Pakistan J. Sci. Ind. Res., Vol. 16, Nos. 1–2, February–April 1973

CHEMOSTERILIZATION OF THE LABORATORY-REARED YELLOW-FEVER MOSQUITO, AEDES AEGYPTI(L) BY SUBSTITUTED PHOSPHINE OXIDES AND METHYLMELAMINES

(Miss) Shams Mohiuddin and Saleem A. Qureshi

PCSIR Laboratories, Karachi 39

(Received November 7, 1972; revised March 2, 1973)

Abstract. Test with 5 substituted bis-(1-aziridinyl)-phosphine oxides indicated that they were active sterilants for third instar larvae of laboratory-reared *Aedes aegypti* (L.) inducing 100% sterility at 2.5 p.p.m. Methylmelamines proved ineffective even up to 10 p.p.m. concentration and are not active chemosterilants for mosquitoes. Toxicity of substituted-phosphine oxides was much higher as compared to methylmelamines. Isopropoxy and butoxy phosphine oxides induced 100 and 91.7% sterility at 1 p.p.m. dose respectively. At higher concentrations phosphine oxides produced abnormal pattern of adult emergence terminating into death.

The susceptibility of larvae of yellow-fever mosquito, *Aedes aegypti* to Apholate, Tepa and Metepa has been amply documented.[1–11] Other studies on the effect of chemosterilants on the reproduction of mosquitoes include those of Bertram,[12] Dame and Schmidt.[13] and White.[14] Recently Madhukar *et al.*[15,16] tested selected aziridinyl compounds including series of alkoxy and (alkylamino)-bisphosphine oxides as well as nine nonalkylating phosphoramides against second instar larvae and pupae of yellow-fever mosquito for their structure-activity interpretations. Besides this no data on the effect of other aziridines on *A. aegypti* is available in up-to-date literature.

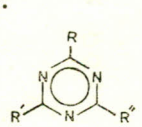
The activity of chemosterilants is known to vary under different experimental conditions, method of application, stage of development of insect, sex, concentration and treatment time, besides having strain specificity. Present study was undertaken to study the sterilization of laboratory-reared *A. aegypti* by phosphine oxides and methylmelamines under our laboratory conditions. The results were compared with Tepa, Hempa and Hemel.

Materials and Methods

The compounds in the first category are substituted bis-(1-aziridinyl)-phosphine oxides. To emphasize their close relationship to tris-(1-aziridinyl)-phosphine oxide (Tepa), these compounds are referred to as bis-(1-aziridinyl)-methoxy phosphine oxide to bis-(1-aziridinyl)-butoxy phosphine oxides with the general structure R—PO (N ⟨)$_2$.

Where R, OCH_3, OC_2H_5, OC_3H_7, iso-OC_3H_7, and OC_4H_9.

The compounds in the second category had the following general structure:



Where	R	R′	R″
	NH_2	NH_2	$N(CH_3)_2$
	NH_2	NH_2	$NHCH_3$
	NH_2	$N(CH_3)_2$	$N(CH_3)_2$
	$NHCH_3$	$NHCH_3$	$NHCH_3$
	$NHCH_3$	$N(CH_3)_2$	$N(CH_3)_2$
	$NHCH_3$	$NHCH_3$	$N(CH_3)_2$
	NH_2	$NHCH_3$	$N(CH_3)_2$

Experimental

Laboratory evaluation were conducted with third instar laboratory-reared larvae of *Aedes aegypti* (L.). In each experiment 100 larvae were exposed in 250 ml water containing various concentrations of chemosterilants at room temperature maintained at 83±3°F and 84±18% R.H. No food was provided for 24 hr, but on the second day of treatment larvae were fed on brewer's yeast and were maintained in the treated water until complete pupation in the parallel control experiment. The larvae were removed from the treated medium, washed in distilled water and transferred to fresh water until complete pupation. Newly formed pupae were removed from the water and isolated in cages for emergence. Mortality and pupation was recorded during and after the treatment.

