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Hazard evaluation for northern bobwhite quail (*Colinus virginianus*) exposed to chlorpyrifos-treated turf and seed

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Abstract

This study evaluated the toxicity effects of chlorpyrifos on bobwhite quail (*Colinus virginianus*) kept in 27 field-exposed large pens arranged over turf in a randomized block design with nine blocks of three pens (16 adult birds per pen). Nine pens were treated with one application of 3.4 kg active ingredient (ai) per hectare followed by a second 3.4-kg ai/ha application 2 weeks later, nine pens with one 6.7-kg ai/ha application, and nine pens with formulation blank. In addition, the seed fed to the birds in the two chemically treated pens was also treated with chlorpyrifos. Mean residue in the grass samples from the first 3.4-kg treatment pens ranged from 306 ± 95 ppm on day 0 to 18 ± 8 ppm on day 14 after treatment. The second 3.4-kg ai/ha treatment grass residues ranged from 361 ± 167 ppm on day 0 to 38 ± 24 ppm on day 14 after treatment. Grass residues from the 6.7-kg treatment pens ranged from 903 ± 310 ppm on day 0 to 9 ± 8 ppm on day 30 after treatment. Half-lives were ~ 2 days and 10 days for grass and seeds, respectively. Whereas the incidence of behavioral deficits was significantly ($P = 0.0156$) higher in the 6.7-kg pens (five females, one male), two of the females could have been the same bird because they were both seen in the same pen on days 23 and 24 after treatment. There was no significant difference in mortality, brain acetylcholinesterase activity, or any other measured parameter among any of the treatments. We conclude that application of chlorpyrifos to turf at 3.4 and 6.7 kg ai/ha is not expected to have chronic deleterious effects on populations of bobwhite quail grazing on treated grass or seeds, provided there is an abundant supply of seeds for the quail to eat.

Keywords: Chlorpyrifos; Pen study; Bobwhite quail; Pyrinex 4E; Turf

1. Introduction

Pyrinex 4E, which contains the active ingredient (ai) chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is used to control a variety of pests on turf as well as many crops (Makhteshim-Agan Inc., 1988). Dosages of Pyrinex 4E per 93 m^2 of turf range from 22 mL (11.1 kg ai/ha) to 89 mL (4.5 kg ai/ha). The 4E formulation contains 48.9% chlorpyrifos, equivalent to 1.2 kg ai/L. Since the anticipated maximal seasonal use rate of Pyrinex 4E is 6.7 kg ai/ha, this rate represents the greatest risk to turf-grazing birds and thus was used as the maximum application rate in this study.

Toxicity of chlorpyrifos to birds is moderate to high. Considering both the acute ($LD_{50} = 13.3$–32 mg/kg) and dietary toxicity ($LC_{50} = 283$–1100 mg/kg) to bobwhite quail, it appears that this bird is one of the more sensitive avian species that has been extensively evaluated (Rexrode, 1984; Smith, 1987; Hudson et al., 1984; Johnson et al., 2001). Because of its sensitivity to chlorpyrifos and also because it grazes on grass (Wiseman, 1977), the bobwhite quail was chosen as an indicator species for the turf study.

Field effects of chlorpyrifos on birds are not at all decisive. For example, there have been at least two reports from the Ecological Effects Branch of the US EPA (1981) of dead geese found on golf courses that had been treated with chlorpyrifos. Additionally, Hurlbert (1977) reported significant mortality of young mallard ducks following treatment with chlorpyrifos. However, Kenaga (1974) sprayed field-confined birds with 4–36 kg ai/ha with no apparent ill effects, whereas Booth et al. (1984) found no effects on waterfowl exposed to chlorpyrifos sprayed on winter wheat. Hence, conclusions about wildlife mortality in the field resulting from

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chlorpyrifos spraying are equivocal. For this reason, additional wildlife studies on this compound have been strongly recommended (Odenkirchen, 1988; Smith, 1987).

Of particular interest to the current study was the potential impact of chlorpyrifos on upland game birds exposed to a contaminated food source resulting from direct spraying of two food substrates. Dietary exposure of insecticides under field-exposed large-pen conditions using a large number of birds and a large number of replicates represents an opportunity to study the lethal and sublethal effects of agrochemicals on selected sensitive birds under controlled field conditions of maximum exposure (Booth et al., 1980a, b).

