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COMPARATIVE RESPONSE OF NESTLING EUROPEAN STARLINGS AND RED-WINGED BLACKBIRDS TO AN ORAL ADMINISTRATION OF EITHER DIMETHOATE OR CHLORPYRIFOS

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ABSTRACT: Red-winged blackbird (*Agelaius phoeniceus*; blackbird) and European starling (*Sturnus vulgaris*; starling) nestlings were dosed with either 2.0 mg/kg body mass chlorpyrifos, 50.0 mg/kg body mass dimethoate, or a propylene glycol carrier *in situ*. Four growth measurements (body mass, culmen, tarsus, wing) were recorded from nestlings to determine if these organophosphorus compounds caused perturbations in development at sublethal concentrations. Blackbird nestlings were more sensitive to chlorpyrifos than starling nestlings were more sensitive to dimethoate than blackbird nestlings. This was in contrast to reported adult LD_{50} values where the reverse was true. Blackbird nestlings were more tolerant of a substantially higher concentration of dimethoate than the adult LD_{50}. The sensitivity of starling nestlings to dimethoate was similar to adults. In contrast, juveniles of both species were more sensitive to chlorpyrifos than adults. After the initial 24 hr, surviving nestlings dosed with either chemical recovered and continued their development. Exposure to dimethoate caused significant depression in starling body mass during the initial 24 hr period. Survivors obtain body mass equal to controls within 48 hr post dosing. The research presented here demonstrates that the simple supposition that passerine nestlings are typically more sensitive to toxins than adults does not always hold true. It also indicates that sensitivity relationships among adults do not necessarily apply to their nestlings.

Key words: Red-winged blackbird, *Agelaius phoeniceus*, European starling, *Sturnus vulgaris*, dimethoate, chlorpyrifos organophosphorus insecticides, nestling sensitivity, experimental study.

INTRODUCTION

Although a great deal of research has been undertaken to determine the impacts of organophosphorus (OP) pesticides on birds, the bulk of the available information is derived from laboratory data. Few studies have been conducted on free-ranging birds, particularly songbirds (order Passeriformes). They are found in nearly all habitats including those where OP's are used. There is a need to develop methodologies that can be used to determine pesticide impacts on this large avian group.

In response to this need, some research has been conducted using the European starling (*Sturnus vulgaris*; starling) and the red-winged blackbird (*Agelaius phoeniceus*; blackbird) to determine the effects of environmental contaminants on passerine species (Schafer, 1972; Schafer and Brunton, 1979; Grue and Shipley, 1981; Grue and Christian, 1981; Grue et al., 1982; Powell, 1984; Grue and Franson, 1986; Stromborg et al., 1988; Kendall et al., 1989; Meyers et al., 1990). Schafer (1972) reported that the starling was consistently more tolerant than the red-winged blackbird to selected OP compounds. This trend of apparent higher tolerance for the starling over those shown for other passerine species has been reported in other studies as well (Schafer and Cunningham, 1972; Schafer and Brunton, 1979). However, the assertion that wild starlings are more tolerant to pesticides than other passerines is based on adult LD_{50} data only. This assertion should be investigated using different life stages in non laboratory situations.

The study presented here examined the differential response of nestling starlings and blackbirds to two OP pesticides using nestling growth rates and survival as measured response variables. Nestlings were used for this study because they may be more sensitive to pesticides than adults (Hudson et al., 1972; Grue and Shipley, 1984). The nestling period is a critical time for altricial birds and it is logical that additional stresses on nestlings during this time period may impair development and fitness. Others have reported reduced mass and growth in young passerine species exposed to OP pesticides (Powell and Gray, 1980; Grue and Shipley, 1984). Krementz et al. (1989) observed that starling body mass and timing of fledging were important indicators of post-fledging survival. Starlings fledging later and at a lower mass than controls had low post-fledging survival estimates in that study. Stromborg et al. (1988) did not observe a relationship between fledgling body mass and post-fledging survival in their study of starlings exposed to an OP insecticide.

The objectives of this study were to (1) compare growth and survival of nestling red-winged blackbirds and starlings following exposure to OP pesticides, (2) document which growth parameter(s) was most indicative of exposure, and (3) comment on the use of adult LD_{50} values for predicting impacts on nestlings.

