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EFFECTS OF LETHAL AND SUBLETHAL CONCENTRATIONS OF THE HERBICIDE, TRICLOPYR BUTOXYETHYL ESTER, IN THE DIET OF ZEBRA FINCHES

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ABSTRACT: Lethal and sublethal effects of dietary triclopyr butoxyethyl ester (TBEE) on zebra finches (*Poephila guttata* Gould) were determined in laboratory experiments conducted between 8 January and 1 May 1991. The 8-day median lethal dietary concentration, LC_{50} (95% confidence interval), of TBEE to zebra finches was 1,923 (1,627 to 2,277) mg/kg. In the sublethal effects experiment, when birds were exposed to 500 mg/kg TBEE in the diet for 29 days, food consumption and body weight were significantly depressed ($P < 0.05$). Similar prolonged exposures to 50 and 150 mg/kg TBEE in the diet had no significant effect on food consumption or body weight ($P > 0.05$). Perch-hopping activity was depressed relative to controls in the 500 mg/kg group, and elevated in the 150 mg/kg group, but neither of these differences was significant ($P > 0.05$). Disappearance of TBEE residues from treated seeds over the 29 day experimental period followed an exponential decay model, with half-lives in the order of 15 to 18 days. On the basis of our observation that TBEE had no significant adverse effects at a concentration greater than the maximum expected environmental concentration, we propose that forestry applications of triclopyr at registered dosage rates pose little risk to wild songbirds.

Key words: Triclopyr, herbicide, bioassay, birds, zebra finch, *Poephila guttata*.

INTRODUCTION

Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) butoxyethyl ester (TBEE) is an auxin type, systemic herbicide that controls broadleaf weeds and many woody plants (Anonymous, 1983). In the United States, triclopyr BEE is registered under the trade name GARLON® 4 (DowElanco, Indianapolis, Indiana, USA) for conifer release and site preparation in forested areas, and can be applied either aerially or by ground-based equipment (Anonymous, 1986). In Canada, the same product is marketed as RELEASE® (DowElanco Canada, Newmarket, Ontario, Canada) herbicide and is currently registered for ground application only, although an aerial registration is being sought by the manufacturer (Campbell, 1991).

Herbicide residues in ripening and ripe berries can be very persistent, declining very slowly or not at all over periods of up to 1 mo (Frank et at., 1983; Roy et al., 1989). There is thus a potential for long-term exposure of wildlife species, including some native songbirds, that eat wild fruit. The acute toxicity of most herbicides to birds is low. For example, for the bobwhite (*Colinus virginianus* L.) the 8-day median lethal dietary concentration (LC_{50} or the concentration of toxicant causing 50% mortality in a test population of subjects) for hexazinone is >10,000 ppm, for TBEE is 9,026 ppm, for 2,4-D (2,4-dichlorophenoxyacetic acid) is >5,000 ppm and for glyphosate is >4,640 ppm (Hill et al., 1975; Sassman et al., 1984). However, the sublethal effects of long-duration exposures to low levels of these pesticides are not well known.

Our objective was to test the hypothesis that TBEE residues accumulating in food as a result of forest spraying pose no significant hazard to exposed songbirds. Two separate experiments were conducted. The first was a subacute toxicity test to determine the 8-day LC_{50} of TBEE to zebra finches (*Poephila guttata* Gould). The second was an experiment to determine the sublethal effects on zebra finches of prolonged exposure (29 days) to TBEE resi-

dues in the diet, as measured by changes in food consumption, and effects on activity and activity patterns.

MATERIALS AND METHODS

The zebra finches used in the tests were from the Forest Pest Management Institute (Department of Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada) aviary. Details regarding the care and maintenance of this colony are provided in Holmes and Boag (1990).