As the pupae were not segregated by sex, the sterilizing activity of the compounds in these experiments is on mixed sexes. The males were provided with 1% aqueous glucose soaked in cotton pads. The females were given the first blood meal from a rat and subsequent blood meals were given on every third day for 15 days. The females were allowed to lay eggs on filter papers kept on the inner side of an enamel bowl containing water. A sample of 100 eggs for each replicate was observed for the hatchability. The corrected % hatchability was calculated by the formula of Crystal.[17]

$$\text{Corrected \% hatch} = \frac{\text{Test \% hatch}}{\text{Control \% hatch}} \times 100$$

Results

Tables 1 and 2 show the data on the toxicity of the candidate compounds to third stage larvae in the breeding water. The toxicity of phosphine oxides (3–7) was more than the substituted-methylmelamine

TABLE 1. EFFECTIVENESS OF SUBSTITUTED-BIS-(1-AZRIDINYL)-PHOSPHINE OXIDES ON THIRD INSTAR LARVAE OF *Aedes aegypti* (L.), EXPOSED TO THE DESIRED CONCENTRATION OF CHEMOSTERILANTS FOR 12 DAYS. (AVERAGE OF 4 REPLICATES).

No.	Ent. No.	Chemical	Concn (p.p.m.)	Mortality (%)	Emergence (%)	Fecundity	Corrected % hatch of eggs from ensuing adults (both sexes treated)
1	24915	Tepa	10	100			
			5	51·1	29·8	O	
			1	8·2	55·9	L	53·5
2	50882	Hempa	100	41·0	42·0	L	0·0
	Phosphine oxides		10	23·1	37·6	N	64·1
3	61342	Methoxy	50	100·0			
			5	39·3	30	O	
			2·5	15·2	62·2	L	30·9
			1·0	8·0	64·0	N	31·8
4	50761	Ethoxy	50	100·0			
			5	35·0	41·8	O	
			2·5	16	62·0	L	2·8
			1·0	8·8	62·7	L	26·9
5	61343	Propoxy	50	100			
			5	25·6	42·0	O	
			2·5	15·5	64·2	O	
			1·0	7·5	65·7	L	30·0
6	61355	Isopropoxy	50	100			
			5	25	45	O	
			2·5	16	56·2	O	
			1·0	7·6	62·2	O	
7	61354	Butoxy	50	100			
			5	19·4	39·5	O	
			2·5	10·6	63·2	L	0
			1·0	6·9	63·7	L	8·3
	Control		—	—	83·3	N	91·3

TABLE 2. EFFECTIVENESS OF METHYLMELAMINES ON THIRD INSTAR LARVAE OF *Aedes aegypti* (L.); EXPOSED TO THE DESIRED CONCENTRATION OF CHEMOSTERILANTS FOR 12 DAYS (AVERAGE OF 4 REPLICATES).

No.	Ent. No.	Chemical	Concn (p.p.m.)	Mortality (%)	Emergence (%)	Fecundity	Corrected % hatch of eggs from the ensuing adults (both sexes treated)
1	22312	N^2,N^2-Dimethylmelamine	50	100			
			25	100			
			10	46·2	44·0	N	89·6
2	50993	N^2-Methylmelamine	50	100			
			25	81·3	3·0		
			10	55·4	36·7	N	91·9
3	51000	N^2, N^2, N^4, N^4-Tetramethyl-melamine	50	95·2			
			25	89·0	7·0		
			10	35·5	43·0	N	86·6
4	51035	N^2,N^4, N^6-Trimethylmelamine	50	49·8	5·0		
			25	40·4	18·0	O	
			10	25·2	64·6	N	100
5	51239	Pentamethylmelamine	50	90·9			
			25	85·0	6·0		
			10	24·2	66·9	N	98·7
6	51234	N^2, N^2, N^4, N^6-Tetramethy-lmelamine.	50	69·9	11·0		
			25	59·5	27·0	O	
			10	23·8	59·5	N	90·2
7	60020	N^2, N^2, N^4-Trimethylmelamine	50	62·2	18·0	O	
			25	40·0	30·0	O	
			10	15·7	73·5	N	97·5
8	50852	Hexamethylmelamine (Hemel)	50	100·0			
			25	44·83	38·50	L	62·71
			10	41·33	41·33	N	78·51
	Control				83·3	N	91·3

N, normal; L, low; O, nil.

