5537	.2878	.5743	.3043	.6789
Lower Limit	.2177	--	.1914	.3134	.3463	.5064	.2722	.5327	.2645	.6085
Upper Limit	.3083	--	.3034	.5556	.3435	.8796	.3906	.8267	1.4800	1.1482
LC_{90}	.2750	--	.2615	.4592	.3081	.7067	.3437	.6930	.5065	.8363
Lower Limit	.2555	--	.2399	.4100	.2865	.6210	.3169	.6232	.3770	.7149

a) nonsignificant regression

Table 10

LD_{50} values (µg malathion per gram of fly) for flies from control and pupae receiving 5,000 R gamma and X-irradiation 2 days before emergence.

TEST	Upper Limit[a]	LD_{50}	Lower Limit[a]
		MALES - CONTROL	
1	20.77	19.64	18.39
2	15.56	13.87	9.97
3	16.47	15.31	13.09
4	36.47	22.22	15.26
5	20.24	19.36	18.34
		MALES - GAMMA IRRADIATED	
1	26.48	24.72	23.11
2	18.78	17.48	15.71
3	52.55	36.15	31.29
4	21.49	20.51	19.46
5	23.46	21.91	20.57
		MALES - X-IRRADIATED	
1	21.26	20.06	18.68
2	11.22	10.31	8.93
3	12.18	11.50	10.87
4	24.66	23.35	22.20
5	23.92	21.07	16.58

a) 95% upper and lower confidence limits

Table 10, Continued

TEST	Upper Limit[a]	LD_{50}	Lower Limit[a]
		FEMALES - CONTROL	
1	19.26	17.81	16.43
2	16.76	15.55	14.21
3	17.29	16.19	15.01
4	20.11	19.05	18.13
5	21.71	20.09	18.82
		FEMALES - GAMMA IRRADIATED	
1	28.38	26.40	24.77
2	20.70	19.12	17.49
3	66.39	41.94	34.83
4	--[b]	--	--
5	45.62	34.69	30.40
		FEMALES - X-IRRADIATED	
1	--	--	--
2	17.57	15.95	13.85
3	27.84	25.42	23.51
4	30.15	27.86	26.16
5	36.65	31.88	29.32

