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Northern Bobwhite Egg Hatchability and Chick Immunocompetence Following a Field Application of Diazinon

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Diazinon is an organophosphate (OP) pesticide commonly used in landscapes inhabited by many bird species (Eisler 1986). This pesticide is highly toxic for generally short periods after its application, but can cause mass mortality in birds and mammals (Eisler 1986). Depending on the type of application, exposure may be via inhalation, ingestion, and dermal absorption (Driver et al. 1991). Signs of acute diazinon exposure in birds include wing spasms, salivation, lacrimation, proventricular ulceration, pleural effusion, and death (Eisler 1986; Hill et al. 1994). Exposure of developing embryos to diazinon may result in teratogenesis (Kitos and Suntornwat 1992) and immunotoxicity (Pruett 1992).

Adults are commonly used to determine toxicity of OP pesticides in avian species (Stromberg 1981; Driver et al. 1991). However, sensitivity of embryos and neonates have received less attention. Previous research approaches have primarily involved immersing or injecting eggs in the laboratory with pesticide solutions and documenting subsequent malformations, growth depression, and mortality (Hoffman and Eastin 1981; Meneely and Wyttenbach 1989). Injection and immersion, however, do not closely mimic exposure of eggs to pesticides during actual field application. Field application of OP pesticides at recommended label rates have resulted in heavy mortality of adult Northern bobwhite (White et al. 1990).

Consequences of sublethal exposure of birds to pesticides during field application as well as in the laboratory are unclear. Prenatal exposure of mammals to diazinon results in significant decreases in immunocompetence (Barnett et al. 1980). Decreased immunocompetence after exposure to diazinon can occur without concurrent alterations in body weight, growth, or food consumption (Street and Sharma 1975; Barnett et al. 1980). Such subtle sublethal effects may not influence survival in a sterile laboratory environment, but may greatly alter survival in pathogen-rich field environments. Unfortunately, few OP pesticides have been tested for immunotoxicity (Pruett 1992). The primary objective of this study was to determine if field application of diazinon at two different recommended label rates would alter egg viability and hatchability, and immune function of 3-week old Northern bobwhite chicks.

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MATERIALS AND METHODS

Twenty-week old Northern bobwhite were obtained from El Reno Gamebird Farm (El Reno,OK) and housed in 15 brooders with 1 male per 4 females per brooder (60 females). Birds were maintained at 18L:6D daylength and 28°C. Eggs were collected daily from each cage over a 5-day period and stored at 10°C in a humidified chamber. On the fifth day, all eggs were randomly assigned by day of collection to one of 36 experimental "nests." A "nest" consisted of six eggs (at least one from each day of egg collection) held in a paper-mache tray designed for egg storage (G.Q.F. Manufacturing, Savannah, GA). One day post-collection, all nests were placed in an insulated holding chamber and transported to the Oklahoma State University Ecotoxicology Field Research Area located approximately 6.5 km W of Stillwater, Oklahoma. Twelve 0.1 ha enclosures at the site contain a homogenous tallgrass prairie habitat consisting of grasses and forbs with scattered shrubs. The site, which supports an indigenous Northern bobwhite population, has no known history of pesticide application.

Three experimental nests were randomly placed throughout each enclosure. Experimental design consisted of 4 replicates of each of 3 treatments of diazinon applied randomly to the enclosures: a high concentration of 4.0 lbs a.i./acre (8X), a low concentration of 0.75 lbs a.i./acre (1X), and a control (not sprayed). Diazinon 4E, a commercial formulation of the OP insecticide diazinon, was obtained (Estes Chemical Co., Oklahoma City, OK), mixed with water, and applied using a CO2 powered sprayer with attached 1.83-m boom. Average wind speed and temperature, and maximum temperature during insecticide application were 1.8 km/hr ,22.6 °C, and 27.2 °C, respectively. Nests were removed from insulated chambers and placed on the ground, and eggs turned on their longest axis immediately prior to spraying. We deliberately removed any vegetative canopy coverage above experimental nests prior to spraying. Experimental enclosures were sprayed by walking at a rate of 7.2 km/hr (diazinon applied at a rate of 10 gal/A). All treatment nests were completely sprayed in the process of spraying each enclosure. It was our design to provide a worst-case scenario where eggs were afforded no foliage protection from spray. Nests were removed from each enclosure one hour after exposure to diazinon spray and returned to the insulated chamber. In the laboratory, eggs were removed from nests and incubated in plastic trays as recommended by Skewes et al. (1990) until hatch. All eggs had dried before they were placed back in the incubator. Upon hatching, chicks were marked and placed in a brooder as described by Wilson and Dugan (1987). Egg viability, egg hatchability, and Type I deformities as described by Kitos and Suntornwat (1992) were recorded for each nest. At 3 weeks-of-age, 48 chicks (N = 20 control, 13 1X diazinon treatment, 15 8X diazinon treatment) were randomly selected to determine the effects of diazinon exposure on overall immune function. Immune function was assessed by pathogenic challenge with *Pasteurella multocida* Type 3, the bacterium which causes avian cholera. Chicks were injected i.m. in the left thigh with 23 colony forming units (CFU) of Avichol® (Scherring-Plough Animal Health Corporation, Omaha, NE), a vaccine strain of *Pasteurella multocida* Type 3, diluted in phosphate-buffered saline. Subsequently, survival of chicks was monitored for 7

