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Toxicity of Anticholinesterase Insecticides to Birds: Technical Grade versus Granular Formulations[1]

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The acute toxicities of 13 granular anticholinesterase insecticides were compared with their technical grade active ingredients by administering single oral doses of chemical to adult Northern bobwhites (*Colinus virginianus*) and evaluating resultant LD_{50} values and dose-response curves. Similar tests with ringed turtledoves (*Streptopelia risoria*) were conducted with five of the granular formulations to check for interspecific differences. The test chemicals were Amaze 15G (isofenphos), Counter 15G (terbufos), Dasanit 15G (fensulfothion), Diazinon 14G (diazinon), Di-Syston 15G (disulfoton), Dyfonate 20G (fonofos), Furadan 10G (carbofuran), Lorsban 15G (chlorpyrifos), Nemacur 15G (fenamiphos), Parathion 10G (parathion), Tattoo 10G (bendiocarb), Temik 15G (aldicarb), and Thimet 15G (phorate). Information is also presented on dose-response relations and their use in hazard assessment, granule size and hazard, response patterns, and toxic signs. The general conclusions were: (1) The organophosphates (fenamiphos and fensulfothion) and the carbamate (aldicarb) were the most toxic of the insecticides tested. (2) The granular formulation and its technical grade active ingredient were of equivalent toxicity, or the granular was significantly less toxic. (3) The dose-response curve enhances hazard assessment. (4) Ingestion of a single granule of Temik 15G was shown to be life threatening to bobwhite-sized birds, and ingestion of fewer than five granules could be lethal to sparrow-sized birds for Dasanit 15G, Diazinon 14G, Dyfonate 20G, Furadan 15G, and Nemacur 15G. (5) It is therefore suggested that the hazard associated with granular insecticides may be more dependent on which species (cf. size and feeding behavior) inhabit a treated area than on the actual application rate. © 1984 Academic Press, Inc.

INTRODUCTION

The acute toxicities of 13 granular anticholinesterase insecticides (Table 1) were compared with their technical grade active ingredients by administering single oral doses of chemical to adult Northern bobwhites (*Colinus virginianus*) and evaluating resultant LD_{50} values and dose-response curves. Interspecies differences were checked for five of the granular formulations by conducting similar tests with ringed turtledoves (*Streptopelia risoria*). The study was conducted because there is no standardized toxicologic data base for wildlife on the array of pesticidal formulations currently registered for use in the United States. Previous studies of this kind have focused on technical grade compounds in order to generate data on many pesticides and species rather than dwell on a few compounds and their formulations, e.g., Tucker and Crabtree (1970), Schafer (1972), Hill *et al.* (1975). It was realized that biological activity could be altered by additives such as solubilizers and

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TABLE 1

TEST CHEMICALS, PURITY, AND GRANULE WEIGHT

Common name	Chemical name[a]	Formulation[b]	Purity	Mean μg/granule	
				Median	Extremes
Aldicarb	2-Methyl-2-(methylthio)propanol O-[(methylamino)carboxyl]oxime	Technical	99% AI		
		Temik 15G	15% AI	536	500, 730
Bendiocarb	2,2-Dimethyl-1,3-benzodioxol-4-ol methylcarbamate	Technical	99% AI		
		Tattoo 10G	10% AI	450	435, 735
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranol methyl-carbamate	Technical	99% AI		
		Furadan 10G	10% AI	320	302, 358
Chlorpyrifos	Phosphorodithioic acid O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)ester	Technical	99% AI		
		Lorsban 15G	15% AI	64	62, 78
Diazinon	Phosphorothioic acid O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]ester	Technical	99% AI		
		Diazinon 14G	14% AI	215	95, 287
Disulfoton	Phosphorodithioic acid O,O-diethyl S-[2-(ethylthio)ethyl]ester	Technical	95% AI		
		Di-Syston 15G	15% AI	94	78, 97
Fenamiphos	Phosphorodithioic acid ethyl 3-methyl-4-(methylthio)phenyl(1-methylethyl)ester	Technical	99% AI		
		Nemacur 15G	15% AI	92	66, 124
Fensulfothion	Phosphorothioic acid O,O-diethyl O-[4-(methylsulfinyl)phenyl]ester	Technical	98% AI		
		Dasamit 15G	15% AI	141	114, 168
Fonofos	Ethylphosphorothioic acid O-ethyl S-phenyl ester	Technical	94% AI		
		Dyfonate 20G	20% AI	197	184, 560
Isofenphos	1-Methylethyl-2-[[ethoxy[(1-methylethyl) amino]phosphinothioyl]oxy]benzoic acid ester	Technical	98% AI		
		Amaze 15G	15% AI	97	64, 178
Parathion	Phosphorothioic acid O,O-diethyl O-(4-nitrophenyl)ester	Technical	98% AI		
		Parathion 10G	10% AI	109	103, 115
Phorate	Phosphorodithioic acid O,O-diethyl S-[(ethylthio)methyl]ester	Technical	93% AI		
		Thimet 15G	15% AI	85	67, 143
Terbufos	Phosphorodithioic acid S-[[1,1-dimethyl-ethyl)thio]methyl]O,O-diethyl ester	Technical	99% AI		
		Counter 15G	15% AI	66	56, 80

[a] Nomenclature is after Chemical Abstracts, 9th Chemical Index.

