原　著

Studies on the Mechanism of DDT Resistance in *Culex pipiens fatigans* III. Effect of Synergists on the Toxicity of DDT. R. L. Kalra (National Malaria Eradication Programme, Delhi, India) Received February 9, 1970. *Botyu-Kagaku* 35, 33, 1970.

4. *Culex pipiens fatigans* の DDT 抵抗性の機構 III. DDT の殺虫力におよぼす共力剤の効力. R. L. Kalra (National Malaria Eradication Programme, Delhi, India) 45. 2. 9 受理

3 系統の DDT 抵抗性 *Culex pipiens fatigans* に対し, DMC (1, 1-bis (*p*-chlorophenyl) methyl carbinol), Warf (*N*-di-*n*-butyl-*p*-chlorobenzene sulfonamide), piperonyl butoxide (3, 4-methylenedioxy-6-propyl benzyl butyl diethylene glycol ether) の *p*, *p*′-DDT および近縁化合物の殺虫力におよぼす効力を調べた. この3種の化合物には DDT 共力効果が認められなかった. この幼虫の体内では *p*, *p*′D-DT が *p*, *p*′-DDE に代謝されることを DMC が阻害しない.

体内での TDE の代謝と *p*, *p*′-DDT の代謝とを比較検討すると, この昆虫における DDT-ase はイエバエやネツタイシマカのそれと異なると思われる.

Kalra *et al*[6] earlier observed the extensive dehydrohalogenation of *p*, *p*′-DDT to *p*, *p*′-DDE in the larvae of both susceptible and resistant strains of *Culex pipiens fatigans*. It was further observed that DMC did not enhance the toxicity of *p*, *p*′-DDT against the larvae of a resistant strain originating from Delhi. Earlier studies on the joint action of synergists and DDT against susceptible and resistant strains of insects by various workers[4,12,16,19~21,24,26] has thrown much light on the mechanism of DDT resistance in various insect species. It is with this object that the detailed investigations on the effect of DMC, Warf anti-resistant and piperonyl butoxide on the toxicity of *p*, *p*′-DDT and related compounds against the larvae of various resistant strains of *C. p. fatigans* were undertaken. The inhibitory effect of DMC on the *in vivo* metabolism of *p*, *p*′-DDT by the larvae was also studied.

Materials and Methods

Insect material

The following strains of *C. p. fatigans* showing different level of tolerance to DDT were used.

DN (Delhi-N): Adult mosquitoes for this strain were originally collected from Delhi in the year 1948. This strain has been under continuous rearing in the laboratory without any intentional contamination of insecticides. Nevertheless, the strain was found to be tolerant to *p*, *p*′-DDT when compared with the susceptible strains.[18]

RR (Rangoon-R): This strain originating from Rangoon (Burma) was selected for *p*, *p*′-DDT resistance at the Department of Zoology, University of Western Ontario, Canada.[29] A sub colony was kindly supplied to National Institute of Communicable Diseases, Delhi and was maintained under selection with *p*, *p*′-DDT.

DR (Delhi-R): The larvae of this strain were originally collected from the areas around Delhi in the year 1964 and were selected with *o*, *p*′-DDT.[5]

All the strains were reared and maintained following the method described by Krishnan[11].

Insecticides:

The insecticides and synergists used were: *p*, *p*′-DDT (2, 2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane), *o*, *p*′-DDT (2-(4-chlorophenyl)-2-(2-chlorophenyl)-1, 1, 1-trichloroethane), *o*-chloro-DDT (2-(2, 4-dichlorophenyl)-2-(4-chlorophenyl)-1, 1, 1-trichloroethane), deutero-DDT (2, 2-bis (*p*-chlorophenyl)-1, 1, 1-trichlororoethane-2d), methoxychlor (2, 2-bis (*p*-methoxyphenyl)-1, 1, 1-trichloroethane), TDE (2, 2-bis (*p*-chlorophenyl)-1, 1-dichloroethane), DMC (1, 1-bis (*p*-chlorophenyl) methyl carbinol), Warf anti-resistant *N*-di-*n*-butyl-*p*-chlorobenzene sulfonamide) and piperonyl butoxide (3, 4-methylenedioxy-6-propyl benzyl butyl diethylene glycol ether). *Toxicological method*:

The toxicity of insecticides, synergists and their

mixtures was determined by following the method recommended by World Health Organisation[30] for assessing the susceptibility of mosquito larvae to insecticides. Lots of 25 larvae were exposed in 250 ml. of water containing the desired concentration of the insecticide and the mortality was recorded after 24 hours of continuous exposure. About 3-4 concentrations were used for each regime and approximately 150 larvae were tested at each concentration. The results obtained were analysed by Probit analysis method[3].