(Table 2, 1–7), but no obvious structure-toxicity relationship was evident within each group. Tepa(10) was much more toxic than phosphine oxides, while Hempa appeared to be less toxic than phosphine oxides (Table 1). Toxicity of substituted-melamines was somewhat similar to that of Hemel (Table 2, 8) at comparable concentrations, except dimethyl(1) and methylmelamines (2) which exhibited a relatively higher toxicity (Table 2).

Phosphine oxides proved to be very effective chemosterilants for mosquitoes and induced complete sterility at 2.5 p.p.m. At 1 p.p.m. methoxy (3), ethoxy (4), propoxy (5), isopropoxy (6) and butoxy phosphine oxides (7) proved more effective than Tepa and induced 68.2, 73.1, 70, 100, 91.7 and 46.5% sterility respectively (Table 1). The fecundity was appreciably decreased at 1 p.p.m. Hempa was ineffective even at 10 p.p.m. and caused 35.9% sterility as compared with 8.7% sterility in the control group.

There is distinct variation of sterilizing activity of phosphine oxides; isopropoxy and butoxy compounds being more effective than other three compounds in this series (Table 1, 3–5).

Following the larval treatment by phosphine oxides, a few adults (3–9%) exhibited an abnormal emergence pattern terminated by death. The frequency of such emergence increased with the dose of the sterilants.

Sterilizing activity of the substituted-melamines was not appreciable even at 10 p.p.m. and the fecundity was found to be normal. The difference of the sterilizing activity within the series was small. The activity of Hemel (8) was slightly higher than candidate melamines (Table 2).

Discussion

Tepa was much more toxic than phosphine oxides (3–7) and Hempa (2) (Table 1). At 10 p.p.m. concentration no third instar larva could pupate up to 27 days and 100% mortality was observed during this period. Dame *et al.*[18] reported that Tepa at 10 p.p.m. reduced adult emergence somewhat when third instar larvae were reared in treated water till pupation. Madhukar *et al.*[15] reported mortality of second instar larvae till their pupation in treated medium at 10 p.p.m. dose of Tepa. This shows that P.C.S.I.R. strain of *A.aegypti* is susceptible to the effect of Tepa as compared with other strains reported by other authors.

Hempa proved much less effective as compared to phosphine oxides. At 100 p.p.m. the per cent mortality and sterility of third instar larvae was 41 and 100 respectively. Hafez *et al.*[19] observed 42% mortality of third instar larvae of *Anopheles pharoensis* reared in treated breeding water till emergence with comparable dose of Hempa. They reported Hempa to be ineffective even at 80–100 p.p.m. when the adult diet was treated. Glancey[20] found Hempa causing complete sterility in *A. aegypti* at 0.5% concentration with a low oviposition rate. This again show that P.C.S.I.R. strain is more susceptable to Hempa as compared to the strain reported by Madhukar *et al.*[16] This comparative study also indicates that much higher concentration of the chemosterilant is required when used in adult diet.

The per cent sterility caused by Hemel was less than Tepa and Hempa at comparable concentration (Table 2). Tepa having closer analogy with phosphine oxides proved to be very effective, inducing complete sterility at 5 p.p.m. Weidhaas[1] exposed third instar larvae of *A. aegypti* until population with 10 p.p.m. of Tepa and noticed 100% sterility in the males and 84% in the females. Madhukar[15] reported Tepa, Metepa, and Apholate more effective than alkoxybis-(1-aziridinyl)-phosphine oxides.

As much closer analogy between Hemel and melamine compounds exists, the toxicity and sterility induced by melamines is much less as compared to phosphine oxides, which appeared even more effective than Tepa at comparable concentrations.