[b] Technical grade chemicals were obtained from Chem Service, Inc., West Chester, Pa., and granular formulations from Richard Balcomb, U.S. Environmental Protection Agency, Washington, D.C.

dispersing agents, but it was believed that tests of technical material gave the best initial estimate of both inherent toxicity and species sensitivity. More intensive study of a pesticide in its various formulations would then be indicated if the compound was found highly toxic or its intended use seemed especially hazardous. However, because hazard is believed to be primarily a function of physical-chemical properties of the parent compound, application rate, and ecosystem target, comparatively little attention has been given to the various formulations of the pesticide. The present study was therefore conducted to determine the oral toxicity of granular insecticides to bobwhites and ringed turtledoves in the form actually encountered in the environment, to provide a means of relating the toxicity of these granulars to existing bobwhite data through concurrent tests of their technical grade active ingredients, and to compare clinical signs and mortality patterns for birds dosed with granular or technical grade insecticides. Bobwhite were selected as the principal test species because of their presumed vulnerability to agricultural insecticides and their historic use as a model for pesticide registration (U.S. Environmental Protection Agency, 1978). Ringed turtledoves were intended to serve as a surrogate of endemic North American doves and are also presumed vulnerable to agricultural insecticides.

METHODS

Animals

Three batches of 10- to 12-week-old bobwhites were purchased from Charles C. Brown (Fayetteville, N.C.). On receipt at the Patuxent Wildlife Research Center the birds were randomized into test groups of five males and five females and conditioned for at least 1 month in stack pens measuring 35 × 100 × 24 cm high. Water and unmedicated gamebird ration (Ziegler Brothers, Inc., Gardners, Pa.) were supplied ad libitum. The light regimen was 10L:14D and the ambient temperature was 26–28°C. The short day was to ensure reproductive quiescence and thereby minimize any sex differences due to reproductive state. Other than occasional culling and replacement of obviously inferior or injured birds, test group integrity was maintained. When the bobwhites were tested at 16–20 weeks of age, the mean weights and coefficients of variation for the three batches were 205 g (CV = 10.5), 197 g (CV = 8.2), and 199 g (CV = 5.5). The overall mean pretest weight was 200 g (SD = 4.2) and neither the batches nor sexes were statistically separable ($\alpha = 0.05$, two-way analysis of variance).

Pairs of adult ringed turtledoves, i.e., >1 yr, were drawn from the Patuxent colony and randomized into modified quail breeding pens measuring 51 × 30 × 20 cm high. Doves were managed the same as were the bobwhites except they were fed Pigeon Chow Checkers (Ralston Purina Co., St. Louis, Mo.). The mean pretest dove weight was 162 g (SD = 4.1) and the sexes were statistically inseparable ($\alpha = 0.05$, paired t test).

Test Protocol

The basic bobwhite test generally followed the avian single-dose oral LD_{50} (median lethal dose) protocol of the Federal Insecticide, Fungicide, and Rodenticide Act (U.S. Environmental Protection Agency, 1978). Adult nonbreeding birds were fasted overnight (1600–1030 hr) and then given five geometrically spaced doses of each technical grade and granular insecticide. Feed was provided immediately posttreatment and all birds were then observed at least twice daily for 1 week for mortality and other evidence of toxicity. The LD_{50}, expressed as milligrams active ingredient per kilogram body wt, and associated statistics (95% confidence interval; slope and standard error of the probit regression curve) were derived by probit analysis (Finney, 1978). LD_{50} values and slopes of probit regressions were compared between technical and granular formulations (and species) by two-tailed t test (Heath *et al.*, 1972). Statistical significance was set at $\alpha = 0.05$.

Assignment of birds and treatment (= dose) to a test group was by random numbers. Test groups, one per dose, were normally of equal sex composition and consisted of 8 or 10 individuals. Two or three control groups, which were equivalent to test groups in all respects except for the absence of insecticide, were provided for both technical and granular insecticides and accompanied each test. Two to four insecticides, including both technical and granular forms, were tested in the same experiment and used common controls.

The dove tests were conducted essentially the same as the bobwhite tests except blocks of four or five pairs served as test groups and tests were restricted to granular formulations.

