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Evaluation of 45 chemicals as chemosterilants in adult male quail *(Coturnix coturnix)*

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Since 1972, the Denver Wildlife Research Center has maintained a screening programme in which coturnix quail are used in a search for compounds that affect reproduction in male birds (Schafer, Guarino & Brunton, 1976). The laboratory programme, operating in conjunction with field studies on the biological and ecological aspects of red-winged blackbirds (*Agelaius phoeniceus*; Bray, Kennelly & Guarino, 1975), is designed to find a safe and effective avian male chemosterilant that can be used to reduce bird damage in agricultural and urban areas. The present paper presents the results of the tests conducted between 1972 and 1974 with 45 compounds of known or suspected chemosterilant activity in birds or rodents.

Methods

Our quail were randomly bred and raised in our facilities from original stock (Random Line 926) obtained from the University of California at Davis. Adult males weighed 110–120 g and females 125–145 g on average.

The chemicals used were technical grade, and were either purchased or were obtained from the sources given in the footnotes to Table 1. For both LD_{50} and reproductive tests, they were administered by gavage with propylene glycol as the carrier.

For each candidate chemosterilant, the acute oral LD_{50} (7-day observation period) was determined in male or female birds by the method described by Schafer, Brunton & De Grazio (1973). An initial determination of chemosterilant activity was made by treating groups of 7 breeding males with a single dose of the candidate substance at about 50% of its LD_{50} value. If excessive mortality of treated birds resulted, a lower treatment level was also run. Compounds producing sterility in breeding males were then tested on groups of 7 males in which testicular regression had been induced by manipulation of the photoperiod (Schafer *et al.*, 1976).

All treated males were individually caged with untreated females of known fertility in a photoperiod of 18 hr light/24 hr, and the reproductive success of the pair was measured over a 35-day period. Since the duration of the spermatogenic cycle in quail is probably between 20 and 25 days (Jones, Kominkova & Jackson, 1972; Jones & Jackson, 1972; Schafer *et al.*, 1976), eggs produced after 20 days should reflect the effects of candidate chemosterilants on the initial phases of sperm development. Males were considered to be permanently sterilized if they showed sterility between 20 and 35 days after treatment (breeding males) or failed to become fertile between 20 and 35 days (quiescent males).

Eggs were collected three times during each 7-day period, incubated by weekly groups for 4 days, and then opened and examined for embryonic development. Individual eggs were classified as fertile, infertile or cracked (cracked eggs were not used in fertility computations). At the end of the 35-day period, males were killed with CO_2, and both testes were extracted and weighed together.

Testicular weights were compared by one-way analysis of variance, and egg fertility data were arc–sine transformed and assessed by two-way analysis of variance with repeated measures (Winer, 1971). For both analyses, means were separated by Duncan's multiple-range test, with $P < 0{\cdot}01$ as the criterion of significance.

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Table. 1. Fertility of eggs produced by female quail after their mates, males in breeding condition, were given oral doses of 45 candidate chemosterilants (common or trivial names in italic)