The activity of substituted phosphine oxides appeared to be increasing with the size of the alkyl substituent. Isopropoxy and butoxy compounds indicated higher sterilizing activity than the methoxy, ethoxy and propoxy compounds. Isopropoxy and butoxy compounds induced 100% and 91.7% sterility respectively even at 1 p.p.m. (Table 1). Madhukar *et al.*[15] noticed isoropoxy compound having much higher sterilizing activity than methoxy, ethoxy, and propoxy bis-(1-aziridinyl)-phosphine oxides when tested against second instar larvae of *A. aegypti*. They tested methoxy, ethoxy and propoxy compounds at 500 p.p.m, while isopropoxy compounds was tested at 25, 50 and 100 p.p.m., and reported their effectiveness less than Tepa. Isopropoxy compound at 50 p.p.m. was reported to give comparable control of reproduction with 5 p.p.m. dose of Tepa.

The sterilizing activity of the substituted bis-(1-azididinyl)-phosphine oxides was quite high at 2.5 p.p.m. concentration (Table 1). This indicates that the phosphine oxides reported here are much more effective than alkoxybis-(1-aziridinyl)-phosphine oxides reported by Madhukar *et al.*[15] La Brecque *et al.*[21] and Chang *et al.*[22] reported methylmelamines to be chemosterilants of houseflies. Methylmelamines proved ineffective even up to 10 p.p.m. indicating that they are not active chemosterilants for mosquitoes.

The abnormal emergence pattern of adults induced by phosphine oxides at higher doses (5–10 p.p.m.) observed by the present authors is also reported by Madhukar *et al.*[15] They reported 1–7% adults exhibiting such abnormality with alkylaminobis-(1-aziridinyl)-phosphine oxides.

Acknowledgements. We express deep appreciations to Dr. A.B. Borkovec, Incharge, Chemosterilant Investigation, Pesticide Chemicals Research Branch, Entomological Research Division, U.S.D.A., for his valuable suggestions and the supply of chemicals.

References

1. D.E. Weidhass, Nature (London), **195,** 786 (1962).
2. D.A. Dame and H.R. Ford, Nature (London) **201,** 733 (1964).
3. E.I. Hazard, C.S. Lofgren, D.B. Woodard, H.R. Ford and B.M. Glancey, Science, **145,** 500 (1964).
4. K.S. Rai, Biol. Bull., **127,**119 (1964).

5. W. Klassen and F. Matsumura, Nature (London), **209,** 1155 (1966).
6. C.L. Judson, Entomol. Expl. Appl., **10,** 387 (1967).
7. R.S. Patterson, C.S. Lofgren and M.D. Boston, J. Econ. Entomol., **60,** 1673 (1967).
8. V.P. Sharma and K.S. Rai, Can. Entomol., **99,** 1116 (1967).
9. M.K.K. Pillai and H.C. Agarwal, Entomol. Exptl. Appl., **12,** 413 (1969).
10. R.B. Turner and N.P. Maheswary, Ann. Entomol. Soc. Am., **6,** 1091 (1969).
11. J.R. Powell and G.B. Craig Jr., J.Econ. Entomol., **63,** 745 (1970).
12. D.S. Bertram, Med. Hyg., **57,** 322 (1963).
13. D.A. Dame and C.H. Schmidt, J. Econ. Entomol., **57,** 77 (1964).
14. G.B. White, Trans. Roy. Soc. Trop. Med. Hyg., **58** 290 (1964).
15. B.V.R. Madhukar, M.K.K. Pillai and A.B. Borkovec, J. Econ. Entomol., **64,** 1024 (1971).
16. B.V.R. Madhukar, M.K.K. Pillai and A.B. Borkovec, J. Econ. Entomol., **64,** 1027 (1971).
17. M.M. Crystal, Ann. Entomol. Soc. Am., **63,** 71 (1970).
18. D.A. Dame, D.B. Woodard and H.R. Ford, Mosquito News, **24,** 1(1964).
19. M. Hafez, A.E. Abdul Nasr and H.S. Salama, J. Econ. Entomol., **62,** 233 (1969).
20. B.M. Glancey, Mosquito News, **25,** 392 (1965).
21. G.C. La Brecque, R.L. Fye, A.B. DeMilo and A.B. Borkovec, J. Econ. Entomol., **61,** 1621 (1968).
22. S.C. Chang, A.B. Borkovec and C.W. Woods, J. Econ. Entomol., **59,** 937 (1966).