Dosing Procedures

Test doses were derived by preliminary tests of three widely spaced doses ($\simeq 5$ bobwhites per dose) of technical grade insecticide and interpolation (or extrapolation) of the LD_{10} and LD_{90} from resultant log probability plots. Three intermediate doses were arranged at constant log intervals between the approximated LD_{10} and LD_{90}. These intermediate doses were then administered between 0830 and 1030 hr as the initial test levels for both technical grade and granular formulations. First day mortalities were used to arrange the final test levels, up or down, by a continuation of the same log increment used between the initial levels. The final two dose levels were administered between 0830 and 1000 hr on Day 3. This two-step dosing scheme was devised to accommodate differences between technical grade and granular formulations, and to reduce the number of birds necessarily poisoned to achieve our experimental objectives. Although this dosing scheme compromised the idealized concept of exact concurrent administration of test substance to all subjects, it did not compromise the integrity of the experiment. All birds were part of the same randomization, dosed at the same time of day, and various ambient factors were comparable throughout the experiment.

All doses were delivered to the proventriculus of both bobwhites and doves in No. 4 gelatin capsules (Eli Lilly Co., Indianapolis, Ind.) with a stainless steel 16.5-cm tricep-forceps. It was not necessary to lubricate either the capsule or tubular straight forceps. Individual doses of technical grade insecticide were prepared by microsyringing serially diluted 50 μl aliquots of acetone–insecticide solution into the capsules and evaporating off the acetone at ambient temperature before capping. Granulars were weighed directly into capsules and immediately capped. All doses were corrected to equivalency on the basis of active ingredient. Percentage active ingredient in technical grade and granular formulations is shown in Table 1. Control doses were prepared the same as above except 50 μl of untreated acetone and 100 mg of untreated clay granules were used.

All doses were prepared and administered on the basis of an assumed mean bird weight of 200 g rather than on individual weights. The averaging method was used because of logistical problems associated with preparation and administration of specific doses for concurrent tests of several hundred individuals. The 200-g mean weight was derived by random subsampling of bobwhites during preliminary range finding trails which showed that weights were normally distributed and the sexes were not of different weights. For confirmation and possible adjustment of actual dose levels, one bird was randomly selected from each 10-bird test group within 2 days preceding each test and weighed to monitor variance within the test population. It was not necessary to adjust any of the bobwhite results but dove LD_{50} values were all increased by a factor of 1.23.

RESULTS AND DISCUSSION

Our two-step dosing scheme, sample sizes of 8 or 10 per test group, and use of average bird weight rather than individual weights as the basis for dosing were efficient, yielded the desired dose–effect relations, and minimized the number of birds poisoned. The 2-day lag between administration of the first three and final two dose levels was especially suited for testing anticholinesterases because all mortalities from carbamates and 96% of those from organophosphates occurred within 24 hr. As a consequence, more than the intended five dose levels were required only twice for bobwhite and once for doves. This first-test success would not have been possible without such a scheme because our initial dose arrangement based on three-dose range finders with bobwhite was actually used for only 6 of 13 technical grade and 3 of 13 granular formulations. Four to five birds of each sex per dose were satisfactory in this study because there were no important sex differences in sensitivity for either bobwhites or doves. Where one sex seemed more sensitive at a given dose, response was either equal between sexes or reversed at the next higher dose. Overall, the pooled sexes gave a dose–dependent relation free of any successive dose reversals in 20 of 26 bobwhite and 3 of 5 dove tests. It was concluded that intertest comparisons could be made without discrimination for each species because body weight and toxic reaction were similar for both sexes during this reproductively quiescent period, all tests were conducted in the same facilities under similar ambient conditions, and no controls died during any study.

Technical Grade vs Granular Formulations: Bobwhite

LD_{50}. For all 13 insecticides, the granular formulation was either of equivalent toxicity or significantly less toxic to adult bobwhites than was its technical grade base (Table 2). (For simplicity, granular formulations are hereafter indicated by "G-" preceding the generic name as necessary). Toxicity ratios between technical and granular forms were characterized as equivalent, or 1× (aldicarb, carbofuran, diazinon, fenamiphos, and fonofos), 1.5× (bendiocarb and isofenfos), 2× (disulforton, fensulfothion, parathion, and terbufos), and 3× (chlorpyrifos and phorate). No single factor, e.g., chemical structure or degree of toxicity, was well correlated with the variable toxicity ratios. When the test insecticides were ranked according to ascending LD_{50}, the apparent order of toxicity was different for technical grade and granular formulations but the most (fenamiphos, fensulfothion, and aldicarb) and least (bendiocarb and chlorpyrifos) toxic compounds were identical in both rankings. Although the other compounds varied between the two rankings, none of the deviations seemed important. The overall coefficient of determination, i.e., technical grade vs granular LD_{50}, was 0.8 ($P < 0.01$). The generalized order of toxicity for technical grade was fenamiphos = fensulfothion = aldicarb > parathion = phorate > diazinon = fonophos = disulfoton = carbofuran = isofenphos = terbufos > bendiocarb > chlorpyrifos. For granulars the order was fensulfothion = fenamiphos = aldicarb > diazinon > carbofuran = parathion = fonofos > isofenphos = phorate > terbufos = disulfoton = bendiocarb > chlorpyrifos. These rankings are based exclusively on LD_{50} values without regard to statistical separations because in the strictest sense, i.e., theory of fiducial probabilities (Finney, 1978), the only statistical breaks ($\alpha = 0.05$) are between technical grade aldicarb and parathion, granular aldicarb and diazinon, and granular bendiocarb and chlorpyrifos. Otherwise, all

