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Toxicity of *p,p'*-DDT, *o,p'*-DDT and Their Mixtures Against Mosquitoes. R.L. KALRA (National Malaria Eradication Programme, Delhi, India) Received September 25, 1969. *Botyu-Kagaku*, 34, 170, (1969).

22. カに対する *p,p'*-DDT, *o,p'*-DDT およびその混合物の毒性

R.L. KALRA (National Malaria Eradication Programme, Delhi). 44. 9. 25 受理

Culex pipiens fatigans, *Aedes aegypti*, *Anopheles subpictus* に対する *p,p'*-DDT, *o,p'*-DDT の殺虫力を dry film, oil solution, topical application 法で検討した．その結果 dry film 法では *o,p'*-DDT は *C.p.fatigans* に対し *p,p'*-DDT より殺虫力が強く，oil solution および topical application 法では，両化合物はほぼ同じ殺虫力を示した．*Aedes aegypti* に対しては，すべての施用法で *p,p'*-DDT は *o,p'*-DDT より殺虫力が強く，*Anopheles subpictus* に対してほぼ同じ殺虫力であった．

p,p'-DDT と *o,p'*-DDT との混合はカの成虫に対して共力作用が認められない．*Aedes aegypti* および *Culex p.fatigans* の *p,p'*-DDT, *o,p'*-DDT に対する感受性を比較すると，*A.aegypti* は *p,p'*-DDT に対して感受性が高く，*o,p'*-DDT に対しては，両種にあまり感受性の差がない．

Yasutomi[22] observed that the joint action of *p,p'*-DDT and *o,p'*-DDT was synergistic against houseflies and body lice. Furthermore, technical DDT has been found to be more toxic than pure *p,p'*-DDT, thereby suggesting the interaction of the isomers[1,16,22]. Kalra and Joshi[10] studied the toxicity of the mixture of *p,p'*-DDT and *o,p'*-DDT against the various species of mosquito larvae. The joint action of the isomers was found to be simple similar against *Culex pipiens fatigans* and synergistic against *Aedes aegypti*. The present communication summarizes the results of the investigation on the relative toxicity of *p,p'*-DDT, *o,p'*-DDT and their mixtures against the adults of various species of mosquitoes and the larvae of *Anopheles subpictus*.

Insect Material

Three to four days old laboratory reared females of *C.p.fatigans* and *Aedes aegypti* were used. Pupae of *Anopheles subpictus* were collected from the areas around Delhi and hatched in the laboratory. The female adult mosquitoes thus obtained were used when 3-4 days old. All the tests were done on glucose fed female mosquitoes. Larvae of *Anopheles subpictus* collected from the field were used as such.

Methods

Dry film method

The insecticides were applied in standard w/v acetone solution to whatman filter papers No. 1 (15cm×12cm), 1.5ml. of the different concentrations of insecticidal solutions were pipetted on the papers and allowed to evaporate com-

pletely. The treated papers were rolled into the plastic vials supplied in the WHO kit for assessing the susceptibility of adult mosquitoes, linning the sides completely. Twenty female mosquitoes were exposed in each vial. Females of *Aedes aegypti* were exposed for 4 hours to the treated papers after which those were transferred to the holding tubes and at the end of 24 hours of holding period the mortality in them was recorded. In the case of *C. p. fatigans* the mortality was recorded at the end of the continuous exposure for 24 hours. About 200 mosquitoes were tested at each concentration.

Oil Solution.

The principle of Busvine and Nash method[2] was followed. Filter papers whatman No. 1, 15cm×12cm were impregnated with various concentrations of the insecticides in B. O. C. white oil. Ethylene dichloride was used as a diluent. The papers were dried for 24 hours after which those were rolled in the plastic vials supplied in WHO kit. Twenty females mosquitoes were exposed in each vial. Females of *Aedes aegypti* were exposed to the treated papers for 1 hour after which those were transferred to the holding tubes and the mortality in them was recorded at the end of 24 hours of holding period. *C. p. fatigans* was exposed for 48 hours and the mortality in them was recorded at the end of exposure. About 160～200 mosquitoes were tested at each concentration.