The 8-day LC_{50} of TBEE for zebra finches was determined according to the method of Hill et al. (1975), with the following modifications. On the basis of the results of preliminary range-finding tests, five concentrations of TBEE were selected to include values both above and below the expected LC_{50}. These were 800, 1,200, 1,800, 2,700 and 4,050 mg of TBEE active ingredient per kg dry weight of seed (ppm). Twelve birds were randomly assigned to each dosage group. Because the number of birds available was limited, it was necessary to include four males and eight females in each group. Test birds were fed acetone-treated seed for 3 days, and then fasted for 3 hr, prior to the start of the test. During the 5-day treatment period, TBEE-treated seed and water were replaced daily. At the end of the 5-day period, the surviving birds were switched to herbicide free diet for an additional 3 days. Controls were treated identically to treated birds, except that they received acetone-treated seed only. Throughout the test, all birds were fed ad libitum. Mortality was recorded daily and birds were weighed on days 0, 5 and 8, or at time of death.

For the sublethal effects experiment, test concentrations were based on the maximum expected concentration of TBEE in fruit. This concentration was estimated using residue data for the herbicides 2,4-D, dichlorprop, picloram and glyphosate (Frank et al., 1983; Roy et al., 1989). Concentrations were corrected for differences in application rate. For example, if a concentration of 10 mg/kg of 2,4-D was observed in fruit following an application at 2.2 kg/ha, then an application at the maximum recommended label rate for RELEASE® herbicide in Canada (3.84 kg/ha) would be expected to result in a concentration of 17.5 mg/kg; calculated as ((10 mg/kg)/(2.2 kg/ha)) × 3.84 kg/ha = 17.5 mg/kg. Using this procedure, and assuming that all five herbicides would behave similarly in the environment, the median and maximum expected concentrations of TBEE in fruit (modeled on data from 24 separate spray applications) were estimated to be about 16 mg/kg and 80 mg/kg, respectively. A concentration halfway between the median and maximum expected concentrations (50 mg/kg) was selected as the lowest dosage for the sublethal effects experiment. The two other concentrations tested were 150 and 500 mg/kg. On the basis of the results of the 8-day LC_{50} test, these higher concentrations were not expected to cause mortality.

Twelve zebra finches were randomly assigned to each treatment group in the sublethal effects experiment. Only male finches were used to avoid any confusion that might result due to differences in behavior between the sexes. Birds were held individually in cages measuring 625 mm long × 275 mm wide × 350 mm high. They were fed fresh acetone-treated seed each day for 8 days, then TBEE-treated seed for 29 days, and finally acetone-treated seed again for 21 days. Controls received acetone-treated seed only. The birds were weighed weekly and the amount of seed consumed by each individual bird was measured daily. Because of the design of the cages, spillage was not a serious problem. A 10 cm high raised lip around the bottom of the cages prevented the loss of any spilled seed. The amount of seed consumed by each bird on each day was calculated by taking the difference between the weight of seeds given to the bird at the start of the day, and the weight of the seeds remaining in the seed dish and recovered from the floor of the cage 24 hr later. Any seed remaining in the seed dish or on the floor at the end of the day was discarded. The perch-hopping activity of individual birds was monitored continuously using a computer-driven activity recorder described by Holmes and Boag (1990). This device counted the number of times each bird hopped between two perches in its cage. Activity measurements were made from 8 days before to 35 days after the initial exposure to the treated seed.

Batches of treated and control diet were prepared immediately prior to the commencement of each experiment. For the 8-day lethal dietary toxicity experiment, batches of seed (450 g) were accurately weighed out on an electronic balance and transferred to a 2 l round bottom flask (RBF). Batches of seed were fortified by six repetitive additions, totalling 33.75 ml, of an appropriate fortification solution. After each addition, the RBF was shaken vigorously by hand for approximately 1 min. Fortification solutions for each test concentration were prepared by dissolving an appropriate volume of GARLON® 4 herbicide in 40 ml acetone. For example, to prepare a batch of seed with a test concentration of 800 μg TBEE per gram of seed (equivalent to 800 mg/kg or ppm), 640 μl of GARLON® 4, containing 667 μg of TBEE per μl of formulation, was dissolved in 40 ml acetone, and 37.5