MATERIALS AND METHODS

The study was comprised of an initial range finding test and a definitive comparative toxicity test. All testing was *in situ*; that is, all nestlings were dosed at their nest or nest box, replaced, and allowed to develop and fledge (comparative test).

Test chemicals

The OP's used in this study were chlorpyrifos [phosphorothioic acid, O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) ester, technical grade, purity = 94%] (Chem Service, Inc., West Chester, Pennsylvania, USA) and dimethoate [phosphorodithioic acid, O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] ester, technical grade, purity = 98%] (Chem Service, Inc.). These chemicals were selected because they are widely used in the United States and have been implicated in avian mortalities (Blus et al., 1989; Stone and Gradoni, 1986). An additional criterion used in the selection of OP's for this study was that adult starlings must have been shown to have a higher LD_{50} value for one chemical and a lower LD_{50} value for the other chemical than those described for adult blackbirds. The reported starling adult LD_{50} value for dimethoate is 32 mg/kg (25 to 41 mg/kg, 95% CL) (Schafer 1972), whereas the blackbird value was 6.6 mg/kg (3.6 to 12 mg/kg, 95% CL) (Schafer, 1972), and for chlorpyrifos the starling LD_{50} value was 5.0 mg/kg (95% CL not reported) (Schafer, 1972) while the blackbird had a reported LD_{50} value of 13.0 mg/kg (95% CL not reported) (Schafer, 1972).

Study site

Blackbirds and starlings used in this study were selected from a free-ranging population nesting at the USEPA Willamette Research Station (Corvallis, Oregon, USA; 44°32′N, 123°15′W). At this station, 48 nest boxes were constructed and erected (Kendall et al., 1989) along the perimeter of the facility to accommodate starlings. Blackbirds nested in emergent vegetation surrounding six 0.1 ha ponds (Meyers et al., 1990). The study was conducted between 1 May and 20 June 1989.

Experiment

A range finding test was conducted during the first two weeks in May 1990 to determine appropriate doses of the chemicals for use in the study. The endpoints of concern in the range finding test were cholinesterase (ChE) depression and mortality. A concentration of each chemical that caused ChE depression equal to or greater than 50% of normal or that caused approximately 50% mortality in one of the species was selected for use in the comparative test.

Cholinesterase activity was determined using a Gilford auto-analyzing spectrophotometer (Ciba Corning Diagnostics Corporation, Cleveland, Ohio, USA) and followed the method described by Ellman et al. (1961) as modified by Meyers et al. (1990). One-way analysis of variance was used to compare mean ChE activity between treatments and control (SAS Institute, 1990).

Testing began using blackbird nestlings at 3 days of age and starling nestlings at 4 days. Age was determined using methods described by Meyers et al. (1990) for red-winged blackbirds and Feare (1984) for European starlings. Different age nestlings were used for this study so that the nestlings were at the same relative developmental stage at the time of exposure as determined by comparison of feather track development.

Ten starling nests and 18 blackbird nests were identified and used in the test. Nest sample size was dependent on nests available between 15 May and 20 June, 1990. Each nest contained three randomly selected nestlings (extras were removed) and each nestling was randomly selected to receive an oral gavage of either chlorpyrifos, dimethoate, or the propylene glycol carrier (control).

Each nestling was identified as to dose by marking a different toe for each dose with indelible ink. Chemicals were dissolved in pro-

TABLE 1. Number of 3-day-old red-winged blackbird and 4-day-old European starling nestlings surviving after a single *in situ* dosage of either dimethoate (50 mg/kg), chlorpyrifos (2.0 mg/kg), or propylene glycol (control).

		Survival by day post-dosage							
Species	n[a]	0.25 (%)	1 (%)	2	3	5	7 (%)	10	13 (%)
Red-winged blackbird									
Dimethoate	18	17 (94)	17 (94)	15	14	11	10 (56)		
Chlorpyrifos	18	11 (61)	10 (56)	10	9	7	5 (28)		
Propylene glycol	18	18 (100)	18 (100)	17	13	11	9 (50)		
European starling									
Dimethoate	10	5 (50)	4 (40)	4	4	4	4 (40)	4	4 (40)
Chlorpyrifos	10	9 (90)	9 (90)	9	9	9	9 (90)	8	8 (80)
Propylene glycol	10	9 (90)	9 (90)	9	9	9	9 (90)	9	8 (80)

[a] Number of nestlings dosed in each group.

pylene glycol and administered at a volume/mass ratio of 5 μl solution per gram of body mass (0.5% of body mass). Nestlings were gavaged in the morning (0800 to 0900 hr) and monitored at intervals of 1, 3, and 6 hr post-dosing. During these intervals observations of nestling condition were recorded and dead birds were collected. To determine if nestlings were intoxicated, nest boxes or nests were tapped lightly to solicit begging response.