Topical application method.

1 μl of the different concentration of insecticides in alcohol was topically applied on the thorax of the anaesthetized insect by means of a micrometer syringe. The insects after application were kept under observation for 24 hours when mortality in them was recorded. About 150 mosquitoes were tested at each concentration of the insecticide.

Larval susceptibility test.

The toxicity of insecticides against mosquito larvae was determined by means of the WHO standard method[21]. Lots of 30 larvae were exposed in 250m*l* of water containing the desired concentration of the insecticides. The mortality of the larvae was recorded after 24 hours of continuous exposure. About 300 larvae were tested at each concentration of the insecticide.

Statistical analysis.

The results obtained were analysed by Probit method[3]. The elucidation of the joint action was done by following Finney[3] and Sun and Johnson[20].

Table 1. Showing the toxicity of *p, p'*-DDT, *op'*-DDT and their mixtures against *C. p. fatigans*.

Method	Insecticide	Exposure period (Hrs.)	Equation of the fitted lc-pr regression line Y=	χ^2(d. f.)	Regression coefficient.	LC_{50} (fiducial limits)
Dry film	*p, p'*-DDT	24	0.1723+1.57 log $10^2\lambda$	2.61(2)	1.57±0.18	11.9 per cent (8.9～15.8)
	o, p'-DDT	24	3.3316+2.59 log $10^2\lambda$	11.5(3)	2.59±0.28	0.044 per cent (0.034～0.056)
	p, p'-DDT+*o, p'*-DDT (80:20)	24	3.0709+1.78 log $10^2\lambda$	6.59(4)	1.78±0.10	0.12 per cent (0.11～0.13)
Topical application	*p, p'*-DDT	—	3.6156+1.55 log 10λ	1.68(2)	1.55±0.17	0.78 μg/mosq. (0.67～0.92)
	o, p'-DDT	—	3.1928+2.03 log 10λ	4.68(2)	2.03±0.17	0.78 μg/mosq. (0.69～0.92)
Oil solution	*p, p'*-DDT	48	1.6530+2.44 log 10λ	7.73(1)	2.44±0.53	2.36 per cent (1.60～3.45).
	o, p'-DDT	48	1.3027+2.44 log 10λ	6.40(1)	2.44±0.50	3.27 per cent. (2.18～4.92)
	p, p'-DDT+*o, p'*-DDT (50:50)	48	1.2220+2.44 log 10λ	0.50(1)	2.44±0.20	3.52 per cent (2.88～4.33)
	p, p'-DDT+*o, p'*-DDT (80:20)	48	1.5496+2.44 log 10λ	3.30(1)	2.44±0.20	2.59 per cent (2.16～3.11)

Results

Toxicity of p,p'-DDT, o,p'-DDT and their mixtures against Culex pipiens fatigans:

The summarised data showing the toxicity of *p,p'*-DDT, *o,p'*-DDT and their mixtures against *C. p. fatigans* are presented in Table 1. The LC_{50} of *p,p'*-DDT when tested by dry film method was found to be 11.9 per cent. The LC_{50} of *o,p'*-DDT in the parallel tests was 0.044 per cent thereby, showing that it was considerably more toxic than *p,p'*-DDT. However, both the isomers were found to be almost equitoxic when tested by topical application and oil solution methods.

The mixture of *p,p'*-DDT and *o,p'*-DDT when tested by dry film method was found to be more toxic than pure *p,p'*-DDT but less toxic than *o,p'*-DDT. As the slopes of log concentration-probit (lc-pr.) regression lines were not parallel, the cotoxicity coefficient was calculated following Sun and Johnson[20]. The value of cotoxicity coefficient was found to be 180, thereby showing synergism.

Toxicological data obtained using oil solution, however, indicated that the mixtures of *p,p'*-DDT and *o,p'*-DDT both in the ratio of 50 : 50 and 80 : 20 were as toxic as *p,p'*-DDT and *o,p'*-DDT. The slopes of lc-pr regression lines of the individual components and mixtures also did not depart significaly from parallelism ($F=0.97$ with $n_1=3$ and $n_2=8$). The results, therefore, indicated that the joint action of *p,p'*-DDT and *o,p'*-DDT was simple similar against *C. p. fatigans*, adults when tested by oil film method.

