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Effects of Simulated Field Spraying of Carbofuran, Carbaryl and Dimethoate on Pheasant and Partridge Chicks

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Insecticides (carbofuran, carbaryl, dimethoate and others) sprayed to control grasshoppers in Alberta during 1984 and 1985 reportedly killed game birds, gulls and passerines. This sparked a concern about the subsequent widespread use of these agents for controlling grasshoppers in or near wildlife habitats. Anticholinesterase insecticides are potentially lethal to wild avian species (Schaefer et al., 1983; Hudson et al., 1984). These insecticides manifest their toxicity by specifically inhibiting acetylcholinesterase (AChE) with consequent impairment of related neuronal functions. A depression in brain AChE activity in birds can indicate exposure to toxic concentrations of these compounds (Hill, 1988). Although field studies to evaluate the impact of insecticide spraying on wild avian species would be preferred, their implementation is costly and data inadequacies are common (Kenaga, 1973).

Because no field data were available to answer the concern over alleged mortality of birds in Alberta, the current study was designed to simulate a worst-case field spraying of insecticides. This report is the only recent study on the simulation of field conditions involving the exposure of game birds to grasshopper control agents. Upland gamebirds were selected for research on field level effects of insecticide spraying because they inhabit agricultural areas where insecticides were used and birds were readily available.

The objectives of the present study were to assess the effects of single and repeat simulated field spraying of carbofuran, carbaryl and dimethoate onto pheasant (Phasianus colchicus) and chukar partridge (Alectoris graeca) chicks.

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MATERIALS AND METHODS

Female pheasant chicks were obtained from Fish and Wildlife Division, Alberta Forestry, Lands and Wildlife (Brooks Wildlife Centre). Chukar partridge chicks of both sexes were purchased from a commercial producer (Black River Game Farm, Pefferlaw, Ontario). Chicks of both species were reared in 3 m x 4 m brooder houses divided into 1.5 m x 2 m pens. A 28% protein gamebird starter ration custom formulated by the Wildlife Centre and water were supplied ad libitum to all birds.

Following hatching, 368 day-old pheasant chicks were weighed and randomly distributed among 4 replicate groups for each of 4 treatments. After acclimation in the pens for 3 d, the surviving chicks were weighed and sprayed with insecticides on d 4. On d 11, the chicks were again weighed and sprayed with insecticides. Mortality and weight gain of chicks were recorded for 3 d following each spraying treatment. The weight gain data for the second spraying included only 3 replicates per treatment because all the birds in one replicate of the carbaryl treatment died following harassment by a hawk during transportation to the spraying site. The pathology diagnosis indicated that death was due to trauma, hysteria and asphyxiation.

Chukar partridge chicks were sprayed with insecticides only once. Day-old chicks were weighed and randomly distributed among 4 treatments within each of 3 replicate groups (14 to 17 chukar chicks per replicate per treatment). Following acclimation for 3 d, the surviving chicks were weighed and sprayed with insecticides on d 4. Mortality and weight gain were recorded for 3 d after spraying. The experimental design for each study was a randomized complete-block design (Steel and Torrie, 1960) with each block (brooder house) containing 1 replicate of the water-sprayed control and 3 insecticide treatment groups.

Commercial formulations of carbofuran (Chemagro Ltd.), carbaryl (Union Carbide Ltd.) and dimethoate (Cyanamid Ltd.) contained 480 g active ingredient (a.i.)/L. For spraying, the insecticide formulations were mixed with water to obtain the following application rates: carbofuran, 132 g a.i. in 100 L/ha; carbaryl, 1200 g a.i. in 45 L/ha; and dimethoate, 432 g a.i. in 45 L/ha. These rates were the maximum permitted for grasshopper control in Alberta (Dolinski, 1986).

Control birds were sprayed with water at a rate of 100 L/ha. A CO_2- pressurized (300 KPa) back-pack sprayer with a 4-nozzle spray boom was used for spraying. The spray volume was altered by changing the nozzles. Chicks were confined in circular wire cages (37 cm in diameter x 15 cm high) for spraying. At 1 hr and 3 d after spraying in each trial, 1 bird from each replicate per treatment was killed by cervical dislocation. Brain tissues were removed and immediately frozen in polypropylene vials and stored at -50°C until processed and analyzed for AChE. The

carcasses were frozen at -30°C for insecticide residue analysis.

