Factors Influencing Discrimination between Insecticide-Treated and Untreated Foods by Northern Bobwhite

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Abstract. Tests were conducted to determine at what dietary concentrations northern bobwhite (Colinus virginianus) chicks (14 days old) could discriminate between pesticide-treated and untreated food using an organophosphate (OP) insecticide, parathion, and two carbamates, carbofuran and methiocarb. Results from subacute dietary LC50 tests (one feeder of treated food per cage) were compared to tests where birds were presented with two feeders (one treated and one untreated, 1:1) or 10 feeders (five treated and five untreated, 5:5; or nine treated and one untreated, 9:1). The dietary concentration above which birds discriminate between feeders by consuming a greater proportion of untreated food is defined as the discrimination threshold (DT). The DT occurred at sublethal concentrations in all 1:1 tests, with little mortality or reduction in food consumption. Little or no discrimination was observed in 9:1 tests, with mortality similar to the LC50 tests. The discrimination response in 5:5 tests was similar to the 1:1 tests for parathion and methiocarb, but with carbofuran the DT was higher than in the 1:1 test and higher mortality was observed. In all tests, mortality was inversely related to total food consumption. No relationship was found between mortality and the amount of active ingredient ingested/bird-day. Consequently, mortality was more a function of ability to locate untreated feeders than amount of chemical ingested. When alternative food choices exist, vulnerability to poisoning can be influenced by the number and relative abundance of those choices, as well as the bird’s ability to detect the chemical.

The ability of birds to detect and avoid toxic foods when nontoxic alternatives are available has been demonstrated with several pesticides (Kenaga et al. 1979; Kononen et al. 1986, 1987; Bennett (in review)). These controlled-feeding tests presented groups of juvenile mallards (Anas platyrhynchos) or northern bobwhite (Colinus virginianus) with a free choice between two feeders—one containing untreated food and the other containing one of several dietary concentrations of test chemical. Under these conditions, birds responded to the presence of many chemicals at sublethal concentrations by decreasing the proportion of treated food consumed without significantly reducing total food consumption, thereby reducing the occurrence of toxic effects.

The field conditions under which wildlife species could utilize the ability to detect and avoid toxic foods are largely unknown. There are several factors acting in the field (e.g., restricted food availability, spacial and temporal patchiness of food, physiological status, and weather) that have been removed in these laboratory tests. Bennett (in review) evaluated the effects of two factors—number of food choices available and relative availability of treated and untreated food sources—on dietary discrimination in northern bobwhite using two organophosphate insecticides. Tests with chlorpyrifos demonstrated that it was detected readily by birds at sublethal concentrations regardless of the number and relative availability of choices, whereas the response in tests with methyl parathion was affected by both the number and relative proportion of food choices. Dietary discrimination information on additional pesticides and additional bird species is needed to understand how birds react to available sensory cues and how responses are affected by changes in feeding scenarios (i.e., changes in the number and relative proportion of choices). The ultimate goal of these
studies was to understand the extent to which behavioral responses of wildlife can modify dietary exposure to chemicals in the field.

The objective of the present study was to further evaluate the role of these factors—number of feeders present and the relative proportion of treated and untreated feeders—on dietary discrimination behavior in bobwhite using three cholinesterase-inhibiting insecticides. A series of feeding tests involving different combinations of treated and untreated food choices was conducted to measure the discrimination response using parathion [O,O-diethyl O-p-nitrophenyl phosphorothioate; technical purity = 98.5%], carbofuran [2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; technical purity = 98.8%], and methiocarb [3,5-dimethyl-4-(methylthio)phenol methylcarbamate; technical purity = 97.7%].

**Experimental Methods**

Northern bobwhite chicks were tested in one of four dietary tests consisting of combinations of chemically-treated and untreated food: 1) one feeder/cage containing treated food (dietary LC50 test); 2) two feeders/cage, one containing treated food and one untreated (1:1); 3) 10 feeders/cage, five treated and five untreated (5:5); and 4) 10 feeders/cage, nine treated and one untreated (9:1). At 10 days of age, birds were weighed and assigned to test cages using a stratified random procedure based on body weight (Steel and Torrie 1960). At 14 days of age, birds were weighed and presented with test diets for 5 days. In each of the tests, cages were assigned randomly to one of six dietary concentrations or a carrier control group. In the 5:5 and 9:1 tests all feeders within a cage contained the same dietary concentration.