	Chemosterilant	Source†	LD_{50} (mg/kg)	Male treatment dose (mg/kg)	Fertility (% of eggs laid that were fertile) Days 1–35	Days 20–35	No. of males dying‡	Wt of testes (g)
	Control	—	—	—	92	91	0	2·854
1.	Acetamide, 2-fluoro-	1	13·3 (♂)	10	93	95	1	2·679
2.	Androst-5-en-3β-ol,17β-[2-(dimethylamino)ethyl]methylamino-dihydrochloride	6	750 (♂)	562	74*	67	3	2·442
				100	74*	63	1	3·358
3.	1-Aziridinecarboxamide, *N,N′*-hexamethylene bis-	2	316 (♂)	178	15*	0*	6	0·347*
				100	20*	2*	1	1·180*
4.	1-Aziridinecarboxamide, *N,N′*-tetramethylene bis-	2	133 (♀)	56·2	27*	11*	0	1·091*
5.	1-Aziridinecarboxamide, *N,N′*-vinylene bis-,(E)-	2	237 (♀)	133	20*	0*	1	0·781*
6.	Benz[a]anthracine, 7,12-dimethyl-	1	>316 (♂)	178	86	85	0	3·244
7.	Benzenesulphonamide, 2,5-dichloro-*N*-(2,4-dinitro-1-naphthyl)-	2	>316 (♂)	316	77*	73	0	3·123
8.	Butyric acid, 2-amino-4(ethylthio)- (DL-*ethionine*)	1	>1000 (♂)	562	96	91	1	3·203
9.	Carbanilide, 4,4′-dinitro-*N,N*-dimethyl-	3	750 (♂)	316	95	94	4	3·193
				178	76*	72	0	3·654
10.	Colchicine	1	42·1 (♂)	31·6	77	89	0	2·770
11.	Dimethanesulphonic acid, *N,N′*-(1-methylethylene)-bis-(2-carbamoylethyl)-	1	>1000 (♂)	562	86	81	0	3·513
12.	1,2,4-Dithiazol-1-ium, 3,5-bis(dimethylamino)-, chloride	2	23·7 (♂)	13·3	86	93	0	2·894
13.	Ethane, 2-bromo-, sulphonic acid Na hydrate	1	>1000 (♀)	1000	91	94	1	3·429
14.	Ethane, 2-chloro-, sulphonic acid Na hydrate	1	>1000 (♂)	1000	88	79	0	3·961
15.	Ethanol, 2-chloro-, methanesulphonate	1	208 (♀)	133	29*	17*	1	1·673
				100	40*	71	0	3·432
16.	2-Imidazolidinone	1	>1000 (♀)	562	85	75	0	3·467
17.	Melamine, hexamethyl-, hydrochloride (*hemel HCl*)	2	>316 (♂)	316	75*	80	0	3·819
18.	Melamine, N^2,N^2,N^4,N^4-tetramethyl-	2	>316 (♂)	316	38*	13*	0	2·658
19.	Methanesulphonic acid	1	1000 (♀)	421	49*	39*	0	2·966
20.	Methanesulphonic acid, acetyl-	1	1000 (♀)	750	87	95	1	3·366
21.	Methanesulphonic acid, amino-	1	>1000 (♂)	1000	81	75	2	3·662
22.	Methanesulphonic acid, ammino-	1	>1000 (♂)	1000	78	74	0	4·023
23.	Methanesulphonic acid, methoxymethyl ester	1	750 (♀)	421	57*	63	3	2·607

Chemosterilant	Source†	LD_{50} (mg/kg)	Male treatment dose (mg/kg)	Fertility (% of eggs laid that were fertile) Days 1–35	Days 20–35	No. of males dying‡	Wt of testes (g)
24. Methanesulphonic acid, methyl ester	1	75 (♀)	56·2	71*	89	2	3·290
25. Methanesulphonic acid, trifluoro-	1	>1000 (♀)	1000	88	89	0	3·602
26. Methanesulphonic acid, trifluoro-, methyl ester	1	>1000 (♀)	1000	84	87	0	3·197
27. 1,3,4-Metheno-2*H*-cyclobuta[cd]pentalen-2-one, 1,1a,3,3a,4,5,5a,5b,6-decachlorooctahydro- (*kepone*)	4	237 (♂)	100	71*	70	1	2·921
28. Phosphine oxide, bis (1-aziridinyl) methylamino-	2	237 (♀)	75	13*	0*	3	0·470*
29. Phosphine oxide, bis(1-aziridinyl) anilino-	2	100 (♀)	56·2	44*	49	0	3·208
30. Phosphine sulphide, bis(1-aziridinyl) amino-	2	100 (♀)	56·2	4*	0*	3	0·590*
			31·6	24*	6*	1	2·677
31. Phosphine sulphide, bis(1-aziridinyl) anilino-	2	>316 (♀)	316	16*	0*	1	0·530*
32. Phosphine sulphide, bis(1-aziridinyl) cyclohexylamino-	2	>316 (♀)	316	23*	3*	4	0·378*
			178	10*	0*	0	0·846*
33. Phosphine sulphide, bis(1-aziridinyl) dimethylamino-	2	75 (♀)	31·6	11*	0*	1	0·923*
34. Phosphine sulphide, tris(1-aziridinyl)- (*thiotepa*)	2	237 (♀)	56·2	14*	0*	1	0·713*
35. Phosphonic diamide, *N,N,N′,N′*-tetramethyl-*P*-(1-pyridinyl)-	2	>316 (♂)	316	88	93	0	3·386
36. Phosphoric acid, trimethyl ester	1	750 (♂)	562	81*	96	0	3·913
37. Phosphoric triamide, hexamethyl- (*hempa*)	1	1000 (♂)	562	70*	83	0	3·805
			316	90	92	0	3·204
38. Pyridine, 1-oxide	1	1000 (♂)	562	91	90	0	3·387
39. 2-Pyridinethiol, 1-oxide	1	178 (♀)	133	77	77	4	4·224
			100	86	100	3	3·163
40. 2(1H)-Pyridinone, 1-methyl-	1	421 (♂)	316	87	94	4	3·455
41. Pyrrolidine, 1-(2,2-diphenylbutyryl-)-2-methyl-	5	316 (♂)	178	88	86	4	3·752
42. Stannane, acetoxytriphenyl-	1	100 (♂)	56·2	59*	83	2	2·798
43. 1*H*-Tetrazol	1	>316 (♂)	316	75*	86	0	3·069
			100	88	93	0	3·665
44. Thiophosphoric triamide, hexamethyl- (*thiohempa*)	2	>316 (♂)	316	28*	0*	0	0·669*
45. Triethylamine, 2,2′,2″-trichloro-	1	133 (♂)	56·2	66*	89	4	2·909