a) 95% upper and lower confidence limits

b) nonsignificant regression

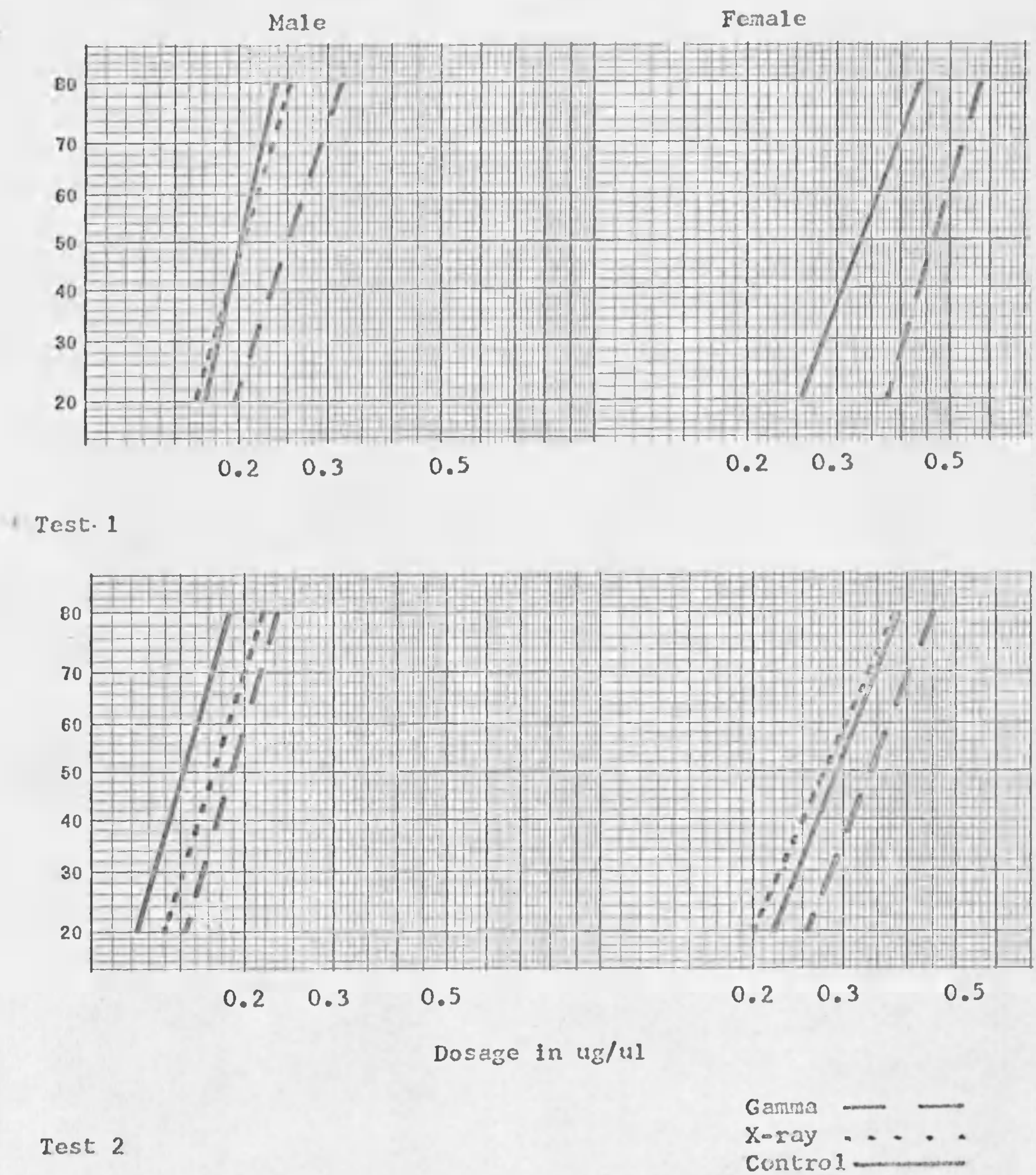
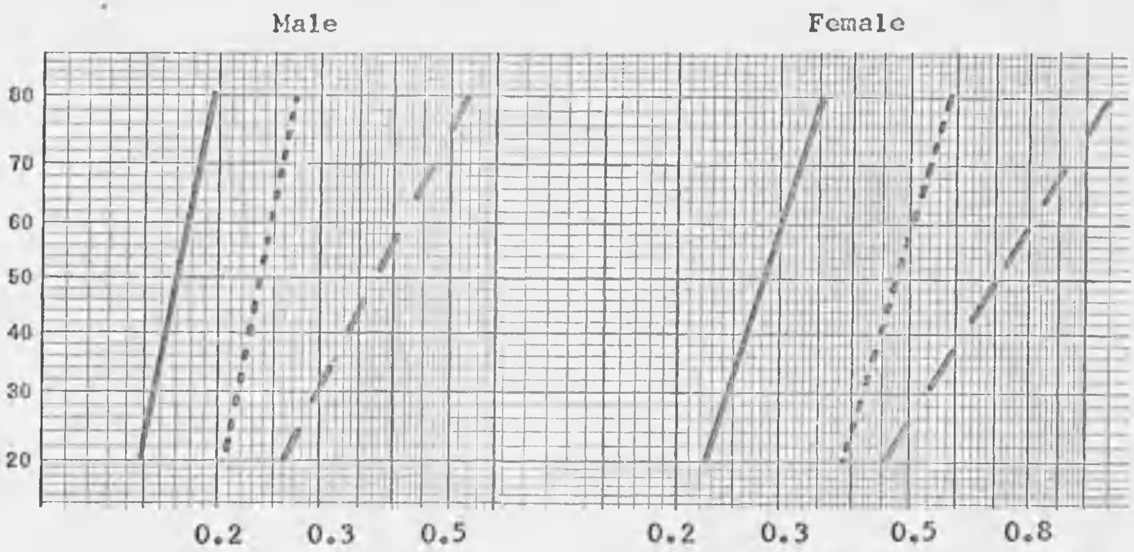
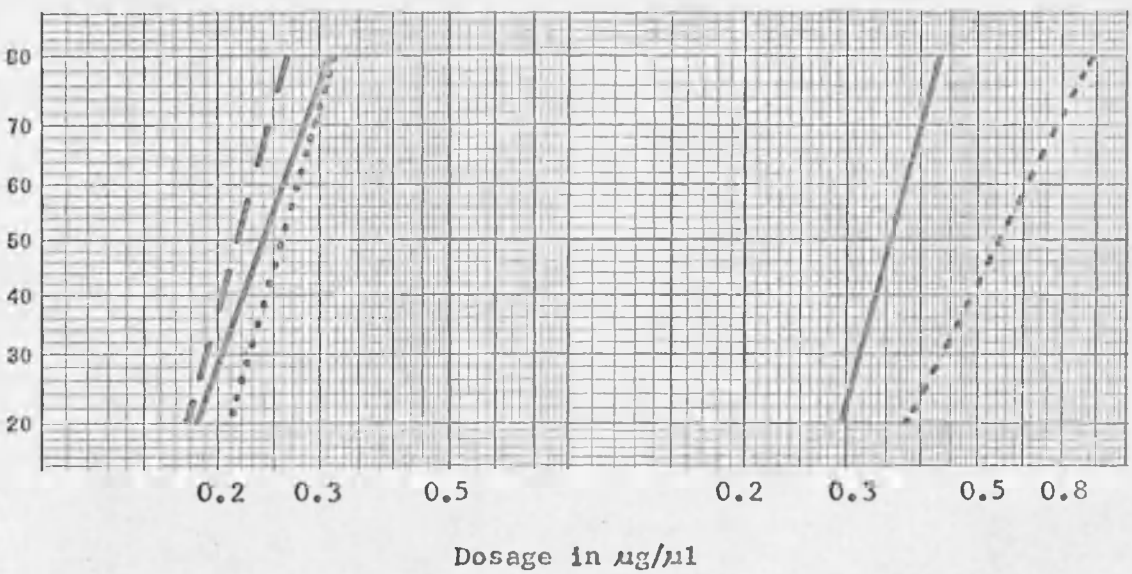