ml of this solution was added to 450 g of seed; calculated as 640 μl × 667 μg/μl × (33.75 ml/40 ml) × (1/450 gm) = 800 μg/g. After fortification, the RBF was shaken by hand for 3 to 4 min. This was followed by evaporation of the acetone carrier from the spiked seeds on a Büchi rotary evaporator (Büchi Laboratoriums-Technik AG, Flawil, Switzerland). Every 10 min, the RBF was removed from the evaporator and shaken vigorously by hand for 1 min to ensure complete mixing and transfer of TBEE residues from the walls of the flask onto the seeds. Total time for evaporation was 30 min. Following rotary evaporation, any remaining acetone was evaporated from the seeds at 20 to 22 C. Dry fortified seeds were transferred into 1 l Erlenmeyer flasks and thoroughly mixed by hand. Fortified seeds were stored in capped 1 l Erlenmeyer flasks at 20 to 22 C for the duration of the experiment.

A similar fortification procedure was used to prepare diets for the sublethal effects experiment, with appropriate modifications for changes in the amount of seed required. For this experiment, fortification solutions were prepared by dissolving an appropriate quantity of GARLON® 4 in 230 μl of acetone. A total of 3,000 g of seed was prepared for each treatment group (50, 150 and 500 ppm) in five batches of 600 g each. Each 600 g batch of seed received 45 ml of fortification solution in six repetitive additions. The five batches of seed for each treatment group were combined and stored in a capped 4 l Erlenmeyer flask at 20 to 22 C for the duration of the experiment.

Control diets were treated with acetone alone (either 37.5 ml of acetone per 450 g of seed or 225 ml per 3,000 g of seed, depending on the experiment) and treated in an identical manner to TBEE-treated seed.

The TBEE concentrations on treated seeds were determined using the following procedure. For each batch of treated seed, moisture content was estimated by drying a 12 g sample of seeds at 130 C in a convection oven (GCA Corp., Chicago, Illinois, USA) until a constant weight was achieved (typically 48 hr). The difference between fresh and dry weight was taken as the moisture content (typically 10 to 12%), and was applied as a correction factor in reporting residue determinations on a dry weight basis. Samples of seed obtained for determination of TBEE residues (3 g) were placed in 40 ml capacity glass vials and frozen prior to analysis. After thawing at 20 to 22 C, 25 ml of an ethyl acetate : hexane (1:1) solvent solution was added to each sample and the mixture was macerated using a Tekmar Tissuemizer (Model SDT-1820, Tekmar Inc., Cincinnati, Ohio, USA). Macerated solids were repeatedly extracted (3 × 25 ml of the solvent solution) by shaking on a mechanical shaker (Eberbach Corp., Ann Arbor, Michigan, USA) for 5 min at 180 oscillations/min. Liquid extracts were decanted through a glass filtering tube containing a plug of glass wool and a bed of anhydrous sodium sulfate (1 g), pooled in a graduated cylinder and brought to a constant volume of 100 ml. Pooled extracts were mixed thoroughly and a 1 ml aliquot was serially diluted in iso-octane so that a 2 μl injection resulted in a peak within the linear range of the detector. Concentrations of TBEE in the seed samples were determined using a capillary column (DB-5 column, 30 m × 0.25 mm, 1.0 μm film thickness; J&W Scientific, Folsom, California, USA), gas-liquid chromatograph (Varian Vista Model 6000 GLC with Varian Model 8000 Autosampler, Varian Model DS604 Integrator and ^{63}Ni electron capture detector; Varian Instruments Group, Palo Alto, California) operating under the following conditions: Column oven (temperature program)—85 C (3 min) to 255 C (7 min) @ 30 C/min; Injection port—200 C; Ionization oven—325 C; Retention time—12.2 min; Carrier gas—ultra high purity nitrogen (1.5 ml/min); Make-up gas—ultra high purity nitrogen (30 ml/min). All samples were compared to an authentic analytical standard of TBEE (0.020 μg/ml) supplied courtesy of DowElanco (Indianapolis, Indiana, USA).