TABLE 2

SINGLE-DOSE ORAL TOXICITY OF TECHNICAL GRADE AND A GRANULAR FORMULATION OF 13 ANTICHOLINESTERASE INSECTICIDES TO ADULT BOBWHITE (POOLED SEXES)

Insecticide	Formulation	Doses/birds[a]	LD_{50}[b]	95% CI	Slope[c]	SE
Phosphate						
Fenamiphos	Technical	5/8	1.0	0.7–1.3	4.9	1.3
	Nemacur 15G	5/10	2.4	1.2–4.6	2.5	0.9
Thiophosphate						
Chlorpyrifos	Technical	5/9	32	24–43	4.6	1.2
	Lorsban 15G	5/10	108**	80–145	4.9	1.3
Diazinon	Technical	5/10	10	7–13	6.5	2.0
	Diazinon 14G	5/10	8	6–11	5.3	1.3
Fensulfothion	Technical	5/10	1.2	1.0–1.6	6.1	1.7
	Dasanit 15G	5/10	2.4**	2.0–2.9	7.8	1.8
Isofenphos	Technical	5/8	13	10–16	7.1	1.8
	Amaze 15G	5/10	19**	15–23	7.2	1.8
Parathion	Technical	5/10	6	4–9	3.7	1.0
	Parathion 10G	5/10	13**	8–21	3.1	0.7
Dithiophosphate						
Disulfoton	Technical	5/10	12	7–19	3.0	0.8
	Di-Syston 15G	5/10	29**	24–34	8.5*	2.0
Fonofos	Technical	5/10	12	10–14	7.3	2.0
	Dyfonate 20G	5/10	14	12–17	9.6	2.5
Phorate	Technical	5/9	7	4–11	4.4	1.2
	Thimet 15G	5/10	21**	14–31	4.5	1.1
Terbufos	Technical	5/10	15	12–19	7.9	1.9
	Counter 15G	5/10	26**	20–34	6.1	1.4
Methyl carbamate						
Bendiocarb	Technical	5/8	21	17–26	8.2	2.4
	Tattoo 10G	5/10	33**	24–44	5.5	1.6
Carbofuran	Technical	8/12	12	7–19	1.8	0.5
	Furadan 10G	8/12	12	9–16	2.7	0.6
Oxime carbamate						
Aldicarb	Technical	7/8	2.0	1.4–2.9	3.2	0.8
	Temik 15G	5/10	2.5	1.6–4.0	3.1	0.7

[a] Doses/Birds: No. of doses tested/mean birds per dose.

[b] LD_{50}: Mg active ingredient per kg body wt in a single oral dose calculated to kill 50% of the test population.

[c] Slope: Probit on log dose.

** Significantly different from technical grade counterpart ($P < 0.01$).

ordered LD_{50} values constitute a continuum. The break between technical grade aldicarb and diazinon is critical because the approximate three-fold separations clearly delineate fenamiphos, fensulfothion, and aldicarb as most toxic of the 13 insecticides tested.

Few comparable studies of acute toxicity were found for quail to compare with results reported herein. All of these studies were based on tests of technical grade chemicals, and only two used bobwhite; all others tested California quail(*Callipepla californica*) or Japanese quail (*Coturnix japonica*). In a study of bobwhite from eight game farms, diazinon LD_{50} values varied from 13 to 17 mg/kg (Hill *et al.*, 1984) compared with 10 mg/kg in the present study. Both of these studies were conducted in the same facilities at Patuxent within 2 months of one another. The difference in LD_{50} between studies was likely a function of differential rates of absorption because in the earlier tests diazinon was administered in a corn oil medium. The other study of bobwhite listed an LD_{50} of 5 mg/kg for carbofuran (Tucker and Crabtree, 1970) which was significantly ($P < 0.05$) lower than our LD_{50} of 12 mg/kg. In the present study and the acute studies of the Denver Wildlife Research Center (Tucker and Crabtree, 1970; Hudson *et al.*, 1984), chemical was administered via gelatin capsule but birds per dose, physiological status of birds, and doses per test were not reported. Comparative LD_{50} values for the present bobwhite study (BW), Japanese quail (JQ), and California quail (CQ) include: aldicarb, BW = 2.0 mg/kg and CQ = 2.6 mg/kg; chlorpyrifos, BW = 32 mg/kg, CQ = 68 mg/kg, and JQ = 16 mg/kg; fensulfothion, BW = 1.2 mg/kg and CQ = 1.7 mg/kg; and parathion, BW = 6 mg/kg and JQ = 6 mg/kg. Ludke (1977) reported a single-dose LD_{50} of 4.0 mg/kg parathion for adult Japanese quail. Although the above comparisons cross species bounds, they generally support one another as to degree of toxicity represented by LD_{50} values, and technical aldicarb, fensulfothion,and parathion were highly toxic to quail. A more distant phylogenetic comparison showed mallard (*Anas platyrhynchos*) LD_{50} values were 0.4, 0.8, 2.3, 4.4, and 83 mg/kg for carbofuran, fensulfothion, parathion, aldicarb, and chlorpyrifos, respectively (Hudson *et al.*, 1972). Again, aldicarb, fensulfothion, and parathion were highly toxic, but carbofuran was even more toxic. In comparison to bobwhites, mallards were more sensitive to fensulfothion (1.5×), parathion (2.6×), and carbofuran (30×), and less sensitive to aldicarb (2.2×) and chlorpyrifos (2.6×). These results corroborate the need for testing multiple species in hazard assessment.

