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# Simulated Field Ingestion of Carbofuran-Contaminated Feedstuffs by Pheasants

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Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is used for grasshopper control in Alberta habitats occupied by pheasants (Phasianus colchicus), and is acutely lethal to pheasants (Hudson et al., 1984). Acute $LD_{50}$ toxicity tests may not be representative of the exposure experienced by birds in the wild. The toxic potential of insecticides to birds in the field will be affected by the residual concentrations, the degradation of residues over time (Hoerger et al., 1972; Kuhr and Dorough, 1976), the ability of birds to detect and avoid contaminated feedstuffs (Kononen et al., 1987) and animal weight and intake (Kenaga, 1973).

This study was designed to simulate a worst-case field scenario where all feedstuffs ingested by pheasants would be contaminated with carbofuran. Pheasant chicks were orally administered declining doses of carbofuran. Juvenile pheasants were confined in outdoor pens where all feedstuffs available were sprayed with carbofuran.

## MATERIALS AND METHODS

One hundred and forty female pheasant chicks were obtained at hatch from stock of the Fish and Wildlife Division of Alberta Forestry Lands and Wildlife, Brooks Wildlife Centre. Chicks were reared for 13 d in a 3 x 4 m brooder house maintained at 31 ± 2°C. Birds were supplied water and a 28% protein starter ration ad libitum. Constant lighting was provided by heat lamps. All chicks were weighed and numbered with a wing band when 13 d old. Ninety chicks (mean body wt 63.7 ± 7.7 g) were then randomly assigned to three carbofuran treatment groups (N=30). Each treatment group consisted of three replicates of 10 birds. Husbandry conditions for the subsequent 6 d exposure period were as described above, except feed was removed 10 hr (2200-0800 hr) prior to orally dosing the birds so that crop contents would not interfere with insertion of the gavage needle.

Two dosage regimes with declining concentrations of carbofuran

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were evaluated in the pheasant chick trial. A commercial carbofuran flowable formulation (FURADAN® 480, Chemagro Ltd.) mixed in tap water was used to prepare daily oral doses. Chicks received six doses of carbofuran over 6 days. In treatment group 1 (expected field dose), chicks were dosed sequentially on two consecutive days with 30, 20 and 10 µg active ingredient (a.i.) carbofuran; in treatment group 2 (maximum challenge dose), the chicks were dosed sequentially on two consecutive days with 60, 40 and 20 µg a.i. Doses were intubated into the crop of each chick with 0.2 mL of tap water. Birds in the control group were administered 0.2 mL of tap water. Birds were observed for 2 hr after dosing each day, and once every 3 hr during the day for signs of intoxication. Each bird's weight was recorded following the 6 d exposure period and after a 4 d post-exposure period. Whole brain samples were collected from chicks that died after dosing, and from two birds from each replicate after the 4 d post-exposure period and immediately frozen in liquid nitrogen. Feed consumption was recorded daily.

In the juvenile pheasant trial, 75 females (mean body wt 321.8 ± 38.4 g) 5 weeks old were moved from indoor rearing pens to three enclosed outdoor wire pens (4.5 x 6.0 x 3.0 m high) with attached brooder houses and conditioned to ground-feeding for 10 d. A grain mixture of 40% cracked corn, 40% cracked wheat and 20% millet was scattered on the ground in each pen and water was supplied by automatic water fountains. For the first 4 d, the birds were outside during the day but housed indoors at night; however, birds were outside all day thereafter. Average ambient temperatures during this period were: low 8.5°C; high 24.0°C. Total precipitation was 22 mm and the natural light was ~ 18 hr light and ~ 6 hr dark.

On d 10, each bird was weighed (mean body wt 382.2 ± 43.8 g) and numbered with a wing band. Sixty birds (body wt range 355–465 g) were selected and randomly assigned to three replicates of 10 birds for each of a control and a carbofuran treatment. Each replicate group was confined in a 4.5 x 6.0 x 3.0 m high wire pen. Pens were separated by a 0.75 m wood partition. Natural vegetation in the pens was predominately crested wheat grass (Agropyron cristatum). One water fountain and 2 kg of the grain mixture were placed in each pen. Birds were acclimatized for 3 d in the exposure pens.