The technique was validated by fortifying three seed samples at each of two nominal concentrations approximating 1,500 and 150 μg/g. Results of the validation test demonstrated essentially quantitative recovery (>99%) and excellent precision (<4% coefficient of variation), irrespective of test concentration. The minimum detection limit for TBEE in seed using this technique was estimated to be less than 1 μg/g.

The TBEE residues in treated seeds were measured periodically in both experiments. In the first experiment, two replicate samples of seed from each concentration batch (800, 1,200, 1,800, 2,700 and 4,050 mg/kg) were collected on days 0 and 5. A single sample was collected from the control on day 5. In the second experiment, three replicate samples from each batch (0, 50, 150 and 500 mg/kg) were collected on day 0, and single samples on days 1 to 14, 17, 18, 20, 21, 24, 26 and 28.

Statistical analyses were performed using the PC version of BMDP Statistical Software (BMDP Statistical Software, Inc., Los Angeles, California). Half-lives ($t_{1/2}$) of TBEE in treated seed were approximated by fitting the data to an exponential decay (first-order kinetics) model ($\ln[C]_t = \ln[C]_0 - kt$, where t = time in days, $[C]_t$ = TBEE concentration at time t, $[C]_0$ = TBEE concentration at t = 0, and k = rate

TABLE 1. Decline in triclopyr butoxyethyl ester residues in treated millet seeds between days 0 and 5 of the experiments.

Experiment	Nominal concentration (mg/kg)	Actual concentration (mg/kg)		Rate of decline[a] (%)
	Day 0	Day 0	Day 5	
Sublethal effects	50	48.4	21.7	55.2
	150	149.1	72.8	51.1
	500	517.2	321.0	37.9
Lethal dietary toxicity	800	792.4	585.4	26.1
	1,200	1,210.0	897.1	25.9
	1,800	1,723.9	1,347.6	21.8
	2,700	2,796.1	2,362.1	15.5
	4,050	4,296.3	3,877.6	9.7

[a] Percentage reduction from original concentration over 5 days.

constant). The LC_{50}'s and associated statistics were derived by probit analysis using POLO-PC software (LeOra Software, Berkeley, California, USA).

RESULTS

Actual measured concentrations of TBEE in seeds on day 0 were within 0.6–6.1% of nominal concentrations (Table 1). Within batch variability (coefficients of variation for replicate samples) ranged from 0.3 to 6.8%. The TBEE residues were not detected in any of the control samples (seeds treated with acetone only).

Between day 0 and day 5, TBEE concentrations declined by 10 to 26% in the lethal dietary toxicity experiment, with the rate of decline inversely proportional to the original TBEE concentration in the seeds (Table 1).

As expected, the level of mortality of zebra finches in the lethal dietary toxicity experiment increased with increasing concentration of TBEE in the diet and with increasing time of exposure. At the end of the 5-day treatment period, one bird had died in the 1,200 mg/kg group (on day 4), four in the 1,800 mg/kg group (one each on days 2 and 3, and two on day 5), 10 in the 2,700 mg/kg group (two on day 1, four on day 2, and two each on days 4 and 5) and all 12 in the 4,050 mg/kg group (three on day 1, six on day 2, and three on day 3). Only one bird died during the recovery period, and that was in the 1,800 mg/kg group on day 7. The calculated 8-day LC_{50} (95% confidence interval) of TBEE was 1,923 (1,627 to 2,277) mg/kg.

All groups, except the controls, lost weight between day 0 and day 5 (or time of death) of the lethal dietary toxicity experiment (Table 2). The mean weight loss of TBEE treated birds was directly proportional to the concentration of herbicide in the diet of each group (r^2 = 63%), and ranged from about 5% for the 800 mg/kg group to 25% for the 4,050 mg/kg group. All of these weight losses were significant ($P < 0.01$), using paired t-tests. During the 3-day recovery period, birds in the 800 and 1,200 mg/kg groups regained lost body

TABLE 2. Weight changes among zebra finches in the 8-day lethal dietary toxicity test of triclopyr butoxyethyl ester.

