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EVALUATION OF 45 CHEMICALS AS CHEMOSTERILANTS IN ADULT MALE COTURNIX

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Summary. Forty-five chemicals were evaluated as single-dose chemosterilants in adult male coturnix with testes in breeding or regressed condition. Of the compounds tested in breeding birds, 12 significantly reduced fertility or produced complete sterility. Eight of these 12 compounds also reduced fertility or produced sterility after they were administered to males with regressed testes. The most active compounds were substituted bis(1-aziridinyl) phosphine sulfides or oxides or tris(1-aziridinyl) phosphine sulfide.

INTRODUCTION

The Denver Wildlife Research Center has maintained for 2 years a screening effort in which coturnix quail (*Coturnix coturnix*) are used to search for compounds that affect avian male reproduction (Schafer *et al.*, 1975). The laboratory program, operating in conjunction with field studies concentrating on the biological and ecological aspects of red-winged blackbird (*Agelaius phoeniceus*) reproduction (Bray *et al.*, 1974), is designed to eventually produce a safe and effective avian male chemosterilant that can be used to reduce bird damage in agricultural and urban areas. This paper presents the results of our tests conducted between 1972 and 1974 with 45 compounds of known or suspected chemosterilant activity in birds.

METHODS

The acute oral LD_{50} (7-day observation period) of each candidate chemosterilant was determined by the gavage method described by Schafer et al. (1973) in male or female coturnix. Propylene glycol was used as the carrier. Following the LD_{50} study, an initial determination of chemosterilant activity was made by treating groups of seven breeding male coturnix with a single dose of the candidate material at 25% to 75% of its LD_{50} value. Compounds producing sterility in breeding males were then tested on males with testes regressed by photoperiod manipulation (Shafer et al., 1975).

All treated males were individually caged with untreated females of known fertility under an 18L:6D photoperiod and the reproductive success of the pair was measured over a 35-day period. Since the duration of the spermatogenic cycle in coturnix is probably between 20 and 25 days (Jones et al., 1972; Jones and Jackson, 1972, Schafer et al., 1975), eggs produced after day 20 should reflect the effects of candidate chemosterilants on the initial phases of sperm development. In the initial test, males showing sterility between day 20 and day 35 were considered to be permanently sterilized. Regressed males failing to become fertile between 20 and 35 days after treatment were also considered to be permanently sterilized.

Eggs were collected three times during each 7-day period and were incubated by weekly groups for 4 days, at which time they were cracked and examined for embryonic development. Individual eggs were classified

as fertile, unfertile, or cracked (eggs in the latter category were not used in any fertility computations). At the end of the 35-day period, males were sacrificed using CO_2, and both testes were extracted and weighed together.

Testes weights were analyzed by a one-way analysis of variance followed by Duncan's Multiple Range Test for Unequal Numbers at 0.01%. Arc-sine transformed egg fertility data were analyzed by two-way analysis of variance with repeated measures (Winer, 1971) followed by Duncan's Test at 0.01%.

The chemicals used were technical grade, and were either purchased or were obtained from the sources footnoted in Table 1.

Additional details of the test procedures and environmental conditions can be found in our previous paper (Schafer et al., 1975).

RESULTS

Of the 45 compounds tested, 12 significantly reduced fertility or produced sterility during the 15-day period beginning 20 days after treatment. All these compounds were either substituted bis(1-aziridinyl) phosphine sulfides or odixes, or substituted bis(1-aziridinyl carboxamides) except for chloroethyl methane sulfonate, hexamethyl thiophosphoric triamide and N^2,N^2,N^4,N^4-tetramethylmelamine. Combined testes weights of birds treated with 10 of the 12 compounds were less than 1 g at the end of the 35-day test period.

Eight of the 12 compounds that reduced fertility in breeding males were also active in producing reduced fertility or sterility in regressed males (Table 2). Five of the eight produced complete sterility. Testes weights of birds dosed with five chemicals were also significantly different from the control, and these five were the same ones that produced complete sterility.

Based on this information, the following compounds were determined to be the most effective in producing sterility in regressed male coturnix: Bis(1-aziridinyl) anilino phosphine sulfide, Bis(1-aziridinyl) (cyclohexylamino) phosphine sulfide, Bis(1-aziridinyl)(dimethylamino) phosphine sulfide, Bis(1-aziridinyl)(methylamino) phosphine oxide, and Tris(1-aziridinyl) phosphine sulfide.