For AChE analysis, the frozen brain samples were thawed and any adhering blood was removed. The tissues were minced and homogenized with 20 volumes (w/v) of 0.1 M Tris-HCl buffer, pH 7.2, containing 1% Triton X-100, using a Polytron Homogenizer (Brinkman). The homogenates were centrifuged at 15000 x g for 30 min and the clear supernatants were collected for AChE analysis. All the above operations were carried out at 0-4°C. AChE activity was measured according to Ellman et al. (1961) and expressed as units per mg protein. Protein was estimated according to Lowry et al. (1951) after precipitation with 10% trichloroacetic acid. Weight gain and AChE data were analyzed by the SPSS* procedure for ANOVA (Nie, 1983).

Insecticide residue analyses consisted of first thawing and extracting surface residues of insecticide by ultrasound agitation of the carcass in methanol for 15 min. The plastic bag which contained the carcass was rinsed with methanol and this material was included in the sample. The extracts were analyzed for insecticide residues using a Hewlett-Packard (HP) 5940 gas chromatograph equipped with a 30 m fused silica DB-5 capillary column and a HP 5970B Mass Selective Detector with malathion as the internal standard. The insecticides were detected using single ion monitoring mode. The selected ions for target compounds were 164 and 149 for carbofuran, 144 and 115 for carbaryl, and 87 and 125 for dimethoate. The detection limit was 1 µg total extractable pesticide based on a final volume of 10 mL. Average recoveries of carbofuran, carbaryl and dimethoate from carcasses spiked with 10 µg of each insecticide were 84.8, 84.5, and 82.0%, respectively.

RESULTS AND DISCUSSION

Spraying carbofuran, carbaryl or dimethoate onto pheasant and chukar partridge chicks at 4 d of age produced no effect ($p>0.05$) on weight gain of treated birds as compared to controls (Table 1). A second spraying of these insecticides on pheasants (d 11) also failed to produce any effect ($p>0.05$) on weight gain in the subsequent 3 d period. There were no significant ($p>0.05$) differences between treatments in bird mortality of either species. One chick died in the control group and 1 chick died in the carbaryl treatment group in the pheasant trial, and 1 chick died in the control group in the chukar trial after spraying at d 4.

The brain AChE activities of control and insecticide treated birds were also not significantly different ($p>0.05$) at any time during the study (Table 2). Inhibition of brain AChE activity by the applied insecticides did not approach >20%, a toxicity criterion proposed by Ludke et al. (1975). It is evident that at the concentrations used the insecticides were either efficiently detoxified by the birds or remained on the external body surface and did not penetrate the body to affect brain AChE

Table 1. Mean weight gain (± 1 SD) of pheasant and chukar partridge chicks in 3 d after spraying with water (control) or insecticides at 4 or 11 d of age.

	Spray Treatment[a]			
Bird Group	Control	Carbofuran	Carbaryl	Dimethoate
Pheasant[b]				
d 4	13.4 ± 0.2 (92)[c]	13.6 ± 0.3 (92)	14.2 ± 0.2 (90)	13.7 ± 0.2 (92)
d 11	14.4 ± 2.3 (60)	15.2 ± 2.3 (60)	15.2 ± 2.0 (59)	14.2 ± 2.5 (60)
Chukar				
d 4	8.3 ± 1.8 (38)	8.8 ± 1.6 (47)	8.0 ± 1.7 (41)	8.7 ± 1.6 (50)

[a]Treatment means for weight gain within a row are not different ($p>0.05$).
[b]Pheasants sprayed on d 4 were again sprayed on d 11 of age.
[c]No. of birds per treatment in parenthesis.

Table 2. Mean (± 1 SD) AChE activity in brain tissue of pheasant and chukar partridge chicks 1 hr and 3 d after insecticide spraying at 4 or 11 d of age.

	AChE Activity (Units/mg protein)[a,b,c]			
Bird Group	Control	Carbofuran	Carbaryl	Dimethoate
Pheasant				
d 4	0.190±0.290	0.215±0.009	0.201±0.005	0.202±0.018
d 7	0.217±0.009	0.178±0.017	0.218±0.008	0.195±0.008
d 11	0.182±0.015	0.156±0.016	0.172±0.004	0.159±0.014
d 14	0.190±0.012	0.180±0.003	0.187±0.025	0.178±0.017
Chukar				
d 4	0.196±0.027	0.187±0.013	0.194±0.005	NS[d]
d 7	0.158±0.001	0.157±0.015	0.178±0.014	0.181±0.015

[a]4 pheasant samples per insecticide treatment per age class, except only 3 pheasant samples for carbaryl at d 11 and 14, and 3 chukar samples.
[b]1 Unit = 1 µmole acetylthiocholine hydrolyzed per min @ 25°C.
[c]Treatment means within age groups are not different ($p>0.05$).
[d]NS = no sample for dimethoate.

activity. Because AChE activity undergoes reversible inhibition with carbamate compounds (Ludke et al., 1975), a lack of inhibiting effects could also be attributed to spontaneous reactivation of the inhibited AChE (Hill and Fleming, 1982).

