Relation Between Temperature and Joint Toxic Action of *p,p'*-DDT with *p,p'*-DMC to Adult House Fly of a DDT Resistant Strain

(Diptera : Muscidae)

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DDT 抵抗性イエバエに対する *p,p'*-DDT と *p,p'*-DMC の 連合致死作用と温度

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Introduction

In the past history of insecticide development studies, intensive works were made to search for appropriate synergists for improving the insufficient effectiveness of DDT against resistant insects. One of chemicals found as promising synergists, DMC is a structurally related compound to DDT but DMC itself is not toxic to insects. The mechanism of synergistic action of DMC for DDT against resistant house flies was studied by Perry et al.[6] and was concluded that DMC competitively inhibits the DDT-detoxification with DDT. On the other hand, it has been reported by many investigators that the temperature coefficient of toxic action of DDT is negative in general in both susceptible and resistant insects. The negative temperature coefficient of joint toxic action between DDT and DMC to resistant house flies was also ascertained by Nagasawa and Hoskins[5] over the range 5° to 35°C. They used the resistant DDT-AS strain which had been kept at the University of California, Berkeley, U. S. A. They, however, did not show a detailed experimental data and only the LD_{50}'s at each temperature were described. In the present paper, the writer wishes to report the results of experiment carried out to study the relation between temperature and the joint toxic action of DDT with DMC to adult house flies of a DDT-resistant strain in Brazil.

Materials and Methods

The resistant house fly used for the present work was the "Biologico-SP" strain which had been inbred for more than 15 years at the Biological Institute of São Paulo, São Paulo, Brazil. It was impossible to get 100 per cent mortality at any dosage of

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p, *p*'-DDT in acetone to adult females by the topical application method at 25°C. Rice bran and brewer's yeast were used for rearing larvae, sugar and water were given as diet to adult flies. The environmental condition of the rearing room was kept at ca 25°C and a relative humidity of 60−70 per cent.

The *p*, *p*'-DDT (2, 2-bis p-chlorophenyl-1, 1, 1-trichloroethane) was rechrystalized from ethanol and had a m.p. of 107−108°C. The *p*, *p*'-DMC (1, 1-bis p-chlorophenyl methyl carbinol) was also rechrystalized from ethanol and had a m.p. of 68−69°C. In the future paragraphs, these two chemicals will be described simply as DDT and DMC. Two chemicals were used together at 1 : 1 (wt/wt) ratio which was found to be optimum by Perry et al.[6] Acetone used as a solvent was chemical pure grade. Five concentrations of the mixture were prepared by 1 : 1 (v/v) serial dilutions of a 2.5 μg/μl solution.

Flies, 4 to 5 days old, were lightly anaesthetized with carbon dioxide and sexed. A group of female flies from 24 to 44 individuals were kept in each glass vial of 9 cm in diameter and 5 cm in height with sugar and water for 24 hours under the environmental condition of the rearing room. A total of 4562 individuals were tested for each of 21 experiments varying in temperature, in dosage, and in control.

Just before the application of chemical, the flies were lightly anaesthetized again with carbon dioxide, and the chemical was topically applied on the mesonotum by use of a micrometer driven syringe. After treatment, each group receiving a given dosage was put back into a glass vial with sugar and water and placed at the chosen temperature. After 24 hours mortality counts were made. All flies in a more or less prostulate condition were counted as dead since recovery is extremely rare after the 24 hours period. As the control, acetone was applied in the same way on an equal group. Correction was made by Abbott's formula when dead individuals were found in the control.

Since the number of test flies was not sufficient to compare the effect of seven temperatures on joint toxic action at a time, three temperatures were tested each day and these were related in a randomized manner in accordance with a balanced incomplete block design of a 7×7 Latin square, each of five dosages being repeated on three days. Thus in seven day's trials each temperature occured once on the same day with every other temperature refers to the temperature at which the house flies were kept after treatment, and not to the temperature before or during treatment which was ca 25°C.

Results and Discussion

The results of experiments, giving the ratio of number of flies used to number of flies killed under each of the seven temperatures, are shown in Table 1. The insufficient effectiveness of DDT on DDT-resistant house flies of the Biologico-SP strain was markedly overcome when the synergist DMC was jointly applied, and the fairly dosage-mortality relations from lower to higher mortalities were obtained under the all temperature conditions tested. Only in the test of 21°C of the block V, there

Table 1. Original counts of mortality of the houseflies, *Musca domestica* (L.), treated topically with a mixture of DDT and DMC in acetone at seven different temperatures.

Block	Temp. °C	Dosage, μg/♀					
		0.156	0.313	0.625	1.250	2.500	Control
I	15	0/33*	2/28	16/36	30/35	33/33	0/43
	18	0/36	2/33	17/37	30/37	28/29	0/38
	24	0/39	2/36	9/36	24/35	36/39	0/32
II	21	0/34	4/37	14/34	26/37	36/36	0/35
	27	1/36	1/37	11/39	22/37	33/36	1/40
	15	0/41	2/38	15/35	28/34	39/39	0/35
III	24	2/36	10/34	19/36	29/30	35/35	0/34
	21	2/38	15/41	24/35	36/37	32/32	0/24
	33	1/32	3/34	8/34	23/34	34/37	1/32
IV	33	0/41	2/40	5/29	25/40	36/40	0/36
	15	0/39	10/33	18/37	32/36	40/40	0/37
	30	0/42	1/36	13/42	29/38	39/40	0/39
V	18	0/39	10/41	23/40	33/36	35/37	0/35
	30	0/39	0/39	1/32	23/37	29/31	0/35
	21	0/40	2/33	4/33	38/38	34/34	2/31
VI	27	0/36	5/41	17/39	34/39	39/39	0/39
	33	0/38	3/40	19/44	28/34	35/35	0/39
	18	3/40	15/36	24/32	37/37	33/33	0/37
VII	30	0/40	3/36	11/35	18/32	30/33	0/35
	24	0/38	5/36	19/38	30/38	36/37	0/32
	27	0/33	4/37	18/38	27/30	34/34	0/37

