Tests of Pesticidal Synergism with Young Pheasants and Japanese Quail

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Wild animals frequently are exposed to more than one pesticide, as has been shown by tissue analyses of numerous specimens (STICKEL 1968). Application of more than one chemical to field and forest is a common practice, and rivers in the United States are known to carry as many as five pesticides simultaneously (WEAVER et al. 1956). Even human foods contain multiple residues (DURHAM 1967, HENDERSON 1965). DUGGAN et al. (1971) reported four to 15 pesticidal chemicals in composite samples from each of 17 classes of human food.

The joint lethality of pesticides to animals may be other than additive. For example, a mixture of malathion and EPN was 10 times as toxic to male rats and 50 times as toxic to dogs as was either compound alone (FRAWLEY et al. 1957). A combination of TOTP (tri-o-tolyphosphate) and malathion was 88 to 134 times as toxic to female rats as either compound alone; 20 percent of about 50 pairs of organophosphates exhibited synergism in tests with female rats (DUBOIS 1961). A mixture of carbaryl and piperonylbutoxide was more than 400 times as toxic to house flies as was carbaryl alone (EL SABAE et al. 1964).

The present study was designed to determine the joint action of pesticides fed in pairs to Japanese quail *(Coturnix coturnix japonica)* and pheasant *(Phasianus colchicus)* chicks, and to compare the results with joint actions reported for mammals and insects. Eighteen chemicals were tested in 13 pairs. Seven of the pairs were selected because of marked synergism reported in other studies and six because of extensive, world-wide usage. The paired chemicals were: aldrin + chlordane, Aroclor 1254 + DDE, chlordane + endrin, DDT + dieldrin, malathion + Co-Ral, malathion + EPN, malathion + parathion, malathion + trichlorofon, phosphamidon + fenitrothion, Guthion + trichlorofon, Aroclor 1262 + malathion, DDE + Ceresan M, and dieldrin + diazinon.

Methods

Tests were made with young Japanese quail (7 or 16 days old), and with young ring-necked pheasants (9 or 14 days old). Chemicals were dissolved in corn oil or propylene glycol and blended into the feed; an equal amount of solvent was added to the feed of the controls. Each test period consisted of 5 days of dosage followed

Bulletin of Environmental Contamination & Toxicology, Vol. 9, No. 4, © 1973 by Springer-Verlag New York Inc.

by 3 days of untreated feed; mortality was recorded for the entire 8-day period. This basic protocol was developed by HEATH and STICKEL (1965) and is in routine use at our laboratories.

Toxicity is expressed as ppm of the chemical in the 5-day diet that, on the average, will be lethal to "k" percent of a population. Letting k = 50 expresses the median lethal concentration, or LC_{50}. (The general lethal concentration is thus noted as the LC_k.)

The chemicals of each pair were tested in the diet both individually and as mixtures which included various ratios of one chemical to the other. Generally three ratios, but as few as one or as many as five, were investigated. Each ratio and individual chemical was fed in concentrations expected to produce 10, 30, 45, 55, 70, or 90 percent mortality, assuming additive joint action. These expectations were based on earlier tests of the individual chemicals. Each dosage was assigned to a group (pen) of 10 birds in a completely randomized experiment. Control groups were included to detect extraneous mortality. The ratios used with each pair of chemicals tested are shown in Table 1. Chemical names of the compounds are shown in Table 2.

TABLE 1

Joint lethal toxicities of pairs of pesticidal chemicals in 5-day diets of Japanese quail or ring-necked pheasant chicks.