Method for metabolic study of DDT:

Batches of 500 larvae were exposed to *p, p'*-DDT and the combination of *p, p'*-DDT and DMC for 24 hours. After exposure, the larvae were thoroughly rinsed with distilled water. The larvae were then ground and extracted with ether in Soxhlet apparatus for 6 hours. After evaporation of the solvent, the residue was dissolved in carbon tetrachloride and passed through celite- sulphuric acid column following the method used by Perry *et al*[21]. The CCl_4 from the elute was evaporated and the amounts of *p, p'*-DDT and *p, p'*-DDE were estimated following Schechter-Haller method[25,10].

Results

Effect of DMC on the toxicity of p, p'-DDT and related compounds.

The LC_{50} values of insecticides when tested alone and in combination with different concentrations of DMC against the larvae of the resistant strains are given in Table 1. The LC_{50} values of DMC alone as obtained in parallel tests are also given.

Table 1. Showing the effect of DMC on the toxicity of *p, p'*-DDT and related compounds against the larvae of the resistant strains of *C. p. fatigans*.

Insecticide	Strain	LC_{50} (ppm) of insecticide with DMC (in ppm). 0.0	5.0	10.0	LC_{50} of DMC (ppm) alone.
p, p'-DDT	DN	0.25	0.40	0.32	10.0
	RR	>40.0 (26.7)	>40.0 (24.0)	>40.0 (18.0)	>10.0 (10.0)
	DR	>40.0 (0.0)	>40.0 (0.0)	>40.0 (0.0)	>10.0 (0.0)
o, p'-DDT	DN	0.015	0.05	0.015	8.3
	RR	0.02	—	0.035	>10.0 (0.0)
	DR	>2.0 (13.3)	>2.0 (0.0)	—	>10.0 (0.0)
o-chloro-DDT	DN	0.013	0.063	0.016	12.6
	RR	0.50	1.26	1.0	>10.0 (0.0)
	DR	<4.0 (56.7)	>4.0 (43.3)	>4.0 (41.7)	>10.0 (0.0)
deutero-DDT	DN	0.0064	0.0080	0.0025	7.9
	RR	0.13	0.11	0.06	>10.0 (0.0)
	DR	>2.0 (25.0)	>2.0 (18.3)	>2.0 (38.3)	>10.0 (6.7)
methoxychlor	DN	0.04	0.045	0.04	>10.0 (0.0)
	RR	0.13	0.11	0.10	>10.0 (0.0)
	DR	4.0	4.0	4.0	>10.0 (0.0)

Figures in parenthesis show the percentage mortality at the indicated concentration.

The LC_{50} value of *p, p'*-DDT when tested in admixture with 5.0 and 10.0 ppm of DMC were 0.40 and 0.32 ppm respectively. The LC_{50} of *p, p'*-DDT alone was 0.25 ppm. The results, therefore did not show any synergistic effect of DMC on the toxicity of *p, p'*-DDT. However, DMC at a concentration of 5.0 and 0.0 ppm rather exhibited some antagonistic effect. DMC itself was also observed to be toxic against the larvae, the LC_{50} value in a parallel test was found to be 10.0 ppm. The LC_{50} value of *o, p'*-DDT was observed to be 0.015 ppm for the larvae of Delhi-N strain. Its LC_{50} value when tested in admixture with 5.0ppm of DMC was 0.05 ppm thereby showing the antagonistic effect. Nevertheless, the LC_{50} value of *o, p'*-DDT when tested in combination with 10.0 ppm of DMC was found to be 0.015 ppm. The LC_{50} value of DMC alone in a parallel test was observed to be 8.3 ppm, thereby suggesting that the effect observed in the case of the mixture of *o, p'*-DDT and 10 ppm of DMC may be due to the toxicity of DMC.

DMC was also not observed to enhance the toxicity of *o*-chloro-DDT, deutero-DDT and methoxychlor against the larvae of Delhi-N strain (Table 1).

