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INTERPRETING POPULATION ESTIMATES OF BIRDS FOLLOWING PESTICIDE APPLICATIONS—BEHAVIOR OF MALE STARLINGS EXPOSED TO AN ORGANOPHOSPHATE PESTICIDE

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ABSTRACT.—We determined activity budgets for 10 pairs of captive male Starlings between 7 May and 18 July 1980. Our objective was to quantify changes in behavior after exposure to an organophosphate (OP) pesticide and to assess the impact of changes in behavior on the interpretation of population estimates of birds following pesticide applications. We observed each pair of males for an hour at 07:30 and 09:30 for four days and classified their behavior into one of four categories: flying, perching, foraging, or singing and displaying. At 06:30 on day 2, one male received a single oral dose of 2.5 mg dicrotophos (3-hydroxy-*N*, *N*-dimethyl-*cis*-crotonamide dimethyl phosphate) per kg of body weight; the other male received an equivalent exposure of corn oil. Changes in the activity budgets of OP-dosed and control males were compared using *t*-tests.

Activity of OP-dosed males was significantly ($P \leq 0.05$) reduced within the 2–4 h following exposure. OP-dosed males spent more time perching (46.1%) than controls and less time flying (−96.6%), foraging (−28.5%), and singing and displaying (−49.5%). The frequency of perching (−75.3%), flying (−83.8%), foraging (−54.1%), and singing and displaying (−59.2%) was significantly reduced. Activity in OP-dosed males returned to normal by 26–28 h posttreatment. Results suggest that movement and vocalization may be significantly reduced in birds exposed to organophosphate and carbamate pesticides. Conventional censusing techniques and population estimating procedures may, therefore, be inadequate to assess changes in bird populations after pesticide applications because of the difficulty in separating decreases in density due to mortality or emigration from reductions in activity.

Organophosphates (OP's) and carbamates are becoming increasingly important as insecticides because of their low potential for accumulation in the environment (Andrilenas and Eichers 1977, Lamoreaux and Newland 1977, Fowler and Mahan 1978). Applications of these pesticides often coincide with peaks in avian abundance and reproductive activity. Census techniques dependent on visual and auditory cues (e.g., Williams 1936, J. T. Emlen 1971) are often used to monitor impacts of these pesticides on bird populations (Finley 1965, McLeod 1967, Doane and Schaefer 1971, Pillmore et al. 1971, Fowle 1972, Moulding 1976, Pearce et al. 1976, Bart 1979, DeWeese et al. 1979, Richmond et al. 1979). Exposure to organophosphates has, however, been shown to reduce activity in captive (Hill 1971, Pope and Ward 1972) and wild (Edwards and Graber 1968) birds. Whether reported reductions in bird populations following OP or carbamate applications (Finley 1965, McLeod 1967, Doane and Schaefer 1971, Fowle 1972, Moulding 1976, Pearce et al. 1976, Bart 1979) were due to emigration, lethargy, or mortality is not clear. Published studies quantifying the effects of sublethal OP or carbamate exposure on bird behavior, particularly song production, are lacking. The objective of this study was to quantify changes in the behavior of captive male Starlings (*Sturnus vulgaris*) after sublethal exposure to an organophosphate pesticide and to assess the impact of changes in behavior on the interpretation of population estimates of birds following pesticide applications.

METHODS

We determined activity budgets for 10 pairs of male Starlings, 1 pair per week, between 7 May and 18 July 1980. Males were housed individually within 2.4 × 3 × 12 m open-wire pens containing a wood nest box (Kessel 1957), two perches, a water pot, and a hanging feeder supplied with commercial turkey starter. Burlap on the sides of the pens provided visual isolation. Birds were acclimated to the pens for 12 days. Grass in the pens was mowed 3 days prior to each trial. Following the acclimation period, we observed each pair of males for an hour at 07:30 and 09:30 for 4 days from blinds and classified their behavior into one of four categories: (1) flying, (2) perching, (3) foraging, or (4) singing and displaying. All periods of inactivity (i.e., time not spent in one of the three other behaviors) were classified as perching; preening was included in this category. The frequency and duration of each behavior were recorded using an Esterline-Angus event recorder. Hours when observations were made corresponded to extremes within which censuses of wild birds have been conducted following pesticide applications (Moulding 1976, DeWeese et al. 1979, Richmond et al. 1979). Food consumption was monitored daily at 06:30. At 06:30 on day 2, one male was given a single oral dose of 2.5 mg dicrotophos (3-hydroxy-*N*, *N*-dimethyl-*cis*-crotonamide dimethyl phosphate) dissolved in corn oil per kg of body weight, a sublethal exposure which has been shown to cause a 50% reduction in brain cholinesterase (ChE) activity in female Starlings (Grue, Powell, and McChesney MS). The remaining male received an equivalent exposure of only the corn-oil carrier. Males were sacrificed at 10:30 on day 4 and frozen (−20°C) prior to brain ChE assays. Differences between changes (Δ = day_1 − $day_{2,3,\text{ or }4}$)

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TABLE 1
BRAIN CHOLINESTERASE ACTIVITY IN OP-DOSED AND CONTROL MALE STARLINGS

	Time postexposure (h)	
	28	52
Control males		
n		11
Mean		21.82[a]
SD		1.77
OP-dosed males[b]		
n	11	10
Mean	11.03	10.78
SD	2.12	1.91
% inhibition	49.5	50.6

[a] μmoles acetylthiochloline iodide hydrolyzed per min per g tissue.
[b] 2.5 mg/kg dicrotophos.

in the activity patterns of OP-dosed and control males were compared using *t*-tests (H_0:$\mu_D = 0$, Snedecor and Cochran 1967:98). Average differences in frequency and total duration of each behavior across the two 1-h observation periods were used in calculating the test statistics for 2–4, 26–28, and 50–52 h postexposure. Differences between changes in the behavior of OP-dosed and control males were considered significant if the probability associated with the test statistic was less than or equal to 0.05.