Figure 6. Dosage-mortality curves for pupae receiving X- or gamma radiation two days prior to peak emergence.

Tests 1 and 2 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae X- or gamma irradiated with 5,000 R two days prior to peak emergence.



Test 3

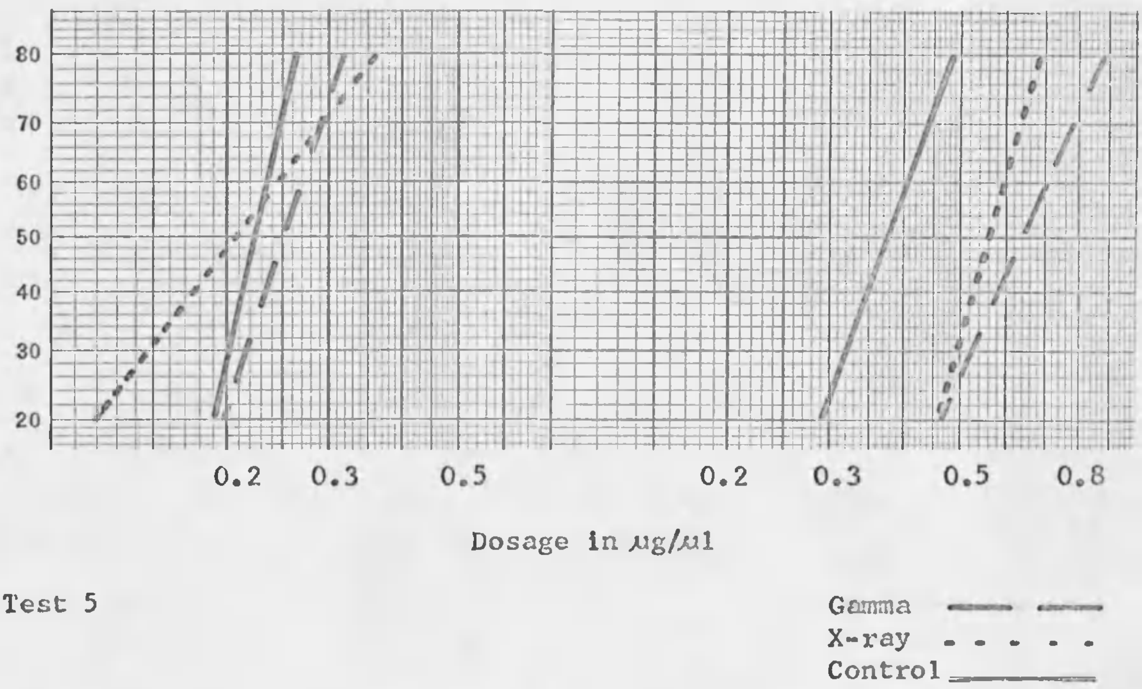


Test 4

Gamma
X-ray
Control

Figure 6, Continued

Tests 3 and 4 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma or X-irradiated with 5,000 R two days prior to peak emergence.