Nominal concentration (mg/kg)	Mean weight (g) ± standard deviation (sample size)[a]		
	Day 0	Day 5	Day 8[b]
Control	13.50 ± 2.20 (12)	13.69 ± 2.11 (12)	13.64 ± 1.98 (12)
800	12.19 ± 1.47 (12)	11.61 ± 1.09 (12)[c]	12.34 ± 1.06 (12)
1,200	12.13 ± 1.09 (12)	10.93 ± 1.36 (12)[c]	12.05 ± 1.09 (11)
1,800	12.67 ± 1.00 (12)	10.37 ± 1.09 (12)[c]	11.77 ± 0.81 (8)[d]
2,700	11.99 ± 0.98 (12)	9.28 ± 0.84 (12)[c]	11.10 ± 0.08 (2)
4,050	12.86 ± 0.78 (12)	9.69 ± 0.66 (12)[c]	—

[a] Birds that did not survive to day 5 or day 8 were weighed at time of death.
[b] Statistics are for birds that survived beyond day 5.
[c] Indicates a value that is statistically different than the corresponding day 0 value at $P < 0.01$ (paired t-tests).
[d] Indicates a value that is statistically different from the corresponding day 0 value at $P < 0.05$ (paired t-tests).

weight, but those in the 1,800 and 2,700 mg/kg groups recovered only partially. By day 8, the only significant difference between initial and final weights was in the 1,800 mg/kg group ($P < 0.05$) using a paired t-test. For the birds that died during the 5-day treatment period, body weight losses ranged from 17.1 to 32.4% (median 24.3%).

Regression equations describing the disappearance of TBEE residues from treated seeds in the sublethal effects experiment were as follows: 50 mg/kg batch, $\ln[C]_t = 6.00 - 0.039(t)$, $r^2 = 0.93$; 150 mg/kg batch, $\ln[C]_t = 4.57 - 0.038(t)$, $r^2 = 0.85$; 500 mg/kg batch, $\ln[C]_t = 3.37 - 0.045(t)$, $r^2 = 0.86$; where t = time in days and $[C]_t$ = concentration in mg/kg at time t. For the 50 mg/kg batch, $t_{½} = 15.4$ days and $k = -0.045$; for the 150 mg/kg batch, $t_{½} = 18.2$ days and $k = -0.038$; and for the 500 mg/kg batch, $t_{½} = 17.8$ days and $k = -0.039$. The slope of the relationship between concentration and time (k) was steeper in the 50 mg/kg batch, than in the other two batches (F-test for equality of slopes, $F_{2,60} = 29.78$, $P < 0.001$). By day 28, TBEE residues had declined 73% in the 50 and 150 mg/kg batches, and 79% in the 500 mg/kg batch.

Within hours of being presented with treated seed, two birds in the 500 mg/kg group displayed marked hyperactivity. The more common response to TBEE poisoning in this group, however, was lethargy. Some birds also had drooping wings and ruffled or fluffed feathers, which may indicate that the birds were hypothermic.

During the pre-treatment period of the sublethal effects experiment, there were no significant ($P = 0.51$) differences in seed consumption among groups (Fig. 1), using a repeated measures analysis of variance (RM ANOVA). A repeated measures analysis of covariance (RM ANCOVA) was performed on the seed consumption data collected during the 29 day treatment period, with subjects grouped according to Dosage (TBEE concentration in the diet) and number of Days of treatment. Differences in seed consumption between individuals were controlled by using the average weight of seeds consumed each day by each bird during the 8-day pre-treatment period as a covariate. Both Dosage and Day had a significant effect on seed consumption ($P = 0.003$ and $P < 0.001$, respectively). However, there was no significant ($P = 0.18$) interaction between Dosage and Day; thus, while both the level and duration of exposure to TBEE were important in determining the magnitude of the effect, these two factors probably acted independently. A contrasts analysis within RM ANCOVA was used to identify which group(s) were responsible for the observed Dosage effect. Seed consumption in the 500 mg/kg group was significantly ($P < 0.05$) lower than all other groups. This was the only significant difference. Seed consumption was depressed most in the 500 mg/kg group during the first treatment week, but remained low over the entire 29-day treatment period (Fig. 1). There were no significant ($P = 0.9735$) differences in seed consumption among groups during the post-treatment (recovery) period (RM ANCOVA).