Growth measurements including tarsus (tibiotarsus length), culmen (bill length from the beginning of feathering on crown to tip of upper mandible), wing (length from proximal end of radial carpal to tip of phalange or tip of the eighth primary feather after eruption), and body mass were recorded on days 0, 1, 2, 4, 6, 8, 10, 13, and 16 post-dosing for starlings and on days 0, 1, 2, 4, 6, and 8 post-dosing for blackbirds. Length measurements were recorded to the nearest mm using a Mac-Cal® digital caliper (Mausner Equipment Company, Inc., East Meadow, New York, USA) and mass to the nearest 0.5 g using a Pesola® precision scale (Rolf Storhmeier, Baar, Switzerland).

All growth measures were compared within and between observation periods for significant differences ($P < 0.05$) between treatment and control nestlings (within a species) using one-way or repeated measures analysis of variance (SAS Institute, 1990) as appropriate. Prior to analysis all data were transformed using the common logarithm ($\log_{10}$) of the data to meet the assumptions for analysis of variance (Box et al., 1978).

RESULTS

For chlorpyrifos the desired 50% depression of brain ChE occurred between 1.25 and 2.5 mg/kg for blackbirds and between 3.75 and 6.5 mg/kg for starlings. Because 100% blackbird (3 of 3) and 67% starling (2 of 3) mortality occurred at 2.5 mg/kg chlorpyrifos and none occurred at or below 1.25 mg/kg, a concentration of 2.0 mg/kg was selected as the test dose for the experiment. With dimethoate there was no appreciable effect on blackbird ChE activity up to 50 mg/kg, but at this dose approximately 50% mortality occurred in starlings. Therefore, a dose of 50.0 mg/kg was selected for dimethoate.

During the comparative test a high incidence of acute mortality to red-winged blackbirds was observed in the chlorpyrifos treatment (2.0 mg/kg) group (Table 1). Of the 18 nestlings treated, seven died within the first 6 hr and one more died within the first 24 hr (44%). Only one European starling (10%) died at this dose of chlorpyrifos during the same period. For blackbirds dosed with 50.0 mg/kg dimethoate 17 of 18 survived the first 24 hr (94%), whereas, five of the starlings died (50%) at this dose.

There were no significant differences among treatments for either species for any of the growth measurements, except that starlings experienced a significant decrease in body mass 24 hr after treatment with dimethoate. Dimethoate-intoxicated starlings exhibited lethargy and lack of begging behavior and did not respond to

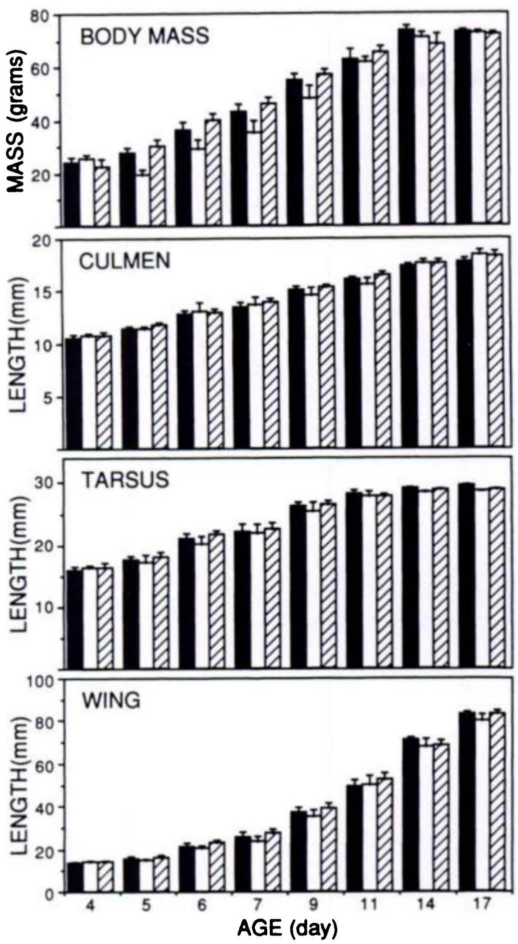

FIGURE 1. Mean (±SE) four growth measurements for European starlings following exposure to 2.0 mg/kg of body mass or chlorpyrifos (solid), 50.0 mg/kg of body mass of dimethoate (open), or propylene glycol (hatch). Sample size varied among treatments. All treatments began with 10 nestlings 4 days old. Final sample size at age 17 was seven for chlorpyrifos-treated group, four for dimethoate-treated group, and eight for propylene glycol control group.