Dose–response lines. Slopes of dose–response lines were compared between technical grade and granular formulations of each insecticide and all pairs were statistically parallel except disulfoton (Table 2). The line for G-disulfoton was 2.8 times as steep as technical disulfoton ($P < 0.05$). No other pairs of slopes were separated by as much as two-fold. Because encapsulated technical grade material may be absorbed more readily than granular material, it was expected that technical grade would yield the steeper slope if differences occurred. But of the nine pairs of slopes arithmetically separated by at least 1.2×, technical grade was steeper five times (bendiocarb, diazinon, parathion, terbufos, and fenamiphos) and granular was steeper four times (carbofuran, fensulfothion, fonofos, and disulfoton). When slopes of dose–response lines were ranked in ascending order there was no apparent relation with similar chemical structures or toxicity for either technical grade or granular formulation. As a group, slopes of technical grade and granular formulations were statistically inseparable (paired t test, $\alpha = 0.05$) but showed significant linear correlation ($r^2 = 0.38$, $P < 0.05$). Although our study was not designed for detailed comparison of dose–response lines, the single-test estimates of slope are adequate for approximation of the amount of insecticide necessary to proportionally change effect (mortality) and thereby provide useful indices of margin of safety (Loomis, 1978). It is cautioned, however, that the probit regression curve is an idealized representation of dose–response and has only limited value for extrapolation outside its range of linearity (±1.0 SD of LD_{50}, i.e., LD_{16} and LD_{84}).

Response patterns and signs. Three general patterns of response were associated with acute anticholinesterase dosing of bobwhite. (1) All deaths occurred within 24 hr and all overt signs subsided within 48 hr (aldicarb and G-aldicarb, bendiocarb, carbofuran and G-carbofuran, G-diazinon, disulfoton, fenamiphos, fonofos and G-fonofos, phorate, and terbufos). (2) All deaths occurred within 24 hr and overt signs persisted beyond 48 hr (G-bendiocarb, diazinon, G-fensulfothion, isofenfos, and G-phorate). (3) Most deaths occurred within 24 hr but some latent mortality, usually within 48 hr was observed (chlorpyrifos and G-chlorpyrifos, G-disulfothion, G-fenamiphos, fensulfothion, G-isofenphos, parathion and G-parathion, and G-terbufos). Although both acute toxicity and its remission occurred rapidly after single-dose exposure to all anticholinesterases tested, the above patterns were characteristic of certain classes of chemical and differed between technical grade and granular formulations. All technical grade carbamates, dithiophosphates, and the phosphate were in the first category, i.e., rapid recovery; whereas, all technical thiophosphates and 9 of 13 granulars had the more prolonged effects associated with the other categories. Two of the remaining granulars that were in the fast action-recovery category were carbamates. In the test of aldicarb, birds were checked at 1, 3, 6, and 24 hr postdosing and 82% of all deaths occurred within the first hr and the rest by 3 hr. Similar checks of bendiocarb-dosed birds revealed 58 and 100% of all deaths occurred within 1 or 3 hr but toxic signs persisted more than 72 hr in survivors of 40 mg/kg. In contrast, all deaths from technical aldicarb and bendiocarb were within 1 hr and all signs of intoxication had subsided by 24 hr. Birds died up to 96 hr postdosing on G-disulfoton, G-chlorpyrifos, and G-parathion. Only a single bird on technical grade chemical died more than 48 hr postdosing. That bird, dosed with 14 mg/kg parathion died during the night of Day 7 and had not exhibited toxic signs preceding its death.