The results obtained in a case of the larvae of Rangoon-R strain showed that *p, p'*-DDT at the concentration of 40.0 ppm caused only 26.7 percent mortality (Table 1). *p, p'*-DDT at 40.0 ppm, when tested in admixture with 5.0 ppm and 10.0 ppm of DMC caused only 24 and 17 percent kill in the larvae thereby showing lack of synergism. Slight antagonistic effect of DMC on *o, p'*-DDT and *o*-chloro DDT was observed whereas no effect was evident with methoxychlor and deutero-DDT (Table 1).

DMC was also not observed to be synergistic for *p, p'*-DDT and related compounds against the larvae of Delhi-R strain of *C. p. fatigans* (Table 1).

Effect of Warf anti-resistant on the toxicity of p, p'-DDT.

Warf anti-resistant and *p, p'*-DDT were tested in the ratios of 1:1, 1:5 and 5:1. The results obtained clearly indicated that Warf anti-resistant did not enhance the toxicity of *p, p'*-DDT against the larvae of any of the resistant strains of *C. p. fatigans* used (Table 2).

Effect of piperonyl butoxide on the toxicity of p, p'-DDT and related compounds:

Piperonyl butoxide at the concentration of 5.0 and 10.0 ppm was tested in admixture with *p, p'*-DDT, *o, p'*-DDT and methoxychlor. At the concentration of 5.0 and 10.0 ppm, piperonyl butoxide alone did not exhibit any toxicity against the larvae. It may be seen from the results that *p, p'*-DDT at the concentration of 40.0 ppm did not cause any mortality in the larvae of Delhi-R strain. Even the addition of piperonyl butoxide did not result any mortality in this strain (Table 3). The toxicity of *o, p'*-DDT and methoxychlor was also not affected. The results did not indicate the synergistic effect of piperonyl butoxide on DDT and related compound even against the

Table 2. LC_{50} values (in ppm) of *p, p'*-DDT alone and in combination with Warf anti-resistant (WARF) for the larvae of resistant strain of *C. p. fatigans*.

Strain	*p, p'*-DDT	*p, p'*-DDT WARF (1:1)	*p, p'*-DDT WARF (1:5)	*p, p'*-DDT WARF (5:1)
DN	0.33	0.31	0.32	—
RR	>20.0 (10.0)	—	—	>20.0 (12.0)
DR	>40.0 (0.0)	—	—	>40.0 (0.0)

Figures in parenthesis indicate the percentage kill at the indicated concentration.

Table 3. LC_{50} values (in ppm) of insecticides alone and in combination with piperonyl butoxide for the larvae of resistant strain of *C. p. fatigans*.

	DN	RR	DR
p, p'-DDT	1.3	>40.0 (35.0)	>40.0 (0.0)
p, p'-DDT+ PB 5.0ppm	1.0	>40.0 (28.0)	>40.0 (0.0)
p, p'-DDT+ PB 10.0 ppm	0.8	>40.0 (32.0)	>40.0 (0.0)
o, p'-DDT	—	—	>4.0 (15.0)
o, p-DDT+ PB 10.0ppm	—	—	>4.0 (3.2)
Methoxychlor	—	0.06	1.0
Methoxychlor+ PB 10.0ppm	—	0.06	1.0

PB=piperonyl butoxide

Figures in parenthesis indicate the percentage kill in the larvae at the indicated concentration.

Table 4. Amount and percentage of *p, p'*-DDT dehydrochlorinated by the larvae of *C. p. fatigans* on exposure to *p, p'*-DDT (0.5 ppm) and *p, p'*-DDT-DMC mixture (0.5 ppm+0.5 ppm).