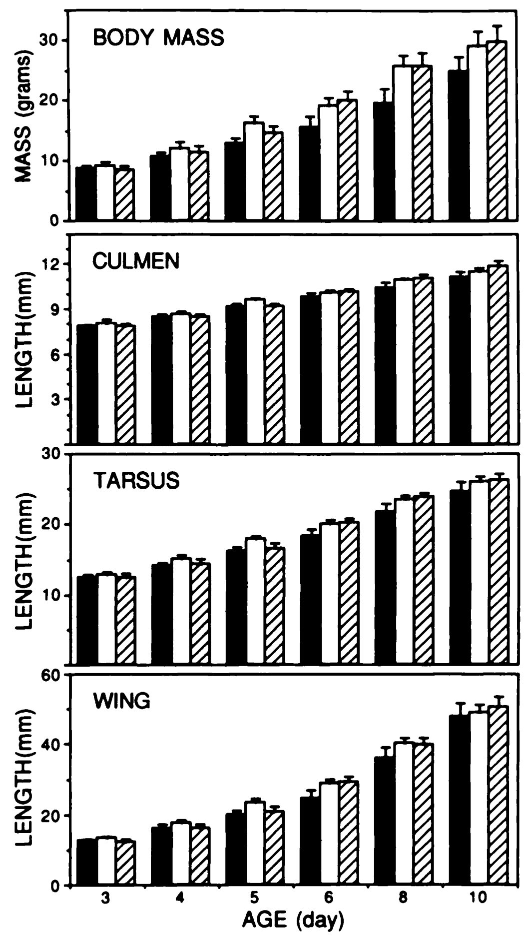

FIGURE 2. Mean (±SE) for four growth measurements for red-winged blackbirds following exposure to 2.0 mg/kg body mass of chlorpyrifos (solid), 50.0 mg/kg body mass of dimethoate (open), or propylene glycol (hatch). Sample size varied among treatments. All treatments began with 18 nestlings at age 3. Final sample size at age 10 was five for chlorpyrifos-treated group, 10 for dimethoate-treated group, and nine for propylene glycol-control group.

tapping on the nest box as did their nest mates. Those starling nestlings surviving the initial dose of dimethoate (40%) recovered and obtained body mass comparable (not significantly different) to controls and their chlorpyrifos treated nest mates within 48 hr after dosing (Fig. 1). Starling nestlings exposed to chlorpyrifos showed no signs of intoxication at 2.0 mg/kg.

Chlorpyrifos-intoxicated red-winged blackbird nestlings exhibited lethargy and lack of begging behavior and did not respond to tapping on the edge of the nest as did their nest mates. The apparent depression in body mass, culmen, tarsus, and wing of chlorpyrifos-treated blackbirds (Fig. 2) was not statistically significant.

DISCUSSION

There were clear differences in sensitivity exhibited between red-winged blackbirds and European starlings in response to the organophosphorus pesticides dimethoate and chlorpyrifos. These differences were evident in the survival data but were not apparent in the growth of individual nestlings. Compound specific species sensitivity could not be predicted for nestlings based on reported adult LD_{50} values (Schafer, 1972). Species sensitivity to chlorpyrifos and dimethoate were reversed from that which was expected based on LD_{50} values for adult blackbirds and starlings. At a dose of 50 mg/kg dimethoate there was no appreciable effect on blackbird nestlings, but at this dose approximately 50% mortality occurred in starling nestlings. This was in contrast to reported adult LD_{50} values of 6.6 mg/kg for blackbirds and 32 mg/kg for starlings (Schafer, 1972) which suggested that blackbirds were more susceptible to dimethoate than starlings. In addition, blackbird nestlings were more tolerant of a substantially higher dose of dimethoate than the adult LD_{50}. Starling nestlings showed a similar tolerance to a dose of dimethoate greater than the adult LD_{50}, however, this was not as pronounced a difference as that exhibited by blackbird nestlings treated with dimethoate.