Compound or Formulation[1]	Species and age[2]	Composition of mixtures[3]	Present Study E/0[4]	Literature Records: E/0, mammals or insects
(organochlorines)				
Aldrin + chlordane	Q 14	80 + 20	1.39	2.08 - mice[5]
		50 + 50	1.22	0.88 - rats
		20 + 80	1.02	KEPLINGER and DEICHMANN (1967)
Aroclor 1254 + DDE	Q 9	90 + 10	0.93	
		50 + 50	0.98	
		10 + 90	0.98	
Chlordane + endrin	Q 14	80 + 20	1.07	2.22 - mice[5]
		50 + 50	1.07	KEPLINGER and DEICHMANN (1967)
		20 + 80	1.11	
DDT + dieldrin	Q 7	90 + 10	0.95	0.85 - rats
		80 + 20	0.95	KEPLINGER and DEICHMANN (1967)
		50 + 50	0.82	1.29 - milkweed bugs TURNER (1955)
		20 + 80	1.02	1.15 - fruit flies FLEMING et al. (1962)

(TABLE 1 continued)

Compound or Formulation[1]	Species and age[2]	Composition of mixtures[3]	Present Study E/O[4]	Literature Records: E/O, mammals or insects
(organophosphates)				
Malathion + Co-Ral	P 14	80 + 20	1.10	2.40 - rats[5]
		50 + 50	1.16	DUBOIS (1961)
		20 + 80	1.13	
Malathion + EPN[6]	Q 14	80 + 20	3.42	50.0-dogs[5]
		50 + 50	2.97	FRAWLEY et al. (1957)
		20 + 80	1.93	10.0-rats[5] FRAWLEY et al. (1957)
Malathion + EPN[6]	P 9	80 + 20	2.77	1.8 - rats[5]
		50 + 50	3.42	DUBOIS (1961)
		20 + 80	2.41	
Malathion + parathion	Q 14	50 + 50	0.92	<additive-rats DUBOIS (1961)
Malathion + trichlorofon[6]	Q 14	50 + 50	2.50	2.2 - rats[5] DUBOIS (1961)
Malathion + trichlorofon[6]	P 9	80 + 20	1.57	
		50 + 50	1.63	
		20 + 80	1.84	
Phosphamidon + fenitrothion	Q 14	20 + 80	1.23	Ca. 3.0 - rats[5]
		50 + 50	1.23	BRAID and NIX (1967)
		80 + 20	1.15	
Guthion + trichlorofon	Q 14	80 + 20	0.95	1.50 - rats
		50 + 50	1.18	DUBOIS (1961)
		20 + 80	1.11	
(organochlorine + other)				
Aroclor 1262 + malathion	Q 16	80 + 20	0.76	
		50 + 50	0.70	
		20 + 80	1.04	
DDE + Ceresan M	P 9	10 + 90	0.88	
		90 + 10	0.82	

(TABLE 1 continued)

Compound or Formulation[1]	Species and age[2]	Composition of mixtures[3]	Present Study E/O[4]	Literature Records: E/O, mammals or insects
Dieldrin + diazinon	Q 7	95 + 5	0.87	1.20 - rats
		75 + 25	0.82	KEPLINGER and
		5 + 95	0.95	DEICHMANN (1967)

1/ See Table 2 for chemical name of active ingredient.
2/ Q = Japanese quail; P = pheasant; numeral = days of age at beginning of test.
3/ Figures show percentages of the LC_{50}'s of the individual chemicals that composed the mixture. The first percentage is for the first chemical listed under "Compound or Formulation".
4/ Expected $LC_{50}(E)$ divided by observed $LC_{50}(O)$. See full explanation in text.
5/ Reported as synergistic by investigators named. All others additive except as indicated.
6/ Combinations significantly synergistic. No others departed significantly from additivity.

TABLE 2

Chemical names of the compounds used in combination against the experimental birds.

Common or registered name	Chemical name
aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene
Aroclor 1254	polychlorinated biphenyl (54% chlorine)
Aroclor 1262	polychlorinated biphenyl (62% chlorine)
Ceresan M	N-(ethylmercuri)-p-toluene sulfonanilide
chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Co-Ral	0,0-diethyl 0-3-chloro-4-methyl-1-oxo-2H-1-benzopyran-7-yl phosphorothioate
DDE	1,1-dichloro-2,2-bis-(p-chlorophenyl) ethylene

(TABLE 2 continued)