Strain	DDT-treatment			DDT-DMC treatment		
	DDT (μg/500 larvae)	DDE	% metabolised	DDT (μg/500 larvae)	DDE	% metabolised
DN	22.5±4.3	18.8±3.8	46.9±1.5	13.1±2.4	17.1±1.2	53.4±1.2
RR	21.2±2.2	24.5±1.0	53.7±3.5	20.5±3.4	24.7±0.9	55.1±4.8
DR	14.5±0.8	25.5±0.3	63.1±1.2	12.7±1.3	22.1±1.5	64.9±0.9

larvae of Rangoon-R and Delhi-N strains of *C. p. fatigans.*

Effect of DMC on the in vivo metabolism of p, p'-DDT

The results obtained on metabolism of *p, p'*-DDT by exposing the larvae under identical conditions to *p, p'*-DDT (0.5 ppm) and *p, p'*-DDT-DMC (0.5 ppm: 0.5 ppm) combination for 20 hours indicated that the larvae of Delhi-N strain were found to be metabolize higher proportion (53.4 percent) of *p, p'*-DDT when exposed to DDT-DMC combination as compared to those exposed to *p, p'*-DDT alone (Table 4). However, no difference was apparent in the actual amount of *p, p'*-DDT metabolised. DMC was also not found to inhibit the metabolism of *p, p'*-DDT to *p, p'*-DDE in the larvae of both Rangoon-R and Delhi-R strains of *C. p. fatigans* (Table 4).

The larvae exposed to high concentration of *p, p'*-DDT-DMC combination (10 ppm : 10 ppm) were observed to pick much less quantity of *p, p'*-DDT as compared to the larvae exposed to *p, p'*-DDT (10 ppm) alone (results given below).

μg. pick up/500 larvae

Strain	*p, p'*-DDT treatment	*p, p'*-DDT-DMC treatment.
Rangoon-R	1222	215
Delhi-R	1372	366

The results given above suggest that the ineffectiveness of DMC as a synergist for DDT could be due to the lower pick up of DDT when present with DMC. The effect of pretreatment with DMC was therefore, studied. Pre-treatment of the larvae with 5.0 ppm of DMC was not observed to increase their susceptibility to *p, p'*-DDT and *o, p'*-DDT. Also pretreatment with DMC was not found to affect the metabolism of *p, p'*-DDT to *p, p'*-DDE in the larvae.

Discussion

Unlike house flies[27] and *Aedes aegypti*[1], DMC was not found to act as a synergist for *p, p'*-DDT against the resistant strains of *C. p. fatigans.* DMC was also not observed to enhance the toxicity of *o*-chloro DDT and related compounds. Abedi *et al*[1] observed the synergistic effect of DMC with *o*-chloro DDT against *Aedes aegypti* whereas the observations made by Perry *et al*[23] did not give any evidence of the synergism of *o*-chloro DDT by DMC in the house flies. The results further indicated the lack of inhibitory effect of DMC on the metabolism of *p, p'*-DDT to *p, p'*-DDE in the larvae of resistant strains of *C. p. fatigans.* DMC has however been reported to be an inhibitor for DDT-ase in case of house flies[14,21] and *Aedes aegypti*[1]. The results of these investigations, therefore, indicated that DDT-ase in *C. p. fatigans* is different from the one present in most strains of the house flies and *Aedes aegypti.* DDT-metabalism in *C. p. fatigans* was not inhibited by DMC and therefore resembles in this characteristic the enzyme present in the strain FC of house fly[16]. Kimura *et al*[8], Fine *et al*[2] and Perry *et al*[22] also did not find any significant decrease in DDE production due to DMC in *Culex tarsalis, T. infestans,* and body lice.

In vivo studies on the metabolism of TDE in relation to *p, p'*-DDT indicated that TDE (30.8 percent) was metabolised at a lower rate than that of *p, p'*-DDT (51.6 percent) in *C. p. fatigans.* These results, therefore further suggest that DDT-ase in *C. p. fatigans* is different from *Aedes aegypti* and house flies and resembles the one reported to be present in FC strains of house flies[16,17].

The present studies revealed the antagonistic effect of DMC on the toxicity of *p, p'*-DDT and

related compounds. The antagonistic effect is considered probably due to the lower pick up of insecticides as the larvae on exposure to the combination of *p, p'*-DDT and high concentration of DMC picked up significantly less quantity of *p, p'*-DDT as compared to the larvae exposed to *p, p'*-DDT alone. Nevertheless, the lack of synergism was not due to the lower pick up as even prior treatment with DMC neither enhanced the toxicity of *p, p'*-DDT nor inhibited its metabolism. Warf anti-resistant proved to be highly effective DDT synergist against DDT resistant house flies[15,26] and *Aedes aegypti*[24] was also not observed to increase the toxicity of *p, p'*-DDT against the larvae of the resistant strains of *C. p. fatigans*.