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TABLE 1

FERTILITY OF EGGS PRODUCED BY FEMALE COTURNIX AFTER THEIR MATES WERE GIVEN SINGLE ORAL DOSES OF 45 CANDIDATE CHEMOSTERILANTS

Chemosterilant	Source[a]	Coturnix LD_{50} (mg/kg)	Male treatment level (mg/kg)	Fertility (% of eggs laid that were fertile)[b] Days 1-35	Days 20-35	Male mortality (no./no. tested)	Testes weight (g)[b]
1. Control	-	-	-	92	91	0/7	2.854
2. Acetyl methanesulfonate	1	1,000 (♀)	750	87	95	1/7	3.366
3. 2-Amino-4(ethylthio) butyric acid (ethionine)	1	>1,000 (♂)	562	96	91	1/7	3.203
4. Aminomethanesulfonic acid	1	>1,000 (♂)	1,000	81	75	2/7	3.662
5. Ammonium methanesulfonate	1	>1,000 (♂)	1,000	78	74	0/7	4.023
6a. Bis(1-aziridinyl) aminophosphine sulfide	2	100 (♀)	56.2	4*	0*	3/7	0.590*
6b.			31.6	24*	6*	1/7	2.677
7. Bis(1-aziridinyl) anilinophosphine oxide	2	100 (♀)	56.2	44*	49	0/7	3.208
8. Bis(1-aziridinyl) anilinophosphine sulfide	2	>316 (♀)	316	16*	0*	1/7	0.530*
9a. Bis(1-aziridinyl)(cyclohexylamino) phosphine sulfide	2	>316 (♀)	316	23*	3*	4/7	0.378*
9b.			178	10*	0*	0/7	0.845*
10. Bis(1-aziridinyl)(dimethylamino) phosphine sulfide	2	75 (♀)	31.6	11*	0*	1/7	0.923*
11. Bis(1-aziridinyl)(methylamino) phosphine oxide	2	237 (♀)	75	13*	0*	3/7	0.470*
12. Bis(dimethylamino) dithiazolium chloride	2	23.7 (♂)	13.3	86	93	0/7	2.894

Table 1.--Continued

	Chemosterilant	Source[a]	Coturnix LD_{50} (mg/kg)	Male treatment level (mg/kg)	Fertility (% of eggs laid that were fertile)[b] Days 1-35	Days 20-35	Male mortality (no./no. tested)	Testes weight (g)[b]
13.	2-Bromoethanesulfonic acid sodium monohydrate	1	>1,000 (♀)	1,000	91	94	1/7	3.429
14.	Colchicine	1	42.1 (♂)	31.6	77	89	0/7	2.770
15.	2-Chloroethanesulfonic acid sodium hydrate	1	>1,000 (♂)	1,000	88	79	0/7	3.961
16a.	2-Chloroethyl methanesulfonate	1	208 (♀)	133	29*	17*	1/7	1.673
16b.				100	40*	71	0/7	3.431
17.	Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one (Kepone)	4	237 (♂)	100	71*	70	1/7	2.921
18.	2,5-Dichloro N(2,4-dinitro-1-naphthyl) benzene sulfonamide	2	>316 (♂)	316	77*	73	0/7	3.123
19.	9,10-Dimethyl-1,2-benzanthracine	1	>316 (♂)	178	86	85	0/7	3.244
20a.	N,N-Dimethyl-4,4-dinitrocarbanilide	3	750 (♂)	316	95	94	4/7	3 193
20b.				178	76*	72	0/7	3.655
21.	1-(2,2-Diphenylbutyryl-) 2-methylpyrrolidine	5	316 (♂)	178	88	86	4/7	3.752
22.	Fluoroacetamide	1	13.3	10	93	95	1/7	2.679
23a.	Hexamethylene bis(1-aziridinecarboxamide)	2	316 (♂)	178	15*	0*	6/7	0.343*
23b.				100	20*	2*	1/7	1.180*

Table 1.--Continued

Chemosterilant	Source[a]	Coturnix LD_{50} (mg/kg)	Male treatment level (mg/kg)	Fertility (% of eggs laid that were fertile)[b] Days 1-35	Days 20-35	Male mortality (no./no. tested)	Testes weight (g)[b]
24. Hexamethylmelamine hydrochloride (Hemel HCl)	2	>316 (♂)	316	75*	80	0/7	3.819
25a. Hexamethylphosphoric triamide (Hempa)	1	1,000 (♂)	562	70*	83	0/7	3.805
25b.			316	90	92	0/7	3.204
26. Hexamethylthiophosphoric triamide (Thiohempa)	2	>316 (♂)	316	28*	0*	0/5	0.669
27. 2-Imidazolidone	1	>1,000 (♀)	562	85	75	0/7	3.467
28. Methanesulfonic acid	1	1,000 (♀)	421	49*	39*	0/7	2.966
29. Methoxymethyl methanesulfonate	1	750 (♀)	421	57*	63	3/7	2.607
30. N,NPR-(1-methylethylene)-bis-(2-carbamoylethyl) dimethanesulfonate	1	>1,000 (♂)	562	86	81	0/7	3.513
31. Methyl methanesulfonate	1	75 (♀)	56.2	71*	89	2/7	3.290
32. N-Methyl-2-pyridone	1	421 (♂)	316	87	94	4/7	3.455
33. Methyl trifluoromethanesulfonate	1	>1,000 (♀)	1,000	84	87	0/7	3.197
34. Pyridine 1-oxide	1	1,000 (♂)	562	91	90	0/7	3.387
35a. 2-Pyridinethiol 1-oxide	1	178 (♀)	133	77	77	4/7	4.224
35b.			100	86	100	3/7	3.163