Comparison of the surface recovered insecticide residues suggests that the birds received high concentrations of the applied insecticides (Table 3). Free ranging wild birds in field habitats would not be exposed to the same degree of spraying as in this study because they would be able to seek cover when insecticides were sprayed or vacate an area being sprayed. The mean values of the recovered residues showed that about 75%, 55% and 70% of carbofuran, carbaryl and dimethoate respectively, had been lost or degraded after 3 d (Table 3). Thus, after 1 week when the 4-d old pheasant chicks were again sprayed (d 11), much of the original concentration of the applied insecticides would have been lost.

Table 3. Mean (± 1 SD) insecticide residues recovered from pheasant and chukar partridge exteriors 1 hr and 3 d after spraying at 4 or 11 d of age.

	Insecticide Residue (µg/bird)[a]		
Bird Group	Carbofuran	Carbaryl	Dimethoate
Pheasant[b]			
d 4	5.6 ± 4.0	6.2 ± 3.3	63.4 ± 46.7
d 7	0.9 ± 0.8	0.7 ± 1.3	6.1 ± 3.2
d 11	23.1 ± 7.2	17.5 ± 7.9	32.9 ± 17.8
d 14	3.8 ± 2.1	4.3 ± 2.0	14.1 ± 12.9
Chukar			
d 4	9.2 ± 2.3	79.1 ± 30.4	NS[c]
d 7	2.1 ± 0.9	11.7 ± 6.5	7.7 ± 4.8

[a]3 samples per insecticide treatment per bird group.
[b]Pheasants sprayed on d 4 were again sprayed on d 11 of age.
[c]NS = no sample for dimethoate.

Few reports are available on the effects of spraying pheasants with insecticides. Zorb and Fouch (1966) reported no effect when adult pheasants were sprayed with malathion and guthion at 1.12, 4.5 and 11.2 kg/ha. Diazinon sprayed at 1.12 kg/ha had no effect on pheasants; however, 3.4 and 5.6 kg/ha produced up to 40% mortality and caused sickness and immobilization of adult birds (Zorb and Fouch, 1966). Black and Zorb (1965) found no effect on pheasants when the birds and the food in pens were sprayed with 1.12, 4.5 and 11.2 kg/ha of malathion. Hill (1979)

dusted Japanese quail (Coturnix japonica) with a 5% carbaryl powder and reported that topical doses of about 150 µg/g body weight had no effect on brain AChE activity and did not induce toxicity symptoms. The lack of effects from the carbaryl spraying in the present study is consistent with the findings for Japanese quail (Hill, 1979).

Carbofuran, carbaryl and dimethoate must be either absorbed, inhaled or ingested if any toxicity is to be manifested. When insecticides were sprayed, 4-d old pheasants and chukar partridges, and 11-d old pheasants averaged 26.2, 17.1 and 57.8 g body weight. The amount (µg) of insecticide recovered per bird (Table 3) expressed as µg/g body weight indicated that total ingestion of the insecticide quantities deposited on the birds would not be acutely toxic. For example, Hudson et al. (1984) reported an LD_{50} value of 4.2 µg carbofuran/g body weight for pheasants 3 months old, but only 0.2 to 0.5 µg/g body weight was recovered 1 hr after spraying in this study. The LD_{50} values of carbaryl are >2000 and 1880 µg/g body weight for 3-4 months old pheasants and 4 months old chukar partridges, respectively (Hudson et al., 1984). These values are much greater than the 0.02 to 0.49 µg of carbaryl per g body weight recovered from pheasants or chukars 1 hr after spraying in this study. Dimethoate residues recovered 1 hr after spraying (range from 0.54 to 2.38 µg/g body weight) were 50 to 6 times lower than the 20.0 µg/g body weight LD_{50} for pheasants 3-4 months old (Hudson et al., 1984).

Young of a species are often more sensitive to toxicants than older animals (Hudson et al., 1984) and differences in age of birds in this study compared to others (Black and Zorb, 1965; Zorb and Fouch, 1966; Hudson et al., 1984) may be important. The absence of any mortality and significant effects on weight gain or depression of AChE activity indicated, however, that the topically applied insecticides in the present study were not assimilated by the birds in toxic quantities. Thus, the absence of effects seen in this study suggests that the acute toxicity of carbofuran, carbaryl and dimethoate to pheasant and chukar partridge chicks in the wild following a practical but worst-case spray application would be minimal. However, simulation of oral toxicities of insecticide-contaminated feedstuffs ingested after a gamebird habitat has been sprayed needs evaluation. This route of exposure in the field would probably be more important than direct topical application.

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