Accordingly, the objectives of the present study were to (1) monitor mortality effects of chlorpyrifos associated with dietary and environmental exposure to bobwhite quail under field-exposed large-pen enclosures over turf, (2) assess the potential of chlorpyrifos to induce behavioral deficits in quail foraging on treated turf; (3) investigate the residue profile of chlorpyrifos in treated grass and, if possible, relate these residues to bird mortality, behavioral deficits, and brain acetylcholinesterase (AChE) levels, and (4) determine whether chlorpyrifos induces body weight loss, reduced organ weight, brain AChE depression, and decreased egg production in surviving birds.

2. Materials and methods

2.1. Chemicals

Pyrinex 4E was supplied by Makhteshim Agan Inc. (Israel) via the Pennwalt Company (Philadelphia, PA), which shipped 11 L of the chemical (batch no. B23-11) directly to Brigham Young University (BYU; Provo, UT). This batch contained 48.9% of the ai chlorpyrifos. The vehicle control consisted of Pyrinex minus chlorpyrifos, which was also supplied by Makhteshim Agan Inc.

2.2. Test site

The study was conducted in Goshen, UT, at a turf farm located ~50 km west of Springville, UT.

2.3. Time table

The investigation was divided into three phases: (1) An acclimation period of 15 days for the birds began when they arrived on May 26, 1988. (2) A chemical application phase of 30 days began on June 11, 1988 for the 6.7-kg ai/ha treatment and the first 3.4-kg ai/ha treatment. The second 3.4-kg ai/ha treatment was applied on June 25, 1988. The field portion of the study was terminated on July 11, 1988, when all surviving birds were sacrificed. (3) An analysis phase (60 days) included behavior, mortality, brain AChE determinations, environmental residues, body and organ weight determinations, and egg laying. All of the appropriate data were analyzed statistically.

2.4. Pens

Hardware cloth-covered pens were constructed from modular panels to cover a minimum of 0.005 ha (50 m^2 per pen). The pen dimensions were 3.1 × 15.2 m, with the top cover at a height of ~1.8 m. A 0.91- by 1.8-m piece of 1.9-cm plywood was placed on the windward (south) side of each pen to reduce violent wind exposure. Rooftop shading was provided in one end of each pen using a nylon mesh shading material. A total of 28 pens was assembled from 3.1-m sections, with 9 pens for the formulation blank controls, 9 pens for the Pyrinex 4E treatment at 6.7 kg ai/ha, and 9 pens for the Pyrinex 4E treatment at 3.4 kg ai/ha + 3.4 kg ai/ha, with each of the two 3.4-kg treatments separated by 14 days. The 28th pen was used as a holding pen for extra birds. The 27 treatment pens were placed in a randomized block design with nine blocks (Fig. 1). Randomization of the treatments and assignment of birds to each pen was done according to a random number generator.

2.5. Birds

Bobwhite quail (*Colinus virginianus*) adults (16-week-old flight-adapted) were obtained from a pen-reared stock from Oakridge Game Farm (Gravette, AK). All birds were flight-conditioned and reared on Gro-scratch feed beginning May 10, 1988 until they were shipped by truck to Provo arriving on May 26, 1988. Each pen was stocked with 16 adult bobwhite quail (8 males + 8 females) 15 days prior to the treatment application. Each bird was weighed, banded (with a numbered leg band), and randomly assigned to one of the 27 pens.

2.6. Bird feed

All feed used in this experiment was Gro-scratch (9% protein containing wheat, milo, sorghum, and cracked corn), a commercial feed obtained from Intermountain Farmers Association (Provo, UT). This feed had been used successfully in a previous large-pen study (Booth et al., 1980a, b).

2.7. General test conditions and procedures

The pens were placed on turf with 15 in one row (end to end) and 12 in another (end to end). The rows were assembled in an east–west direction. The pens were separated east and west (end-wise) by ~1.5 m, and north and south by ~0.9 m. The pens were arranged so that five blocks were located on the north and four