The results also did not indicate the synergistic effect of piperonyl butoxide on the toxicity of *p, p'*-DDT and *o, p'*-DDT. Unlike *Aedes aegypti*[9] and house flies[28], piperonyl butoxide was not found to be synergistic for methoxychlor against the larvae of *C. p. fatigans*. The methylenedioxy phenyl compounds are known to act as synergists by inhibiting more particularly the hydroxylation of DDT. Lack of synergism of DDT and related compounds with piperonyl butoxide suggested the absence of the role of α-hydroxylation by mixed function oxidases as a mechanism of resistance.

Summary

The effect of DMC, Warf anti-resistant and piperonyl butoxide on the toxicity of *p, p'*-DDT and related compounds against the larvae of various resistant strains of *Culex pipiens fatigans* was investigated. None of the compounds tested showed any synergistic effect. DMC was also not found to inhibit the *in vivo* metabolism of *p, p'*-DDT to *p, p'*-DDE in the larvae of *C. p. fatigans*.

The results on the investigations on comparative *in vivo* metabolism of TDE in relation to *p, p'*-DDT and effect of synergists like DMC and Warf anti-resistant suggest that DDT-ase in *C. p. fatigans* is different from the one reported to be present in house flies and *Aedes aegypti*.

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Les effets toxiques d'insecticides variès sur les imagos du Moth Fly. Akifumi Hayashi et Masayoshi Hatsukade (Laboratoire d'Entomologie Appliquèe, Compagnie Pharmaceutique Taisho, Toshimaku Tokyo) Reçu le 20 Mars, 1970. *Botyu-Kagaku* 35, 38, 1970. ((Avec un français résumé 43)

5. オオチョウバエ *Telmatoscopus albipunctatus* Williston 成虫の数種殺虫剤に対する感受性について

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45. 3. 10 受理

最近，オオチョウバエが不快害虫として問題になっているが，駆除方法についての研究がなされていないので基礎的な実験を行なった．

オオチョウバエの成虫を駆除する場合，油剤の直接噴霧法によるならば pyrethrins が最も効果的で，DDVP がこれに準ずるものであることが明らかになった．残留噴霧法によるならば DDVP, Diazinon が最も効果的で，pyrethrins がこれに準ずることがわかった．

また，オオチョウバエの発生源ならびに棲息場所からみて，DDVP 樹脂蒸散剤の効果の高いことがわかった．

オオチョウバエ *Telmatoscopus albipunctatus* Williston幼虫の殺虫剤感受性については林ら(1969)[2]の報告があるが，成虫についての報告は少ない．著者らは，これらの駆除対策をたてる目的で基礎的な実験を行ない，知見を得たので報告する．

本文に入るに際し，御指導をいただいた神奈川県衛生研究所の森谷清樹博士に御礼申し上げる．また，研究に際し，種々御便宜をいただいた当社の常務取締役井川俊一博士，研究部長田中一郎博士に謝意を表する．

I. 実験材料および方法

供試昆虫 実験にもちいたオオチョウバエ *Telmatoscopus albipunctatus* Williston は1968年11月に神奈川県衛生研究所より譲渡をうけ，その後，当研究室において林ら (1969)[2]の記載した方法で累代飼育中の羽化後2日から3日目の成虫である．

供試薬剤 実験にもちいた殺虫剤は DDVP, Baytex, Diazinon, Malathion, Sumithion, γ-BHC, Allethrin, pyrethrins, Phthalthrin, および IBTA の10種類で，いずれも工業用純度の原体である．

実験方法 実験は (1)0.5 m³ 箱型装置法，(2)ろ紙接触法，(3)局所施用法の3方法で行なった．

(1) 0.5 m³ 箱型装置法：林ら (1968)[1] によって詳細が報告されている方法で，エアゾール製剤と DDVP 樹脂蒸散剤の実験を行なった．

実験にもちいたエアゾールは pyrethroids 系では 0.2%，有機燐剤，有機塩素剤は 0.5%，IBTA は 2.0 % の濃度でもっとも通常の方法で調製されたものである．

DDVP 樹脂蒸散剤 (1枚中に DDVP が 5% 含まれている製品) は 1 m³ に1枚，3 m³ に1枚，6 m³ に1枚，の3薬量で実験を行なった．実験は 0.5 m³ 箱型