* Significantly different from the control values at $P \leqslant 0{\cdot}01$.

† Source of chemicals as follows (reference to company names does not imply endorsement of commercial products by the U.S. Government): 1, purchased; 2, USDA—Beltsville, Maryland; 3, Merck & Co., Rahway, New Jersey; 4, Allied Chemical Co., Morristown, New Jersey; 5, Upjohn Co., Kalamazoo, Michigan; 6, G. G. Searle & Co., Chicago, Illinois.

‡ 7/group except for groups tested with chemosterilants 3 (6) and 44 (5).

Table 2. Fertility of eggs produced by female quail after their mates, males with artificially regressed testes, were given single oral doses of 12 chemosterilants

	Chemosterilant	Male treatment dose (mg/kg)	Fertility (% of eggs laid that were fertile) Days 1–35	Days 20–35	No. of males dying†	Mean days to first fertile egg	Fertile pairs at end of test (no. fertile/no. surviving)	Wt of testes (g)
	Control	—	29	66	0	18	6/7	2·957
3.	1-Aziridinecarboxamide, *N,N′*-hexamethylene bis-	100	15	30	0	21	3/7	3·008
4.	1-Aziridinecarboxamide, *N,N′*-tetramethylene bis-	56·2	3*	7*	1	32	2/6	1·106
5.	1-Aziridinecarboxamide, *N,N′*-vinylene bis-, (E)-	133	4*	2*	0	33	1/7	1·121
15.	Ethanol 2-chloro-, methanesulphonate	133	10	20	0	29	5/7	2·108
18.	Melamine, N^2,N^2,N^4,N^4-tetramethyl-	316	5*	16	1	29	3/6	2·080
28.	Phosphine oxide, bis(1-aziridinyl) methylamino-	56·2	0*	0*	0	—	0/7	0·501*
30.	Phosphine sulphide, bis(1-aziridinyl) amino-	31·6	9	10*	1	20	2/6	1·159
31.	Phosphine sulphide, bis(1-aziridinyl) anilino-	316	0*	0*	1	—	0/6	0·297*
32.	Phosphine sulphide, bis(1-aziridinyl) cyclohexylamino-	100	3*	0*	1	—	0/6	0·876*
33.	Phosphine sulphide, bis(1-aziridinyl) dimethylamino-	31·6	5*	0*	2	—	0/5	0·475*
4.	Phosphine sulphide, tris(1-aziridinyl)-	56·2	0*	0*	0	—	0/7	0·291*
44.	Thiophosphoric triamide, hexamethyl-	178	6*	15	2	30	3/5	2·278

* Significantly different from the control values at $P \leqslant 0{\cdot}01$.
† 7/group.

Additional details of the test procedures and environmental conditions are given by Schafer *et al.* (1976).

Results

Of the 45 compounds tested in breeding males, 13 resulted in significantly reduced fertility or sterility (Table 1). Of these active compounds, 6 were substituted bis(1-aziridinyl) phosphide sulphides or oxides, and 3 were substituted bis(1-aziridinecarboxamides). The combined testicular weights of the birds treated with 9 of the 13 compounds were less than 1 g at the end of the 35-day test period, compared with an average weight of 2·854 g for the controls.

When the 12 compounds that reduced fertility in the breeding males by at least 70% were tested in males with regressed testes (Table 2), 3 resulted in reduced fertility and 5 resulted in complete sterility. These five, all of which were substituted (1-aziridinyl) phosphines, also significantly reduced testicular weights and were considered to be the most effective in producing sterility under the conditions of our test. Studies are now being conducted with thiotepa, the most active of the five, on male starlings (*Sturnus vulgaris*), red-winged blackbirds, and female quail to broaden our knowledge of the chemosterilant activity of this compound under a variety of test conditions.

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