Common or registered name	Chemical name
DDT	1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane
diazinon	0,0-diethyl 0-(2-isopropyl 4-methyl-6-pyrimidinyl) phosphorothioate
dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene
endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene
EPN	0-ethyl 0-p-nitrophenyl phenylphosphonothioate
fenitrothion	0,0-dimethyl 0-(4-nitro-m-tolyl) phosphorothioate
Guthion	0,0-dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl]phosphorodithioate
malathion	S-[1,2,-bis(ethoxycarbonyl)ethyl] 0,0-dimethyl phosphorodithioate
parathion	0,0-diethyl 0-p-nitrophenyl phosphorothioate
phosphamidon	2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate
trichlrofon	0,0-dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate

If the joint effect of two chemicals is additive, the mortality produced by any mixture of the pair will be the same as that of an equivalent amount of either chemical alone. For example, if the LC_{50} of compound A is 100 ppm and that of compound B is 200 ppm, then 50 ppm of A combined with 100 ppm of B should produce approximately 50 percent mortality if the two are additive. Significantly greater mortality would indicate synergism; lesser mortality would suggest independent action or possibly antagonism.

Using methods proposed by WADLEY (1945, 1949), we determined the individual LC_{50}'s from the current tests and used these values to calculate the relative toxicities of the two chemicals. The

ratio of one LC_{50} to the other was used to convert the concentrations of one chemical in the mixture to equitoxic concentrations of the other, which was termed the "principal" chemical. (Either chemical may be designated as principal.) The converted concentrations of the second chemical and the actual concentrations of the principal chemical in the mixture were summed to give adjusted concentrations. The dosage response curves for the mixtures were plotted on log-probability paper in terms of the adjusted concentrations, and the observed LC_{50}'s were estimated from the curve. Each observed LC_{50} was compared with the LC_{50} that would have been obtained from the mixture if the action were additive. WADLEY (1949) states that if an observed mortality curve for a mixture (plotted as probits against log concentrations) is at least three standard errors below its expected value, there is strong evidence of synergism. He gives approximate standard errors for the log of the ratio of expected to observed concentrations for various regression coefficients and sample sizes.

Results and Discussion

The joint lethality of two of the pairs of chemicals--malathion + EPN, and malathion + trichlorofon--was shown to be synergistic to chicks of both Japanese quail and ring-necked pheasants. The joint action of the other pairs appeared to be additive. Findings are presented in Table 1.

Since feeding was *ad libitum*, a necessary condition for a valid test of synergism is that birds fed a diet containing a pesticidal mixture of given expected toxicity consumed that diet at essentially the same rate as did birds receiving the corresponding diets containing the single pesticides. Although food consumption was difficult to measure precisely due to spillage, we could detect no marked differences in consumption within equitoxic dietary concentrations.

Results of tests of 11 of the same pairs of chemicals against mammals or insects also are presented in Table 1. Eight of the 11 pairs were reportedly synergistic to rats, mice, dogs, or two species of insects. FRAWLEY et al. (1957) reported that malathion + EPN was 50 times as toxic to dogs and 10 times as toxic to male rats as expected, whereas we found the mixture to be approximately three times as toxic as expected to both Japanese quail and ring-necked pheasants.

The results of this study suggest that pesticides are not likely to prove lethally synergistic to birds, insofar as one can judge from two species, and that when synergism does occur it will not be especially severe.

Acknowledgement

We wish to express our appreciation to R. G. Heath for advice and assistance throughout this study.

Summary

Thirteen pairs of chemicals involving 18 pesticides and two polychlorinated biphenyl preparations were each fed for 5 days to Japanese quail or ring-necked pheasant chicks 7 to 16 days of age. Malathion + EPN, and malathion + trichlorofon were moderately synergistic in tests with both species, whereas joint toxicities of the other chemicals tended to be additive. Comparisons with other studies of joint action of pesticides against mammals and insects suggest that the two species of birds tested are less susceptible to synergism than are mammals or insects. The results also suggest that the likelihood of a factor of synergism greater than three in birds is not great.

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