*Number of flies killed/number of flies used

was no responsed flies at the lowest dosage, mortalities at the next two lower dosages were very small and there were no survivors from either of the two higher dosages. After the correction by Abbott's formula, only one observation indicated an effective mortality for computation, which lead to its omission. The dosage-mortality relations obtained were analysed by the standard method (Bliss[1]) with corrected probits and weights after the dosages were multiplied by 10 and converted to logarithms to avoid negative values and facilitate computation. The analysis has been done in this modified scale and in the final step reconverted to the original dosages by subtracting 1.000 from the log-dose killing 50 per cent of the house flies (log-LD_{50}) at each temperature.

The analysis of variance in Table 2 shows that the three dosage-mortality regression lines in each day determined together are parallel within the experimental error and these three regression lines can be adequately represented by their combined slope, and that 20 individual dosage-mortality regression lines can be represented by a single combined slope of $b_c=3.663$, since the seven combined slopes did not also differ from day to day within the limits of the experimental error. No consistent non-linear trend is also proved in Fig. 1 in which the individual curves were calculated by the method of Bliss and Marks[2] from the empirical probits and equal weights and were shifted horizontally on the x axis until their mean probits fell along a single line

Table 2. Analysis of variance of the slopes of the curve relating probit kill and log dosage based upon weighted empirical probits.

Variance due to	Degrees of freedom	Sum of squares	Mean square	F
Combined slope for all tests, b_c	1	1361.72	1361.72	
Differences between daily slopes	6	3.88	0.65	1.35
Daily slopes	7	1365.60		
Differences between individual slopes	13	18.62	1.43	2.99
Individual slopes	20	1384.22		
Deviation from straight lines	60	28.70	0.48	
Total	80	1412.92		

Table 3. Toxicity in terms of the log-LD_{50} (+1.000) for each series. The missing value* of Table 3 was estimated to complete the balanced incomplete block experiment data (Cornish[3]), then calculation was proceeded.

°C	I	II	III	IV	V	VI	VII	V_s	W_s	Y_s
15	0.832	0.855		0.746				2.433	−3.036	2.252
18	0.858				0.745	0.568		2.171	−0.898	2.117
21		0.879	0.693		0.855*			2.427	−0.828	2.378
24	0.973		0.619				0.830	2.422	0.082	2.427
27		1.014				0.815	0.802	2.631	0.852	2.682
30				0.923	1.077		0.975	2.975	−0.106	2.968
33			0.991	1.020		0.846		2.857	3.934	3.092
T_s	2.663	2.748	2.303	2.689	2.677	2.229	2.607	17.916	0.000	17.916

passing through the mean probit for all series of $\bar{y}=4.756$ and $b_c=4.001$.

Following the computational instruction given by Moore and Bliss[4], the data shown in Table 3 were analysed and the mean toxicities were adjusted on the basis of all available information. The values V_s and T_s along the margin of Table 3 are the sums of the entries for each temperature and for each day, respectively. The value W_s is a multiple of the daily discrepancy between the three days on which a given temperature was tested and the four days on which it was omitted. Each of the Y_s in Table 3 represented the sum of three determinations and was divided by 3 to obtain the final estimate of y_s for the log-LD_{50}'s for each temperature. The adjusted mean log-LD_{50}'s at seven temperatures have been listed in Table 4, and in the third column of Table 4 these have been expressed as the dosage in μg/♀ which killed 50 per cent of flies under the condition of experiment. The standard error of the mean and the just significance difference (LSD) have been added to the data. The log-LD_{50}'s are definitely decreased with the increase of temperature, and the result well coincides with the previous data that Nagasawa and Hoskins[5] obtained in the DDT-AS strain.

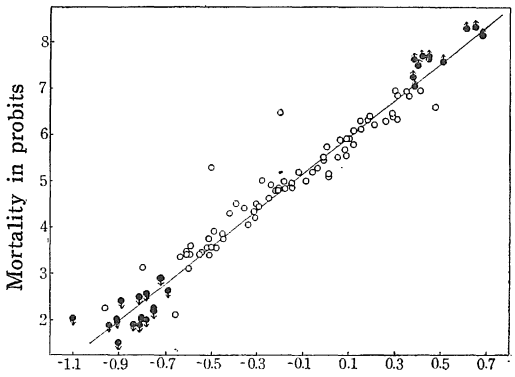

Fig. 1. Dosage-mortality curves for all tests of 7 temperatures following adjustment of the log-dosage to bring the means for the individual curves into a single straight line. Solid circles indicate corrected probits for 0 or 100 per cent mortality, all other values representing empirical probits.

Table 4. Relative toxicity of DDT synergized by DMC to the DDT-resistant houseflies at seven different temperatures for differences between days.

Temp. °C	log-LD_{50} (+1.000)	LD_{50}(μg/♀)
15	0.751	0.564
18	0.706	0.508
21	0.793	0.621
24	0.809	0.644
27	0.894	0.784
30	0.990	0.977
33	1.031	1.074
SE	0.043	
LSD	0.142	

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Summary

The high DDT resistance of adult house fly of the Biologico-SP strain in Brazil was mostly overcome by adding DMC in 1 : 1 ratio by weight. Temperature coefficient of joint toxic action between DDT and DMC was negative in general over the range 15°−33°C.