The response recorded for nestlings exposed to chlorpyrifos was quite dissimilar to that exhibited for dimethoate. The adult LD_{50} reported for chlorpyrifos (13 mg/kg) (Schafer, 1972) was more than six times higher than the dose (2.0 mg/kg) that produced 44% mortality in nestling blackbirds. Because this dose caused high mortalities among blackbird nestlings (10 of 18 died within 24 hr), it was concluded that the blackbird nestlings were more sensitive to chlorpyrifos than adults. The data for starling nestling response to chlorpyrifos did not allow for any definitive conclusions regarding differences between adult and nestling tolerance, although results from the range finder indicated that more than 50% of the starling nestlings would be killed by doses at or above 2.5 mg/kg compared to an adult LD_{50} of 5.0 mg/kg (Schafer, 1972). In our study nestling starlings were more tolerant of chlorpyrifos than blackbird nestlings.

The research presented here demonstrates that the supposition that passerine nestlings are typically more sensitive to OPs' than adults does not always hold true. This is in concurrence with the results of Hudson et al. (1972) for mallard (*Anas platyrhynchos*). It also indicates that one should not suppose that sensitivity relationships among adults apply to their nestlings.

Mortality occurred primarily during the initial 24 hr period post-dosing. Nestlings that survived this period appeared to recover, as indicated by begging response, and continued their development maintaining or regaining body mass and growth rates similar to controls. Recovery from a sublethal dose of OP in our study appeared to be similar to the reports of others for starling, common grackle (*Quiscalus quiscula*), and dark-eyed junco (*Junco hyemalis*) for a variety of OP's (Grue and Shipley, 1984; Stromborg et al., 1988; Grue, 1982; Zinkl et al., 1981).

Starlings dosed with dimethoate had depressed body mass 24 hr after dosing but were able to regain mass equivalent to controls within 48 hr after dosing. Loss of body mass in starlings after dosing with an OP was also observed by Grue and Shipley (1984) and Stromborg et al. (1988). Unlike those studies, our starlings obtained body mass similar (difference not significant) to controls within 48 hrs after dosing. This may have been an artifact of small sample size, variability in body mass measurements and age of nestlings in our study. Body masses of blackbirds in our study were not significantly different from controls, but means for chlorpyrifos treated blackbirds were lower than either controls or dimethoate treated nestlings after dosing. Powell (1984) found that some red-winged blackbirds exposed to a single application of the OP fenthion had a 15% slower growth rate during their first 3 to 4 days of life but noted that overall growth for those surviving to fledging age was the same as controls.

Stromborg et al. (1988) noted that nestlings surviving severe OP exposure appeared to gain mass faster than controls. Our observations agree with this conclusion. To obtain levels comparable to controls nestlings would need to increase their rate of gain. We also agree with Stromborg et al. (1988) that recovery of body mass by nestlings must be dependent on the age at initial exposure and duration and magnitude of exposure.

The study presented here and those of others (Stromberg et al., 1988; Powell and Gray, 1980; Powell, 1984; Grue and Shipley, 1984) have demonstrated that exposure to an OP can cause depression in nestling body mass. The importance of transient decrease in nestling body mass is unknown and the effects of depressed fledging body mass are unclear. Results from those studies testing the effects of depressed fledging mass are contradictory. Stromborg et al. (1988) cited many of these studies and noted that "in spite of these contradictory results, a positive relationship between fledging mass and survival is generally assumed to exist in species for which studies have not been conducted." Little is known about the long term effects of OP-exposure on passerines and it may be that after surviving an exposure, recovery is complete and there are no detectable long term effects. This was the case for adult red-winged blackbirds that showed no difference in over-winter survival after receiving a sub-lethal dose of methyl parathion the previous breeding season (Meyers et al. 1990).

One goal of this project was to evaluate selected measurements for describing differences in growth among treatment groups. Because growth parameters (culmen, tarsus, wing) were not significantly different among treatments, a quantitative evaluation of these measures was not possible. However, indications from our research and that of others (Powell and Gray, 1980; Powell, 1984; Stromberg et al., 1988; Krementz et al., 1989) suggest that body mass may be an informative character for use in evaluating impacts of OP exposure, especially as it relates to postfledging survival.

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