On d 1 of the exposure period the birds (7 weeks old) were weighed and 3.5 kg of the grain mixture used for conditioning was broadcast on the ground in each of the six pens. The carbofuran treatment pens were sprayed using a 3-nozzle spray boom (275 kPa) with carbofuran at a rate equivalent to 132 g a.i./ha in 100 L water/ha. Control pens were sprayed with water at 100 L/ha. The birds, the vegetation, any insects and the supplemental grain mixture were sprayed. One water fountain was put in each pen after spraying. Each bird's weight was recorded after 5 d. All birds were observed constantly for the first

4 hr after spraying and twice each day thereafter. Average ambient temperatures during the 5 d exposure period were: low 11°C; high 24°C. Total precipitation was 0.3 mm, and lighting was natural as described before.

A paper tray containing 15 g of the grain mixture was placed in each pen before spraying to provide samples for monitoring degradation of carbofuran. The trays were placed in an adjacent pen with no birds for 5 d. A 5 g sample from each tray was taken 2 hr and 5 d after spraying and stored at -30°C for carbofuran residue analysis.

At the end of the 5 d exposure period, six birds from each treatment group (two per replicate) were killed by cervical dislocation. Crop and gizzard contents were collected and stored at -30°C for carbofuran residue analysis. Whole brain samples were taken and immediately frozen in liquid nitrogen. The remaining birds were given a 24% protein grower ration ad libitum, and 2 kg of the "clean" grain mixture was scattered on the ground in each pen. After a 3 d post-exposure period, birds were again weighed. Average ambient temperatures were: low 11°C; high 24°C. Precipitation was 6.3 mm.

Carbofuran residues were extracted by ultrasound agitation of the samples for 15 min in methanol. Chromatographic analysis of the extracts was as described by Somers et al. (1991). For acetylcholinesterase (AChE) analysis, the brain samples while frozen were minced and immediately homogenized and processed in cold (0-4°C), as described previously (Somers et al., 1991). AChE activity was measured according to Ellman et al. (1961) and expressed as units per mg protein. Protein was measured according to Lowry et al. (1951) after precipitation with 10% trichloroacetic acid. Although it is difficult to rule out any changes (e.g. reactivation) in carbofuran-inhibited AChE activity in storage and/or processing, studies in this laboratory (unpublished) with one-half of the same brain samples have shown no difference in AChE inhibition in extracts prepared from tissues analyzed either immediately after collection or after storage at -50°C for 2 weeks.

Both trials were designed as randomized complete-block experiments (Steel and Torrie, 1960). Statistical analyses used the general linear model for 2-way analysis of variance and a Least Significant Difference means test ($p<0.05$) (SAS, 1985).

## RESULTS AND DISCUSSION

Two carbofuran dosing regimes administered to pheasant chicks to simulate the decline of residues on natural feedstuffs had no effect ($p>0.05$) on weight gain or feed conversion (g feed:g weight gain) during the 6 d exposure period (Table 1). "Boss" birds tend to consume more feed than timid birds, particularly with a restricted feeding program (Summers and Leeson, 1976).

Thus, limiting the daily feed available probably contributed to the variation in the weight gains and feed:gain ratios (Table 1) of treatment groups. Restricting feed, however, facilitated the gavage procedure, and gavaging ensured that all birds received the specified carbofuran dose each day.

Table 1. Mean (± SD) response of pheasant chicks administered declining oral doses of carbofuran simulating 6 d of field ingestion and during a 4 d post-exposure period.