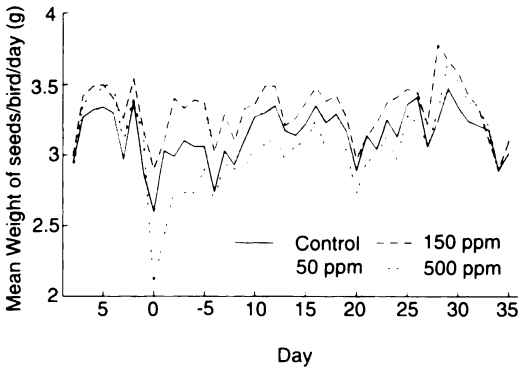

FIGURE 1. Seed consumption by zebra finches over the 44 day period of the sublethal effects experiment. Birds were provided with triclopyr butoxyethyl ester treated seed from day 0 to 28 (inclusive).

The lower seed consumption observed in the 500 mg/kg group was accompanied by a reduction in body weight in this group (Fig. 2). Using paired t-tests, the mean body weight of birds in the 500 mg/kg

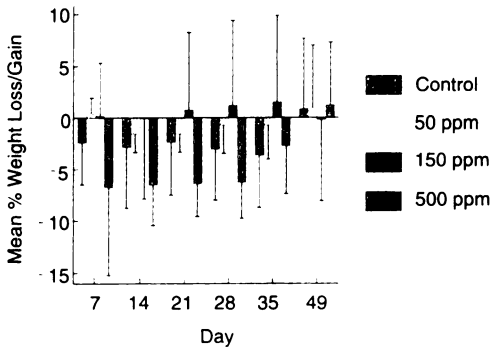

FIGURE 2. Mean percent weight loss/gain by zebra finches fed triclopyr butoxyethyl ester contaminated diets (relative to pre-treatment weights) in the sublethal effects experiment. An asterisk symbol indicates that the average body weight of the group on that day was significantly different from the group's average pre-treatment body weight (paired t-test, $P < 0.05$).

group was significantly ($P < 0.05$) lower than the average pre-treatment body weight of birds in this group on all sampling days between day 7 and day 35 post-treatment. By day 49 (3 wk post-treatment), however, the mean body weight of birds in the 500 mg/kg group had returned to normal. The only other significant weight losses observed were in the 50 mg/kg group on days 14 and 21, and in the controls on day 35. The small but consistent decrease in the body weight of controls (2 to 4%) between days 7 and 35 was puzzling, since there was no prolonged reduction in feeding activity in this group (Fig. 2). Seed consumption by controls, however, was somewhat reduced in the first week following treatment.

One bird in the 500 mg/kg group died on day 5 post-treatment. The body weight of this bird was depressed by about 32% at the time of death.

During the pre-treatment period (days -7 to -1), there were no significant ($P = 0.47$) differences in perch-hopping activity among groups (RM ANOVA). For the 29-day treatment period (days 0 to 28), a RM ANCOVA was performed on the activity data, with Dosage and Day as the two independent variables. Differences in activity between individuals were controlled by using the mean activity level (hops/hr) of each bird during the 7 days immediately preceding treatment as a covariate. Again, there were no significant ($P = 0.14$) differences among dosage groups, but there was a significant ($P = 0.0089$) interaction between Dosage and Day (RM ANCOVA), which was not observed during the pre-treatment period (RM ANOVA, $P = 0.30$). This significant ($P = 0.0474$) interaction persisted through days 29 to 35 of the post-treatment period (RM ANCOVA). The average coefficient of variation (CV) for all groups combined during the pre-treatment period (days -7 to -1) was 49.9%, and during the treatment period (days 0 to 28) was 59.8%.