days. Differences in percent egg viability and hatchability and chick deformity among treatments were tested using a one-way analysis of variance with replication (Norusi 1990). Differences in mortality among treatment groups due to Avichol® challenge were tested using a Z-test for binomial proportions (Ott 1988).

RESULTS AND DISCUSSION

We observed no ($P > 0.6$) differences among treatment groups with respect to percent egg viability, and percent egg hatchability (Table 1); as both were within normal ranges (Schom and Abbott 1974). In the early avian embryo the most obvious OP-induced abnormalities are slower development, folding or undulation of the notochord and neural tube, and unilateral retardation of the cranial sense organs (Meneely and Wyttenbach 1989). No visible Type I malformations were evident in chicks in this study. Likewise we detected no decrease ($P > 0.4$) in immunocompetence of diazinon-exposed chicks as measured by percent mortality to Avichol® (Table 1). Small sample size, however, may have limited the power of our test to detect subtle differences in mortality among treatments.

Table 1. Effects of field sprayed diazinon exposure on percent egg viability and hatchability (Means ± SEM; n= 36 nests), and resistance to Avichol® challenge of Northern bobwhite.

			Avichol® Challenge	
Treatment	Percent Viability	Percent Hatchability	Number Challenged	Percent Mortality
Control	70.92± 5.05	58.42 ± 5.99	20	25.0
Diazinon				
1X	71.41± 4.81	54.92 ± 6.51	13	30.8
8X	76.41± 5.19	61.08 ± 6.23	15	33.3

Mechanisms of resistance of avian species to *P. multocida* involves both cellular and humoral immunity (Lamont et al. 1987) and exposure of birds to immunosuppressive drugs or immunotoxins is known to decrease resistance to *P. multocida* (Rocke and Yuill 1984). Because diazinon treatment in our study did not affect resistance of Northern bobwhite chicks challenged with *P. multocida,* we conclude that immune system development relevant to P. *mulfocida* resistance was normal. Other investigations involving immersion or injection of eggs in the laboratory with diazinon solutions have documented subsequent malformations, growth depression, and mortality (Hoffman and Eastin 1981; Meneely and Wyttenbach 1989). Injection and immersion, however, do not closely imitate exposure of eggs to pesticides during actual field application. It appears that diazinon dissolved in a water vehicle at manufacturer-recommended concentrations posed no detrimental effect to Northern bobwhite embryos when exposed during field application. Future investigations should determine if the relatively thick shell membranes of

Northern bobwhite eggs (Hoffman 1990) provide protection against diazinon exposure.

Exposure of breeding Northern bobwhites to 150 ppm diazinon in feed also results in no adverse effects in egg hatchability or survival of chicks to 2-weeks-of-age (Stromberg 1981). Similarly, egg hatchability and chick survival of Northern bobwhite eggs sprayed with azodrin (Schom et al. 1979), an OP pesticide dissolved in a saline vehicle, do not differ from controls. Pesticides applied in oil vehicles may be much more toxic in comparison to pesticides applied using a water vehicle (Hoffman and Eastin 1981). However, hatching and fledgling success of red-winged blackbirds (*Agelaius phoeniceus*) was not affected by aerially sprayed fenthion dissolved in a diesel oil vehicle (Powell 1984). Eggs may also be chronically exposed to organophosphates if feathers of incubating hens become contaminated (Kilbride et al. 1992).

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