Toxicity of p,p'-DDT, o, p'-DDT and their mixtures against Aedes aegypti.

The summarized data showing the toxicity of *p,p'*-DDT, *o'p*-DDT and their mixtures against *Aedes aegypti* are given in Table 2. The results indicated that *p,p'*-DDT with LC_{50} of 0.0063 was 27 times more toxic than *o,p'*-DDT when tested by dry film method. By topical application method and oil solution also, *p,p'*-DDT was found to be considerably more toxic than *o,p'*-DDT. *p,p'*-DDT was found to about 9 times more toxic when tested by topical application and 45 times more toxic when tested by oil solution method than *o,p'*-DDT.

The mixture of *p,p'*-DDT and *o,p'*-DDT was found to be less toxic than pure *p,p'*-DDT when tested by dry film or oil solution methods. The LC_{50} of the mixture was found to 0.078 per cent as compared to 0.0063 of pure *p,p'*-DDT when the insects were tested by exposing to dry deposit. The LC_{50} of the mixture when tested by

Table 2. Data showing the toxicity of *p,p'*-DDT, *o,p'*-DDT and their mixtures against *Aedes aegypti*.

Method	Insecticide	Exposure Period (Hrs.)	Equation of the fitted lc-pr regression line Y=	χ^2(d.f.)	Regression coefficient	LC_{50} (fiducial limits)
Dry film	*p,p'*-DDT	4	$-0.5120+3.06 \log 10^4\lambda$	13.5(3)	3.06±0.32	0.0063 per cent (0.0058~0.0073)
	o,p'-DDT	4	$-0.9869+1.89 \log 10^4\lambda$	9.6(2)	1.89±0.32	0.17 per cent (0.13~0.21)
	p,p'-DDT+*o,p'*-DDT (5:95)	4	$-3.8465+3.06 \log 10^4\lambda$	3.34(1)	3.06±0.24	0.078 per cent (0.072~0.084)
Topical	*p,p'*-DDT	—	$0.6989+3.23 \log 10^3\lambda$	4.12(2)	3.23±0.36	0.021 μg/mosq. (0.019~0.025)
	o,p'-DDT	—	$-0.3428+2.34 \log 10^3\lambda$	2.07(2)	2.34±0.31	0.19 μg/mosq. (0.18~0.20)
Oil solution	*p,p'*-DDT	1	$1.2254+3.82 \log 10\lambda$	0.22(2)	3.82±0.24	0.97 per cent (0.89~1.05)
	o,p'-DDT	1	$2.1411+1.08 \log 10\lambda$	0.5(2)	1.08±0.24	44.4*per cent (10.1~196.8)
	p,p'-DDT+*o,p'*DDT (50:50)	1	$1.6800+2.75 \log 10\lambda$	16.85(2)	2.75±0.58	1.6 per cent (1.2~2.1)

* By extrapolation.

oil solution method was found to 1.6 per cent as compared to 0.97 per cent of pure *p, p'*-DDT. The cotoxicity coefficient of the mixture was found to be 97 when tested by dry film method, thereby indicating simple similar action. When the mixture was tested by oil film method, the cotoxicity coefficient of 117 was obtained. This, however, could not be considered significant as the concentration of *p, p'*-DDT in the mixture that caused 50 per cent kill in the adults of *Aedes aegypti*, did not differ significantly from the LC_{50} of pure *p, p'*-DDT.