The perch-hopping activity of birds in the 500 mg/kg group was depressed relative to controls throughout the entire 29 day treatment period (Fig. 3). The mean ($\pm$SD) difference between treatment (500 mg/kg) and control groups during this period was 35 $\pm$ 15%. In contrast, birds in the 150 mg/kg group were more active, relative to the other groups and to their own pre-treatment activity level, from day 8 through day 32 (Fig. 3). The mean ($\pm$SD) difference in activity between this group and the controls over the 29 day treatment period was 22 $\pm$ 19%. Neither of these differences were statistically significant, however.

DISCUSSION

In both experiments, birds were exposed to TBEE residues in seeds that declined over time. This was intentional since this approach is more realistic. The disadvantage is that the bioassays are less repeatable than if a constant concentration of TBEE had been maintained over time. We chose the more realistic exposure, because our primary aim was to provide a hazard assessment for birds exposed to TBEE residues in the field.

The rate of disappearance of TBEE residues from treated seeds was inversely related to concentration (Table 1). This observation is contrary to first-order kinetics,

which would predict a similar rate of decline of TBEE residues regardless of initial concentration (Sparks, 1989). The fact that the batches of seed were stored in sealed flasks may have been at least partly responsible for this lack of conformity to the model. If the primary mode of degradation in the flasks was microbial, then it may be that, as the concentration of herbicide in the flask increased, the system became saturated; that is, the ratio of processors or processing sites to herbicide molecules was reduced. In such a system, the disappearance of TBEE residues might be better described by an equilibrium model, in which two or more processes compete with one another in an isolated or closed system, and the equilibrium rate constant is a function of substrate concentrations (Spain, 1982).

In the sublethal effects experiment, half-lives ($t_{1/2}$) of TBEE residues in seed were in the order of 15 to 18 days. On the basis of these $t_{1/2}$ values, TBEE would be classified as nonpersistent (Sparks, 1989). In general, $t_{1/2}$ values are smaller for field than for laboratory studies (Sparks, 1989). This is due to the greater number of factors affecting pesticide disappearance in the field (Rao and Davidson, 1980). Thus one might expect TBEE residues to be even less persistent under field conditions.

In the laboratory, TBEE concentrations in treated seed were reduced by 70 to 73% in 28 days. There are no data in the literature on the persistence of TBEE residues in wild fruits. In general, herbicide residues in ripening or ripe berries decline slowly or not at all (Frank et al., 1983; Roy et al., 1989). Frank et al. (1983) found that 2,4-D residues in raspberries declined by 89 to 95% in 21 to 28 days following spraying, but that, in most cases, residues in blueberries did not decline at all over periods ranging from 23 to >31 days. If TBEE behaves similarly to 2,4-D, then our study would represent a worst-case scenario for raspberries, but would underestimate the effects of a prolonged exposure to contaminated blueberries.

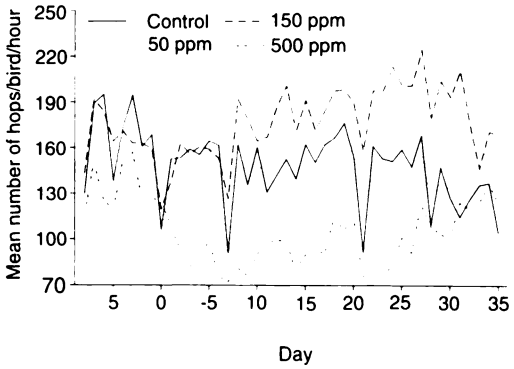

FIGURE 3. Perch-hopping activities of zebra finches over the 44-day period of the sublethal effects experiment. Birds were provided with triclopyr butoxyethyl ester treated seed from day 0 to day 28.

In our study, the 8-day LC_{50} of TBEE to zebra finches was 1,923 mg/kg. According to Sassman et al. (1984), the 8-day LC_{50} of TBEE to mallards (*Anas platyrhynchos* L.) is >10,000 mg/kg, and to bobwhite is 9,026 mg/kg. Thus, the zebra finch is almost five times more sensitive than the bobwhite, and at least five times more sensitive than the mallard, to TBEE. An inverse correlation between body size and sensitivity to pesticides is a frequently observed trend in avian toxicology (Hill, 1971).