All insecticides were dissolved in corn oil in a 65°C water bath. The corn oil solution was mixed 2:98 (w/w) with Purina® gamebird starter diet for 10 min. Control diets contained corn oil in the same proportion.

Birds were housed in brooder cages measuring 70 × 91 × 22 cm, and maintained at 35°C with a 12 hr light:12 hr dark cycle. LC50 and 1:1 tests used rectangular galvanized steel chick feeders, while 5:5 and 9:1 tests used 500-ml rectangular polyethylene bottles with four 2.5-cm holes in the sides for feeding. Bottles were arranged in two rows of five aligned from front to back in each brooder and held in place by metal frames. Since birds can quickly develop preferences among feeder positions, feeders were moved daily to reduce the association of toxic effects with a particular location and to force birds to respond to stimuli provided by the food. The position of feeders in the 1:1 test was changed on a random schedule. In order to ameliorate the effects of potential feeder position bias in the 5:5 and 9:1 tests, each bottle feeder spent equal time in each of the five positions in the tray during the 5-day treatment period, using a stratified random schedule. Food and water were provided ad libitum, and each individual feeder contained more than the total amount of feed consumed daily by the control groups.

Food consumption, clinical signs of toxicity, and mortality were recorded daily in the LC50 test during the 5-day treatment and 3-day post-treatment periods. Food consumption (corrected for spillage) was calculated as grams consumed per bird-day, where bird-day equaled the average number of birds alive at the beginning and end of each 24-hr period. Individual body weights were recorded on days 0, 5, and 8. The same measurements were taken in the food choice tests, except that all surviving birds were asphyxiated with CO2 at the end of the 5-day treatment period.

In the LC50 tests, the median lethal concentration (LC50) and 95% confidence interval were calculated by probit analysis using the Statistical Analysis System computer software package (SAS Institute Inc 1982). In the food choice tests, the dietary concentration above which birds discriminated between treated and untreated foods by consuming a higher proportion of untreated food was defined as the discrimination threshold (DT). The DT was calculated as the intersection of a two-phase regression using the log of the ratio of untreated to treated food consumption over the 5-day test period as the dependent variable and log of concentration of treated food as the independent variable (Bennett and Schafer, in press). At concentrations below the DT the expected value of the response, y = log (untreated/treated), should equal 0, reflecting no preference for either food choice. At higher concentrations the regression has a positive slope, indicating a dose-related increase in the probability of selecting untreated food. In the 9:1 tests, the dependent variable was modified to y = log ((untreated/treated)/(1/3)), to account for the change in relative availability of the two food types so that the expected value for y equaled 0 at concentrations below the DT. This method was based on the assumption that the two-phase regression is an appropriate model.

Food consumption and body weight data were analyzed by fitting linear regression models to various dose ranges and selecting the best fit by using the criterion of the highest coefficient of determination. Dietary concentrations were transformed to log (concentration + 1). Regression slopes are presented ± one standard error.

**Results**

**Parathion**

The LC50 test with parathion produced an LC50 value (95% confidence interval) of 177 ppm or mg/kg (154, 212). Hill et al. (1975) calculated an LC50 of 194 ppm for the same age bobwhite. Mean body weights of control birds increased 60% during the 5-day treatment period, while decreasing in all dietary concentrations that resulted in one or more deaths (Table 1). The relationship of dietary concentration (x) to percent weight change (y) was $y = 2.43 - (1.19 ± 0.16) \log(x + 1)$, $79 < x < 251$, $R^2 = 0.93$. All bird mortality occurred between days 3 and 6, with an average loss of $37\% ± 1$ (SE) of their body weight during treatment (range: 21–43% loss). Total food consumption was reduced in all treated groups and generally decreased with increasing dietary concentration [$y = 15.8 - (5.9 \pm 1.4) \log(x + 1)$, $79 < x < 251$, $R^2 = 0.80$] (Figure 1). The greatest ingestion of active ingredient occurred in the 126 ppm group, the lowest concentration producing mortality, with chemical ingestion decreasing thereafter (Table 1).