Toxicity of p, p'-DDT, o, p'-DDT *and their mixtures against Anopheles subpictus.*

The summarized data showing the toxicity of *p, p'*-DDT, *o, p'*-DDT and their mixtures against the females of *Anopheles subpictus* as obtained by topical application method are given in Table 3. The results indicated that *p, p'*-DDT and *o, p'*-DDT were equitoxic, the LD_{50} of 0.50μg/mosquito was obtained. The ld-pr regression lines of the individual components as well as their mixtures did not depart significantly from parallelism (F=2.9651 with n_1=3 and n_2=8). Also the LD_{50} of the mixtures of *p, p'*-DDT and *o, p'*-DDT when combined in the ratio of 80:20 and 50:50 were not found to differ significantly from the individual components. The results, therefore, showed that the joint action of these isomers was simple similar.

The results obtained against the larvae of *A. subpictus* are given in Table 4. Both *p, p'*-DDT and *o, p'*-DDT were found to be equitoxic even against the larvae of *A. subpictus*. Their joint action was also found to be simple similar as the LC_{50} of the mixtures did not differ from the individual isomers.

Discussion

p, p'-DDT is known to be generally the most toxic of DDT isomers against insects. Yasutomi[21] and Sakai[17] however, observed, *o, p'*-DDT to be more toxic than, *p, p'*-DDT against resistant bodylice and turnip aphid respectively. The

Table 3. Data showing the toxicity of *p, p'*-DDT, *o, p'*-DDT and their mixtures against *A. subpictus* adults (Topical application method).

Insecticide	Equation of the fitted ld-pr regression line Y=	χ^2(d. f.)	Regression coefficient	LD_{50}-μg/mosquito (fiducial limits)
p, p'-DDT	3.7531+1.79 log 10λ	2.92(2)	1.79±0.20	0.50 (0.43~0.58)
o, p'-DDT	3.7422+1.79 log 10λ	1.61(2)	1.79±0.20	0.50 (0.43~0.59)
p, p'-DDT+*o, p'*DDT (50:50)	3.8679+1.79 log 10λ	0.43(2)	1.79±0.20	0.43 (0.37~0.49)
p, p'-DDT+*o, p'*-DDT (80:20)	3.7201+1.79 log 10λ	0.74(2)	1.79±0.20	0.52 (0.45~0.59)

Table 4. Data showing the toxicity of *p, p'*-DDT, *o, p'*-DDT and their mixtures against *A. subpictus* larvae.

Insecticide	Equation of the fitted lc-pr regression line Y=	χ^2(d. f.)	Regression co-efficient	LC_{50} (ppm) (fiducial range)
p, p'-DDT	3.5924+1.55 log 10λ	8.46(2)	1.55±0.30	0.81 (0.66~0.99)
p, p'-DDT	3.6794+1.55 log 10λ	10.95(2)	1.55±31	0.71 (0.53~0.95)
p, p'-DDT+*o, p'*-DDT (50:50)	3.5781+1.55 log 10λ	1.35(2)	1.55±0.10	0.83 (0.80~0.97)
p, p'-DDT+*o, p'*-DDT (80:20)	3.4629+1.55 log 10λ	4.93(2)	1.55±0.10	0.98 (0.88~1.08)

present investigations indicated that *o,p'*-DDT was considerably more toxic than *p,p'*-DDT against *C. p. fatigans* when tested by dry film method. Both the isomers were, however, found to be equitoxic when applied topically or as oil solution. Tests against *Aedes aegypti* revealed that *p,p'*-DDT was much more toxic than *o,p'*-DDT whereas both the isomers were equitoxic against *Anopheles subpictus*.

The different order of toxicity of the isomers as observed against *C. p. fatigans* and *Anopheles subpictus* may be either due to species specificity or selection of specific resistance in these strains to *p,p'*-DDT. In the case of house flies specific resistance to *p,p'*-DDT due to the presence of DDT-ase has been demonstrated[7,13,15]. Kalra *et al*[11,12] also observed the extensive metabolism of *p,p'*-DDT to *p,p'*-DDE and practically insignificant conversion of *o,p'*-DDT to *o,p'*-DDE in *C. p. fatigans*. Kalra and Pal[9] observed that *Anopheles subpictus* also metabolized *p,p'*-DDT to *p,p'*-DDE. *Anopheles subpictus* used in the present investigation was collected from the areas where this species has already been reported to be DDT resistant[18,19]. The adults of *C. p. fatigans* were also resistant as the LC_{50} of *p,p'*-DDT for this strain (2.36 per cent with 48 hours of exposure) was more than the estimated base line value for the susceptible population of this species (LC_{50} of *p,p'*-DDT 0.5~0.72 per cent with 24 hours of exposure).[14] It, therefore, appears more likely that the increased or same level of toxicity of *o,p'*-DDT in comparison to *p,p'*-DDT was due to the intensification of the defence mechanisms specific for *p,p'*-DDT in the resistant strains of *C. p. fatigans* and *Anopheles subpictus* used in the present investigation.