Test 5

Figure 6, <u>Continued</u>

Test 6 of dosage-mortality curves for topically applied malathion on adult house flies from control pupae and pupae gamma or X-irradiated with 5,000 R two days prior to peak emergence.

more rapid than in the insect (70-80% is degraded in 30 minutes), with malaoxon production correspondingly lower. They suggested two degradative pathways for malathion in insects: that owing to phosphatases attacking the P-S-C site and that owing to carboxyesterases attacking the carboxyester group, $COOC_2H_2$.

O'Brien (1967) states that phosphatase and carboxyesterase cleavage exists universally in organophosphates and in those compounds containing a carboxyester group, COOR, as in malathion. Cleavage of the latter group may constitute the predominant degradative pathway. With malathion, carboxyesterase activity predominates in mammals, while phosphatase and carboxyesterase activity are about equal in insects. Krueger and O'Brien's work (1959) supports this since, of the total identified products, the percentage of carboxyesterase products at 30 minutes was 57% in the American cockroach, 49% in the German cockroach, 36% in the house fly, and 77% in mice. Therefore, in cockroaches carboxyesterase action accounted for up to a half of the degradation, while in the house fly, carboxyesterase action was far less.

The mouse is more resistant to malathion than the house fly and insects in general because of the greater carboxyesterase action in the mouse. This contention is further supported by Matsumura and Brown (1961) in their work with malathion resistance in the mosquito, Culex tarsalis. They showed that the carboxyesterase level in larvae of a malathion resistant strain of this mosquito was three times higher in the resistant than in the susceptible strain. Correspondingly lower malaoxon levels were present in the resistant strain after malathion poisoning. Backcrossing studies showed that resistance is genetically inseparable from increased carboxyesterase activity.

It appears, therefore, that there is a direct relationship between insect resistance to malathion and carboxyesterase levels. The effects of irradiation may be to stimulate, either directly or indirectly, the production or activity of these degrading enzymes such that their overall potential for attacking and rendering the malathion molecule ineffective is enhanced. This may be one way of explaining the results of increased tolerance to malathion of irradiated house flies.

SUMMARY AND CONCLUSIONS

The effects of gamma irradiation on house fly pupal emergence were tested on pupae irradiated with different doses of gamma rays, ranging between 2.0 KR and 25.0 KR, on varying days before emergence. The dosage-mortality curves show that emergence increases as the age of the pupae at time of irradiation increases. A second series of emergence studies examined adult emergence from control pupae and from pupae irradiated with 12,000 R or 15,000 R at 2, 3, 4, 5, and 6 days before emergence. The greatest emergence for the irradiated flies occurred in pupae irradiated at 2 days before emergence with no emergence from those irradiated at 5 and 6 days before emergence.

The results from the combined effects of pupae subjected to gamma irradiation (5,000 R) on varying days before peak emergence, and followed by topical treatment of adults with malathion, are as follows:

1) Females irradiated 2 days before emergence were more tolerant to malathion than non-irradiated females. Males similarly treated showed only a slight tolerance to malathion.

2) Males from pupae irradiated 3 days before emergence showed an increased tolerance to malathion over the controls. Females were slightly more tolerant.

3) Males from pupae irradiated 4 days before emergence were significantly more tolerant to malathion than the controls. Females were only slightly more tolerant.

In summary, these data show that male pupae, irradiated 4 days before emergence, are significantly more tolerant than when irradiated as older pupae. With females the reverse is true. Female pupae irradiated at 2 days before emergence were more tolerant to malathion than when irradiated earlier.

It is suggested that the increased resistance of irradiated pupae may be attributed to increased carboxyesterase levels.

In comparing the influence of gamma and X-rays on the toxicity of malathion to pupae irradiated at 2 days before emergence, it was found that gamma irradiation had a greater effect on tolerance to malathion than X-irradiation. However, this may be due to the margin of error in the delivery dosages of the X-rays.

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