| | Carbofuran Oral Dose Regime | | |
|---|---|---|---|
| Variate | Control[a] | Expected field[b] | Maximum challenge[c] |
| Initial wt (g) | 63.3± 7.9 (N=30) | 64.0±7.8 (30) | 63.2± 7.7 (30) |
| Exposure period | | | |
| Wt gain (g) | 18.4±13.6 (30) | 21.8±6.3 (30) | 18.5±11.1 (29) |
| Feed:gain | 4.3± 2.4 ( 3) | 2.9±0.6 ( 3) | 3.7± 1.9 ( 3) |
| Mortality (%) | 0 | 0 | 3.3 |
| Post-exposure period | | | |
| Wt gain (g)[d] | 36.8± 8.1[x] (30) | 41.9±7.3[y] (30) | 37.8± 7.1[x] (29) |
| Feed:gain | 2.2± 0.1 ( 3) | 2.0±0.1 ( 3) | 2.2± 0.1 ( 3) |
| Mortality (%) | 0 | 0 | 0 |
| AChE (unit/mg protein)[e] | 0.228±0.017 (6) | 0.232±0.023 (6) | 0.231±0.021 (6) |

[a] Control birds were administered 0.2 mL tap water/d.
[b] Each 2 d dose was 30, 20, and 10 µg carbofuran a.i./bird.
[c] Each 2 d dose was 60, 40, and 20 µg carbofuran a.i./bird.
[d] Means with different superscripts are different ($p < 0.05$).
[e] 1 unit=1 µmole acetylthiocholine hydrolyzed per min @ 25°C.

One bird (42.5 g) died within 10 min of dosing with 60 µg carbofuran (1.41 µg/g) on exposure d 1. AChE activity in brain tissue was 0.188 units/mg protein, or 17.5% less than in control birds. Three other birds of this treatment group weighing 55.5, 57.1 and 60.8 g were immobilized within 10 min and comatose after 15 min on d 1 but did not die. Signs of intoxication included lethargy, nutation, wing-drop, loss of righting-reflex, paralysis, opisthotonos and coma. These three birds which received an average of 1.04 µg carbofuran/g body weight were in remission within 35 min of entering coma. Thus, 1 µg carbofuran/g body weight appears to be a critical dose

for the 14-d old pheasant. No signs of intoxication were induced in these three birds after dosing with 60 µg carbofuran on d 2. No gross effects on behavior were observed in other birds dosed with 60 µg carbofuran on exposure d 1 or d 2. None of the other daily doses of carbofuran affected behavior. Observed recovery on d 1, and the lack of effects of the 60 µg carbofuran dose on d 2, agrees with reports by Hicks et al. (1970) and Hudson et al. (1984) that indicated no cumulative effects of carbofuran.

A significant ($p<0.05$) weight gain by birds in the expected field dose regime treatment during the 4 d post-exposure period (Table 1) was probably not dose related. Feed:gain ratios of about 2:1 (Table 1) were comparable with known feeding efficiencies for pheasants of this age consuming a commercial starter ration (Summers and Leeson, 1976). No mortality occurred in the post-exposure period (Table 1). AChE activity in brain tissues from the control and carbofuran treatments (Table 1) was the same ($p>0.05$) at the end of the post-exposure period. These post-exposure observations also suggest no cumulative effects of carbofuran on pheasants.

Consumption of carbofuran-sprayed feedstuffs by juvenile pheasants for 5 d had no significant effect ($p>0.05$) on weight gain, mortality, or brain AChE activity (Table 2). No signs of

Table 2. Mean (± SD) response of juvenile pheasants ingesting feedstuffs for 5 d in pens sprayed with carbofuran to simulate field ingestion of contaminated feedstuffs.

| Variate | Control[a,b] | Carbofuran[b] (132 g a.i./ha) |
|---|---|---|
| Initial wt (g) | 413.3 ± 24.2 (N=30) | 409.5 ± 29.9 (30) |
| Exposure period | | |
| Wt gain (g) | 25.2 ± 13.2 (30) | 20.7 ± 10.3 (30) |
| Mortality (%) | 0 | 0 |
| AChE (units/mg protein)[c] | 0.312 ± 0.065 ( 6) | 0.273 ± 0.022 ( 6) |
| Post-Exposure Period | | |
| Wt gain (g) | 37.4 ± 20.2 (24) | 38.1 ± 20.8 (24) |
| Mortality (%) | 0 | 0 |

[a] Control pens were sprayed with water @ rate of 100 L/ha.
[b] Treatment means for each variate are not different ($p>0.05$).
[c] 1 unit=1 µmole acetylthiocholine hydrolyzed per min @ 25°C.