The data further revealed that dry deposit of *o,p'*-DDT was considerably more toxic than the oil solution. Georghiou and Giddon[5] also observed organochlorine insecticides, dieldrin and DDT, considerably more toxic as dry deposits than as oil films and this was considered most likely due to the difference in the availability of the active ingredient to the test organism. The different testing methods indicated the difference in the relative order of toxicity of *p,p'*-DDT and *o,p'*-DDT against *Culex p. fatigans*.

All the methods, however, indicated the same order of toxicity of the isomers against *Aedes aegypti*. Hadaway and Barlow[6] observed the wide variation in the ratio of intrinsic toxicity, determined by the topical application of solution and the contact toxicity of dry crystalline deposits on adult mosquitoes within a group of closely related insecticides. However, Georghiou and Metcalf[4], in general, observed a close relationship between the two methods *i.e.* topical application and dry film. The type of the deposit and the site of application, therefore seem to be important for the differential pick-up and absorption of the different isomers in *C. p. fatigans*. Nevertheless the factors responsible need elucidation.

Kalar and Joshi[10] earlier observed that *o,p'*-DDT was more toxic than *p,p'*-DDT against the larvae of *C. p. fatigans*. The dry film method and larval test, therefore, gave comparable results against *C. p. fatigans*. Nevertheless, the topical application method as well as the larval test indicated both isomers equitoxic against *Anopheles subpictus*. These results, therefore further demonstrate that different species behave differently.

Kalra *et al*[12] observed that the larvae of *Aedes aegypti* were more susceptible to *p,p'*-DDT and less susceptible to *o,p'*-DDT as compared to the larvae of *C. p. fatigans*. The present investigations indicated that the adults of *Aedes aegypti* were also much more susceptible than *C. p. fatigans* to *p,p'*-DDT. Parallel tests with the adults of *C. p. fatigans* and *Aedes aegypti* did not reveal much difference in their susceptibility to *o,p'*-DDT (results given below):

LC_{50} values of *o,p'*-DDT.

		Aedes aegypti	*C. p. fatigans*
Adults	Dry film	0.02 per cent (24hrs exposure)	0.05 per cent (24hrs exposure)
	Topical application	0.2 μg	0.25 μg
Larvae		0.8 ppm	0.02 ppm.

Studies on the joint action of *p,p'*-DDT and *o,p'*-DDT against the adult mosquitoes did not reveal any significant synergism. The action seems to be simple similar. The action was also

found to be simple similar against the larvae of *Anopheles subpictus*. Kalra and Joshi[10] observed the action to be of dissimilar type against the larvae of *Anopheles subpictus* collected during the month of November. The larvae used in the earlier studies were highly resistant to *p, p'*-DDT, the LC_{50} of *p, p'*-DDT was 27.5 ppm. Hewelett and Placket[8] suggested that apart from the "sites of action" the "sites of loss" also play an important role in the type of the joint action exhibited. The toxicological data obtained during the course of present investigations as well as the studies reported so far on the metabolism of DDT in mosquitoes, however, give sufficient indications of the presence of specific sites of loss for *p, p'*-DDT in the strains of *C. p. fatigans*, *Aedes aegypti* and *Anopheles subpictus*. The relative importance of detoxification, however, has not been established so far. It is also likely that the toxicological technique used was not sensitive enough to manifest these differences when the mixtures were tested to elucidate the type of the joint action.