Overt signs were similar for bobwhite on all 13 insecticides. This was probably because all of the compounds were anticholinesterases that initially attack the nervous system in the same way, manifest their toxicity within minutes of exposure, and, if death does not ensue, recovery is rapid. In general, progression and intensity of overt signs followed a common sequence and the time to each phase was dose dependent for organophosphates but not for carbamates. The usual sequence preceding death was (1) reduction of activity; (2) mild tremoring and feather fluffing; (3) huddling together, labored breathing, salivation, occasional lacrimation, and reduced reactivity to investigator; (4) ataxia and near immobility; (5) wing-beat convulsions; and (6) death. Death was characterized by prostration with wings and legs fully extended. Because the birds were penned together it was difficult to make repeated observations on specific individuals and document peculiarities of response to specific insecticides. However, other studies of birds have generated extensive lists of signs associated with acute toxicity of at least seven insecticides reported herein, and support all of our observations (Tucker and Crabtree, 1970). For birds that survived anticholinesterase dosing, some never showed signs of intoxication or they progressed to the third category and were hyperreactive to investigator presence. Tremoring was the usual overt sign after 24 hr postexposure. At highest doses, birds often died within 10 to 30 min on all insecticides. Recovery was not necessarily dose dependent but signs usually persisted longest for highest doses, and longer for granulars than technical grade counterparts. As mentioned earlier, birds dosed with technical grade carbamates exhibited signs almost immediately regardless of dose, but they again appeared normal within the day. In contrast, Tucker and Crabtree (1970) reported that although some birds died from carbofuran within 5 min, others continued to show signs as long as 7 days posttreatment.

Ringed Turtledoves vs Bobwhites: Sensitivity to Granular Insecticides

Ringed turtledoves were 1.7 times more sensitive than bobwhites to carbofuran ($P < 0.05$), bobwhites were 1.5 times more sensitive than doves to chlorpyrifos ($P < 0.05$), and the two species were equally sensitive to fensulfothion, parathion, and phorate (cf. LD_{50} values in Tables 2 and 3). Although these results seem erratic and are based on only five comparisons, the toxic ranking of the compounds for doves remained the same as for bobwhites and the quantitative differences between species were well within bounds reported in other studies. Tucker and Haegele (1971) tested 16 technical grade pesticides with six species of birds and found that LD_{50} differed more than two-fold between species pairs over one-half of the time and more than threefold about one-third of the time. Their tests of Japanese quail and rock doves (*Columba livia*), the most similar phylogenetic comparison to our study, showed quail more sensitive to six of nine organophosphates and doves more sensitive to three of four carbamates. The median differences between species, in either direction, were 1.7× and 2.3× for organophosphates and carbamates. In another study, species' differences of two- to fivefold were consistently reported between LD_{50} values for a wide variety of species (Tucker and Crabtree, 1970). The present study therefore demonstrated that when doves and bobwhites were tested under comparable conditions, their sensitivity to acute anticholinesterase exposure was similar. Thus, assuming ringed turtledoves are suitable surrogates for other columbids of similar size and feeding behavior, results of standard bobwhite tests should provide a reasonable basis for assessing pesticidal hazard to endemic dove populations.

Slopes of the dose–response lines were statistically parallel for all five granulars tested against ringed turtledoves (Table 3). Interspecies comparisons showed slopes of corresponding dose–response lines were statistically inseparable between doves and bobwhites, but little correlation existed between slope and species ($r^2 = 0.11$, $P > 0.05$). Overall, the slopes of dose–response lines for the five corresponding anticholinesterases averaged a little steeper ($\bar{x} = 4.6$ vs 3.8, $P > 0.05$, paired t test) but were less uniform (CV = 43.7 vs 10.0) for bobwhites than for doves (cf. Tables 2 and 3).

TABLE 3

SINGLE-DOSE ORAL TOXICITY OF FIVE GRANULAR FORMULATIONS OF ANTICHOLINESTERASE INSECTICIDE TO ADULT RINGED TURTLEDOVES (POOLED SEXES)

Formulation	Doses/birds[a]	LD_{50}[b]	95% CI	Slope[c]	SE
Thiophosphate					
Dasanit 15G	6/8	2.1	1.6–2.6	3.9	1.0
Lorsban 15G	5/8	157	123–200	4.4	1.2
Parathion 10G	5/10	12	9–16	3.6	0.8
Dithiophosphate					
Thimet 15G	4/8	17	9–34	3.4	2.1
Methyl carbamate					
Furadan 10G	5/8	7	5–9	3.7	1.0

[a] Doses/birds: No. of doses tested/mean birds per dose.
[b] LD_{50}: Mg active ingredient per kg body wt in a single oral dose calculated to kill 50% of the test population.
[c] Slope: Probit on log dose.

Ringed turtledoves responded to granular anticholinesterases virtually as described for bobwhites. Again, carbofuran toxicity ran its course within 24 hr, 90% of all organophosphate-mediated deaths occurred within 24 hr, and all deaths after 24 hr were from parathion (48 hr) and chlorpyrifos (120 hr). Only three doves, all on chlorpyrifos, showed toxic signs beyond 48 hr. Overt signs of anticholinesterase poisoning were similar for doves and bobwhites except doves sometimes regurgitated within 10 to 15 min after exposure to both organophosphates and the carbamate.