Table 1.--Continued

Chemosterilant	Source[a]	Coturnix LD_{50} (mg/kg)	Male treatment level (mg/kg)	Fertility (% of eggs laid that were fertile)[b] Days 1-35	Days 20-35	Male mortality (no./no. tested)	Testes weight (g)[b]
36a. SC 12998	6	750 (♂)	562	74*	67	3/6	2.442
36b.			100	74*	63	1.7	3.351
37. Tetramethylene bis(1-aziridinecarboxamide)	2	133 (♀)	56.2	27*	11*	0/7	1.091*
38. N^2,N^2,N^4,N^4-Tetramethylmelamine	2	>316 (♂)	316	38*	13*	0/7	2.658
39. Tetramethylpyridine phosphonic diamide	2	>316 (♂)	316	88	93	0/7	3.386
40a. Tetrazole	1	>316 (♂)	316	75*	86	0/7	3.069
40b.			100	88	93	0/7	3.665
41. 2,2',2"-Trichloro triethylamine	1	133 (♂)	56.2	66*	89	4/7	2.909
42. Trifluoromethanesulfonic acid	1	>1,000 (♀)	1,000	88	89	0/7	3.602
43. Trimethyl phosphate	1	750 (♂)	562	81*	96	0/7	3.913
44. Triphenyltin acetate	1	100 (♂)	56.2	59*	83	2/7	2.798
45. Tris(1-aziridinyl) phosphine sulfide	2	237 (♀)	56.2	14*	0*	1/7	0.713*
46. Vinylene bis(1-aziridinecarboxamide)	2	237 (♀)	133	20*	0*	1/7	0.781*

Table 1.--Continued

[a] Source of chemicals as follows:

1. Purchased
2. USDA - Beltsville, Maryland
3. Merck & Co., Rawhay, New Jersey
4. Allied Chemical Co., Morristown, New Jersey
5. Upjohn Co., Kalamazoo, Michigan
6. G. G. Searle & Co., Chicago, Illinois

[b] Numbers followed by an asterisk are significantly different from the control at P = 0.01.

TABLE 2

FERTILITY OF EGGS PRODUCED BY FEMALE COTURNIX AFTER THEIR MATES WITH ARTIFICIALLY REGRESSED TESTES WERE GIVEN SINGLE ORAL DOSES OF 13 CHEMOSTERILANTS

Chemosterilant	Treatment level (mg/kg)	Fertility (% of eggs laid that were fertile)[a]		Mortality (no./no. tested)	Average time to first fertile egg (days)	Fertile pairs at end of test no./no. survivors	Testes weight (g)[a]
		Days 1-35	Days 20-35				
1. Control	-	29	66	0/7	18	6/7	2.957
2. Bis(1-aziridinyl) aminophosphine sulfide	31.6	9	10*	1/7	20	2/6	1.159
3. Bis(1-aziridinyl) anilinophosphine sulfide	316	0*	0*	1/7	-	0/6	0.297*
4. Bis(1-aziridinyl) (cyclohexylamino) phosphine sulfide	100	3*	0*	1/7	-	0/6	0.876*
5. Bis(1-aziridinyl) (dimethylamino) phosphine sulfide	31.6	5*	0*	2/7	-	0/5	0.475*
6. Bis(1-aziridinyl) (methylamino) phosphine oxide	56.2	0*	0*	0/7	-	0/7	0.501*
7. 2-Chloroethyl methanesulfonate	133	10	20	0/7	29	5/7	2.108
8. Hexamethylene bis(1-aziridinecarboximide)	100	15	30	0/7	21	3/7	3.008
9. Hexamethylthiophosphoric triamide	178	6*	15	1/7	30	3/5	2.278
10. Tetramethylene bis(1-aziridinecarboxamide)	56.2	3*	7*	1/7	32	2/6	1.106
11. N^2,N^2,N^4,N^4-Tetramethylmelamine	316	5*	16	1/7	29	3/6	2.080
12. Tris(1-aziridinyl) phosphine sulfide	56.2	0*	0*	0/7	-	0/7	0.291*
13. Vinylene bis(1-aziridinecarboxamide)	133	4*	2*	0/7	33	1/7	1.121

[a] Numbers followed by an asterisk are significantly different from the control at P = 0.01.