intoxication or overt differences in behavior were displayed by birds on the carbofuran treatment. On exposure d 1, carbofuran residues in 3 grain samples averaged 3.55 ± 2.77 µg carbofuran/g, but declined to 1.39 ± 0.75 µg during 5 d under ambient conditions. However, <0.1 µg carbofuran was detected per g of crop-gizzard content sampled after 5 d exposure. Physiological degradation after ingestion may be responsible for greatly reduced amounts of carbofuran in crop-gizzard contents. Weight gain of birds in the control and carbofuran treatments (Table 2) were comparable ($p>0.05$) during 4 d post-exposure, and no birds died. Birds in both treatment groups gained more weight than during the exposure period (Table 2), probably because commercial feed was available.

Rapid environmental disappearance of carbofuran (Hoerger and Kenaga, 1972; Kuhr and Dorough, 1976) will mitigate adverse effects on wildlife. Reviewing data provided by Kuhr and Dorough (1976) and NRC (1979) indicates that the 33% decrease in doses every 2 d used in this pheasant chick study would be realistic. In addition to environmental degradation in the field, the daily dose of carbofuran will be limited by feed intake. For example, it was assumed for the chick study that a 14-d old pheasant would consume 15 g of feed daily (Summers and Leeson, 1976; Hill and Camardese, 1981) and all feedstuffs would contain 2 µg carbofuran/g. That expected field dose of 30 µg carbofuran was not toxic to 14-d old pheasants. The maximum challenge carbofuran dose (60 µg) regime would require ingestion of 30 g of feed/bird/d, or about 45% of body weight for a 14-d old pheasant. Irrespective of the fact that this much feed would not be consumed daily, gavaging chicks with the declining carbofuran doses of the 60 µg regime induced limited toxicity.

Carbofuran (3.55 µg/g) recovered from grain sprayed in the juvenile pheasant trial suggests that the 2 µg/g estimate used for gavaging in the chick trial was too low. A calculated daily dose, using 15 g feed/d contaminated with 3.55 µg carbofuran/g (53.3 µg carbofuran/bird/d), would be equivalent to ~0.8 µg carbofuran/g body weight for a 14-d old pheasant. Chicks in this study were not affected by carbofuran when the dose was <1.0 µg/g body weight. Thus the two dosing regimes used in the chick trial to simulate field ingestion and degradation of carbofuran appear to encompass doses to be expected from spraying carbofuran in the field.

Feed consumed by juvenile pheasants in pens sprayed to simulate field ingestion of carbofuran could not be determined. Using feed intake data from Summers and Leeson (1976), ingestion of 40 g of feedstuffs/d contaminated with 3.55 µg carbofuran/g would be equivalent to ~142 µg/bird/d or ~0.35 µg/g body weight, which was much lower than the $LD_{50}$ of 4.12 µg/g body weight for 3-4 month old pheasants reported by Hudson et al. (1984). The maximum daily dose in the present study would

subsequently decline, as evidenced by the degradation of carbofuran on grain samples over the 5 d test period. Young birds are more sensitive to toxicants (Hill and Camardese, 1981; Hudson et al., 1984), so the lack of effects on juvenile pheasants in this simulation study is not surprising considering that 1 µg carbofuran/g body weight was not toxic to the 14-d old chicks.

Juvenile pheasants in this study and wild pheasant chicks would consume small portions of contaminated feed during the day, rather than a single dose as in this chick study. This feeding behavior and the rapid metabolism of ingested carbofuran (Hicks et al., 1970; Westlake et al., 1981) would reduce carbofuran toxicity in the field. Finally, unlike the 14-d old and juvenile pheasants in this study, free-ranging wild pheasants could move to habitats not sprayed with carbofuran. These factors, in addition to feed intake and environmental degradation of carbofuran, would serve to reduce the toxicity of carbofuran sprayed in natural habitats.

Indirect field consequences, such as a reduction of feed base, resulting from spraying carbofuran cannot be simulated. The present simulation of direct toxicity effects of carbofuran on pheasants suggests, however, that consumption of carbofuran-contaminated feed by 14-d old and juvenile pheasants in the field would not be toxic.

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