Granular Insecticides and Hazard

Method and rate of application, systemic properties, stability under different physical conditions, and toxicity of the active ingredient to selected vertebrates are among the factors evaluated before an insecticide is registered for a particular use. Comparative tests of various formulations with nontarget species are not usually part of this evaluation; therefore, hazard assessment is essentially a mathematical exercise based on the assumption that important toxicological differences are simply a function of rate of application. Although none of the granulars tested in the present study were more toxic than their technical grade active ingredient, mathematical manipulations without experimental validation seem inadequate because neither slope of dose–response lines nor toxic rankings of granulars were consistently related to their technical base. Conclusions about either variable are therefore tentative because tests were not replicated. However, because all tests were of anticholinesterases and most were of similar toxicity, i.e., $LD_{50} < 15$ mg/kg, therein lies a common bond of conservatism, and if compounds eliciting toxicity through alternative mechanisms had been included, the differences between technical and granular forms may have been even more erratic or pronounced.

Acute hazard evaluations are often made without regard for dose–response lines. This critical omission may have been simply because most approximate methods of LD_{50} determination do not provide an estimation of slope (e.g., Thompson, 1947; Weil, 1952), or because desired effect levels, e.g., LD_{01}, are in the nonlinear region of the dose–response curve. As an alternative, it has become popular to use some fraction of the LD_{50}, say $\frac{1}{10}$, to denote a particular level of hazard associated with potential exposure. Suppose the desired level of $\frac{1}{10}LD_{50}$ was intended to coincide with the LD_{01}, a reference value often used to denote hazard (but of limited statistical precision), then the corresponding dose–response slope would approximate 2.3 which is less than 30 of 31 slope estimates in the present study. Our average bobwhite LD_{01} values were 36 and 39% of the LD_{50} for technical and granular forms. For the steepest slopes (bendiocarb and G-fonofos), the LD_{01} values were 52 and 57% of the LD_{50}, and for the shallowest (carbofuran and G-fenamiphos), the LD_{01} values were 5 and 12% of the LD_{50}. Thus the $\frac{1}{10}LD_{50}$ was generally a much more conservative criterion for hazard assessment than was the calculated LD_{01}. This concept is illustrated for bendiocarb and carbofuran in Fig. 1.

Whereas the $\frac{1}{10}LD_{50}$ can be approximated from tests of as few as six to eight animals, a statistically acceptable ($\alpha = 0.05$) dose–response curve often requires over 50 animals. Although the curve has limited statistical value outside its linear range, it does have interpretive value in addition to approximation of the amount of insecticide necessary to proportionally change effect or estimation of reference values such as the LD_{01}. It can also provide information about rates of absorption,

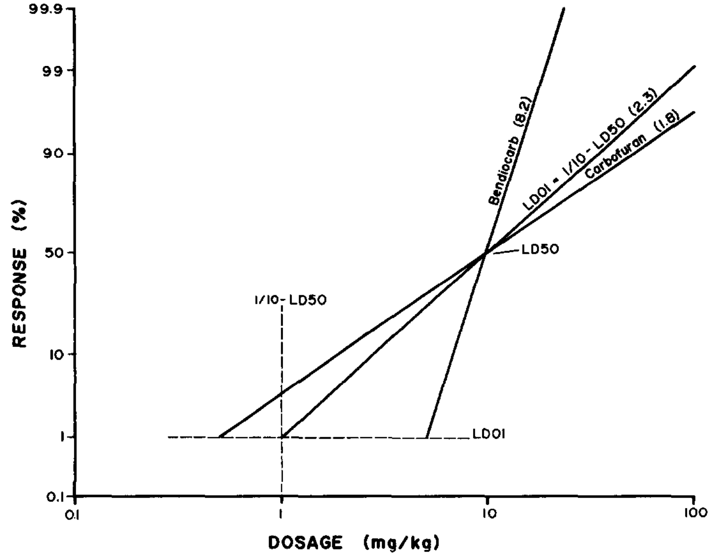

FIG. 1. Dose–response lines of bendiocarb and carbofuran for bobwhites and a hypothetical line constructed where the LD_{01} and $\frac{1}{10}LD_{50}$ coincide (slopes are shown in parentheses). LD_{50} values are arbitrarily corrected to 10 mg/kg. The "buffer" and "hazard" zones depict the relation between $\frac{1}{10}LD_{50}$ and experimental estimations of effect levels.

metabolism and excretion, mechanism of action of the substance, sex, population and species differences, and homogeneity of the test population (Klaassen and Doull, 1980; Loomis, 1978).