Summary

The relative toxicity of *p, p'*-DDT and *o. p'*-DDT against the adults of *Culex pipiens fatigans*, *Aedes aegypti* and *Anopheles subpictus* was determined. Toxicological methods used were dry film, oil solution and topical application. Dry film method indicated that *o, p'*-DDT was considerably more toxic than *p, p'*-DDT against *C. p. fatigans*. *o, p'*-DDT and *p, p'*-DDT were found to equitoxic when tested by oil solution and topical application methods. Against *Aedes aegypti p, p'*-DDT was observed to be considerably more toxic than *o, p'*-DDT by all the methods. Both the isomers were equitoxic against the larvae and adults of *Anopheles subpictus*.

The mixtures of *p, p'*-DDT and *o, p'*-DDT did not indicate any significant synergism against adult mosquitoes.

Comparative susceptibility of the adults of *Aedes aegypti* and *Culex p. fatigans* revealed that *Aedes aegypti* were much more susceptible to *p, p'*-DDT whereas there was not much difference in their susceptibility to *o, p'*-DDT.

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Studies on Phenylphenol Derivatives with Biological Activity. Part V. Miticidal Activity and Effect on Oxidative Phosphorylation. Hong-Ming CHENG[1*], Morifusa ETO, Eiji TANIGUCHI, Shozo KUWATSUKA[2*], Yasuyoshi OSHIMA[3*] and Masaru KADO[**] (Department of Agricultural Chemistry, Kyushu University, Fukuoka and **Kumiai Chemical Co., Shimizu, Japan) Received September 26, 1969. *Botyu-Kagaku*, 34, 176, 1969.

23. フェニルフェノール誘導体の化学構造と生物活性に関する研究（第5報）殺ダニ性と酸化的リン酸化に対する影響とについて

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34種のフェニルフェノール誘導体を合成し，ニセナミハダニ *Tetranychus telarius* (Linnaeus) に対する殺ダニ性を検討した．その中で，4-クロル-2-フェニルフェノールおよび6-ニトロ-4-クロル-2-フェニルフェノールは強い殺成虫力を有し，4-フェニルフェニルアリルエーテルは殺卵力にすぐれていた．これら誘導体の殺ダニ性は置換基の種類と置換位置によって影響されるようである．

一方，マウス肝臓ミトコンドリヤを用い，その呼吸作用とATPase活性に対するフェニルフェノール類の影響を検討した．4-ニトロ-6-クロル-2-フェニルフェノールは ATPase の活性を高め，酸化的リン酸化のアンカップラーと考えられた．

Introduction

Previous studies in our laboratories have demonstrated that several derivatives of phenylphenols have not only herbicidal but also fungicidal and bactericidal activities, indicating that they have a broad spectrum of biological activities[1~4]. This paper deals with the relationship between structure and miticidal activity of phenylphenol derivatives. As a result, three of these tested chemicals were proved to have relatively high toxicity to the adults or eggs of *Tetranychus telarius* (Linnaeus).

In view of the mode of toxic action of these derivatives, it is of interest to study further the effects on mitochondrial oxidative phosphorylation and ATPase activity. It is well known that the toxic action of dinitrophenols is attributed to their abilities to uncouple the oxidative phosphorylation and to elicit ATPase activity in mitochondria[5]. The present investigation also reports that 4-nitro-6-chloro-2-phenylphenol and 4, 6-dinitro-2-phenylphenol act on mitochondria in the similar manner with unsubstituted dinitrophenol, indicating that *o*-aryl nitro- and chlorophenols are the uncouplers of oxidative phosphorylation.

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Experimental

Miticidal activity tests

For adult mites: Each of test chemicals was provided as a 10 percent wettable powder. The determination of miticidal activity was carried out according to a dipping method. Thirty to eighty adults of *Tetranychus telarius* were parasitized on the leaves of kidney beans (French bean) which were cultured in a green house and then were treated by dipping the leaves into a 0.1 percent test solution for a few seconds. Two days after the treatment, the mortality of the mites was determined. Only compounds whose mortality values were above ninety percent were further subjected to determine LC_{50} values.

For eggs: Ovicidal activity tests were also carried