In a study of the effects of high dietary concentrations of glyphosate herbicide on zebra finches, Evans and Batty (1986) found that birds that died as a result of a 5-day exposure to 5,000 mg/kg glyphosate lost an average of 28% of their body weight. In our study, the 29 birds that died from exposures to TBEE ranging from 500 to 4,050 mg/kg lost an average of 24% of their body weight. Evans and Batty (1986) suggested that their birds may have died of starvation. Support for this hypothesis was provided by Hill (1972), who found that house sparrows lost an average of 21% (range 17 to 25%) of their body weight when they were starved to death.

Evans (1985) and Evans and Batty (1986) reported that zebra finches avoid diets containing herbicides. In the case of the herbicide Nufarm LV Ester 40, this refusal to eat treated seeds was attributed to taste

aversion (Evans, 1985). Further, it was determined that it was not the formulation ingredients in Nufarm LV Ester 40, but rather the active ingredient, iso-octyl 2,4-dichlorophenoxyacetate, itself that caused the taste aversion. In the case of insecticides, decreased food consumption in birds has been attributed to either anorexia (Grue, 1982) or conditioned taste aversion (Evans, 1985). Our study was not designed to distinguish among these various possible causes of reduced food consumption.

There were no statistically significant differences in the activity levels of birds in the different treatment groups. However, the activity of birds in the 500 mg/kg group appeared to be depressed by exposure to TBEE (by about 35%), whereas birds in the 150 mg/kg group appeared to be more active (by about 22%) following treatment. The inability of the statistical tests to demonstrate these apparent differences may be explained by the high level of variability in the activity data. On the basis of the coefficients of variation observed during the pre-treatment (50%) and treatment (60%) periods, and with a sample size of 12 individuals per group, it is possible to calculate the smallest true difference between groups that could have been detected by an analysis of variance approach (Sokal and Rohlf, 1981). At a significance level of 0.05, these would have been 75% and 90%, respectively. To detect differences of 22 and 35% during the treatment period, sample sizes of 194 and 76 would have been required, respectively.

There are at least two possible explanations for the observation of increased activity in the 150 mg/kg treatment group. Triclopyr could act as a stimulant when present at some low level in the diet. This is probably not the case, however, at least not in a direct sense, since activity levels remained high in the 150 mg/kg group for a few days even after the birds were returned to an untreated diet. Alternatively, the basal metabolic rate of the birds might have been elevated to deal with the added demands of detoxifying and eliminating the TBEE residues, and a side-effect of this increase in metabolic rate was increased activity. In the 50 mg/kg group, there was no increase in metabolic rate or activity because normal detoxification mechanisms could easily handle the lower toxic load.

Sharp reductions in the activity levels of all groups (50, 150 and 500 mg/kg, and controls) were observed at weekly intervals from day 0 through to day 35 (Fig. 3). Similar drops were seen in seed consumption (Fig. 1). The timing of these drops corresponded to the days when the birds were weighed. The weighing involved removing each bird from its cage for a period of about 2 to 3 min. During this time the bird was held in a cloth bag. The entire process for all 48 birds required about 2 hr to complete and was done during the dark phase of the photoperiod (14L:10D) just prior to turning on the lights. Although casual observation did not suggest any significant effect on the birds, this apparently was not the case. The birds appear to have been stressed, and this resulted in lower than normal activity levels, and as a consequence lower energy and food requirements. The ability of the behavioral assay to detect these differences illustrates the sensitivity of this system.

The behavioral assay used in this study appeared to be sensitive for detecting sublethal effects of pesticides in the diets of birds. With TBEE, effects on the activities of zebra finches were observed at concentrations that were ¼ (depression; 500 mg/kg) to $\frac{1}{12}$ (elevation; 150 mg/kg) of the 8-day dietary LC_{50} for this species (1,923 mg/kg). Because activity was unaffected in birds exposed for 29 days to a concentration of TBEE (50 mg/kg) that was close to the absolute maximum concentration expected in contaminated foods (80 mg/kg), we believe that triclopyr applications at registered dosage rates pose little risk to forest songbirds.

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