In a practical sense, the steepness of the dose–response curve can be reduced to a qualitative hazard index based on the ratio between some constant LD value, e.g., LD_{01} or LD_{16}, and the LD_{50}. The smaller the ratio, the more hazardous the substance because it takes proportionally smaller amounts to increase effect, and thereby reduces the acceptable margin of error associated with an environmental application of the pesticide. The $\frac{1}{10}LD_{50}$ criterion protects against this source of hazard because the steeper the slope, the larger the difference between a given LD value and the $\frac{1}{10}LD_{50}$ (Fig. 1). In contrast, compounds eliciting shallow slopes have a greater inherent safety because it takes proportionally more chemical to increase effect. However, low levels of these compounds may not be without associated hazard because they may induce effects below the $\frac{1}{10}LD_{50}$. Carbofuran is such a compound as its $\frac{1}{10}LD_{50}$ for bobwhites extrapolated to LD_{04}. The lowest dose tested was 3.0 mg/kg which corresponded with the extrapolated LD_{14} but actually killed 20%. Agricultural applications of both flowable and granular carbofuran have been implicated in large-scale bird kills (Flickinger *et al.*, 1980).

The distinctive hazard to wildlife from granular pesticides seems to be ingestion of intact granules, and is therefore a function of interaction between species sensitivity, body weight, and granule weight. However, many species of birds may not be able to tolerate ingestion of even one granule depending on the insecticide and amount of active ingredient. Thus, hazard associated with granulars may be more dependent on which species (and their feeding behavior) inhabit a treated area than on actual application rate. For example, various seed eaters such as small doves (BW = 100–130 g), rails (BW = 65–90 g), and sparrows (BW = 15–35 g) all use habitats that could be treated with any of the granulars tested in our study. Assuming sensitivity similar to adult bobwhite, ingestion of a single granule would be clearly life threatening for only Temik 15G. Extrapolation from the bobwhite dose–response curve showed a single granule containing 90 μg of aldicarb corresponds with the LD_{06}, LD_{14}, and LD_{70} for birds weighing 115, 80, and 25 g, respectively. Birds of similar sensitivity weighing over 100 g would probably not be affected by a single granule of any of the granulars tested. But, because of different granule sizes and toxicities, ingestion of fewer than five granules could be lethal to significant numbers (e.g., 50%, of sparrow-sized birds), i.e., Dasanit 15G, Diazinon 14G, Dyfonate 20G, Furadan 15G, Nemacur 15G, and Temik 15G. Size-related susceptibility to granular insecticides may even be exacerbated in juveniles compared with adults because of incomplete development of various detoxicating systems, protective barriers, or excretory processes. A final question regarding hazard is whether granules are randomly, i.e., accidentally, or selectively ingested. If ingestion is random, then application rate is critical; but if it is selective, then extreme caution must be exercised wherever the granular formulations are used. Of course the entire question of hazard is a function of availability, which, in turn, may vary according to method of application, e.g., drilling vs broadcast application, etc.

CONCLUSIONS

This series of single-dose experiments with adult bobwhites provided a direct comparison of the acute oral toxicity (LD_{50}) of 13 granular formulations of insecticides with a single stock of bobwhites. Information was also acquired on dose–response relations and their use in hazard assessment, toxicologic relations between granular formulations and their technical grade active ingredient, granule size and hazard, response patterns and recovery, and species differences. Although bobwhite was the principal test species, the following conclusions regarding anticholinesterase substances should have generalized application to gallinaceans and other predominantly seed-eating birds.

1. Our two-step dosing scheme, i.e., 2-day lag between administration of the first three and final two dose levels, and use of an average bird weight as the basis for dosing were efficient methods of achieving our defined experimental objectives.
2. The organophosphates, fenamiphos (Nemacur 15G) and fensulfothion (Dasanit 15G), and the carbamate, aldicarb (Temik 15G), were clearly the most toxic of 13 anticholinesterase insecticides administered orally to adult bobwhites.
3. Of 13 anticholinesterases tested, the granular formulation and its technical grade active ingredient were of equivalent toxicity five times and the granular was significantly less toxic than technical eight times. Therefore, alternative granular formulations of the insecticides reported herein and other granular anticholinesterases will probably also be of equivalent or lesser toxicity than their technical grade active ingredient. This generalization cannot be projected to granulars eliciting toxicity via other modes of action or to flowables or other liquid formulations of anticholinesterases.
4. The dose–response slope added an additional dimension to hazard assessment by providing a basis for denoting compounds requiring extreme caution against "over-treatment" and those likely to produce effects at doses below the $\frac{1}{10}LD_{50}$.

5. Of the 13 granulars tested, ingestion of a single granule would be clearly life threatening to birds of bobwhite size (assuming a similar order of sensitivity as bobwhite) for only Temik 15G. But ingestion of fewer than five granules could be lethal to sparrow-sized birds for Dasanit 15G, Diazinon 14G, Dyfonate 20G, Furadan 15G, and Nemacur 15G.

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