Immediately following the 10 trials, we dosed an additional 10 male Starlings with 2.5 mg dicrotophos per kg of body weight to determine the level of brain ChE inhibition at 28 h postexposure. Males were housed together in one of the four adjacent pens used for the behavioral trials. These birds were sacrificed 28 h after exposure and frozen. Brain ChE activity was determined colorimetrically using methods described by Ellman et al. (1961), as modified by Deiter and Ludke (1975) and Hill (1979).

RESULTS

Brain ChE activity of OP-dosed males was inhibited an average of 49.5% 28 h following exposure and an average of 50.6% 52 h postexposure (Table 1). Compared to controls, the activity of OP-dosed males was significantly reduced within the 2–4 h after exposure (Table 2). Frequency of perching (−75.3%), flying (−83.8%), foraging (−54.1%), and singing and displaying (−59.2%) was significantly lower in OP-dosed males than in controls (Fig. 1). OP-

TABLE 2
ACTIVITY BUDGETS OF OP-DOSED AND CONTROL MALE STARLINGS

			Average change posttreatment					
	Pretreatment average		2–4 h		26–28 h		50–52 h	
Activity	OP-dosed[a]	Control	OP-dosed	Control	OP-dosed	Control	OP-dosed	Control
Perching								
Frequency[b]	173.4 ±90.0	190.4 ±109.5	−123.1[d] ±96.0	8.3 ±99.0	−32.3 ±83.6	−9.4 ±128.2	−1.9 ±79.1	−3.4 ±98.3
Total duration[c]	1721.3 ±579.0	1983.9 ±603.7	607.0[d] ±1135.9	−214.6 ±566.6	−140.5 ±635.0	−167.3 ±562.5	−85.2 ±674.5	−268.7 ±567.0
Foraging								
Frequency	44.3 ±17.5	42.3 ±20.5	−24.0[d] ±19.3	−0.1 ±13.9	−2.2 ±19.7	2.5 ±17.1	−7.0 ±13.1	−0.4 ±15.1
Total duration	1261.2 ±669.4	1134.7 ±564.0	−76.6 ±1111.0	255.0 ±589.6	242.6 ±630.4	170.9 ±557.0	45.1 ±730.6	236.2 ±565.6
Flying								
Frequency	135.2 ±67.6	156.4 ±112.2	−104.2[d] ±73.1	10.5 ±112.0	−24.5 ±61.6	−3.2 ±126.5	−8.4 ±53.9	4.8 ±104.6
Total duration	207.4 ±121.5	243.3 ±195.0	−159.9[d] ±126.9	48.2 ±216.3	−47.3 ±96.4	−5.4 ±230.2	−25.1 ±87.3	7.7 ±211.6
Singing and displaying								
Frequency	42.1 ±51.7	22.8 ±31.6	−36.8[d] ±47.8	−6.4 ±20.2	−9.2 ±23.9	−1.6 ±12.3	4.2 ±26.3	−0.3 ±12.0
Total duration	433.7 ±544.0	262.2 ±397.8	−395.1[d] ±523.7	−109.0 ±250.6	−74.5 ±238.1	−25.5 ±224.8	45.4 ±195.3	8.3 ±191.5

[a] 2.5 mg dicrotophos per kg of body weight.
[b] Frequency per hour ± standard deviation.
[c] Total duration per hour in seconds ± standard deviation.
[d] Change significantly different from controls, *t*-test, $P \leq 0.05$.

TABLE 3
DAILY CONSUMPTION OF FOOD (G) BY OP-DOSED AND CONTROL MALE STARLINGS

	Pre-treatment	Time postexposure (h) 24	48
Control males			
n	10	10	10
Mean	25.5	25.0	28.0
SD	5.8	5.8	5.9
OP-dosed males[a]			
n	10	10	10
Mean	23.1	13.7[b]	26.1
SD	5.9	10.5	6.0

[a] 2.5 mg dicrotophos per kg of body weight.
[b] Difference between OP-dosed and control males significant, $P \leq 0.05$, paired *t*-test.

dosed males also spent significantly more time perched (46.1%) than controls, and significantly less time flying (−83.8%) and singing and displaying (−49.5%, Fig. 1). Though males exposed to the organophosphate spent less time foraging on the ground (−28.5%) compared to controls, the difference was not statistically significant. However, consumption of food within the hanging feeders by OP-dosed males was significantly lower (−38.7%) than that of controls within the 24 h after dosing (Table 3). Differences between changes in the behavior of OP-dosed and control males 26–28 and 50–52 h postexposure were not statistically significant.

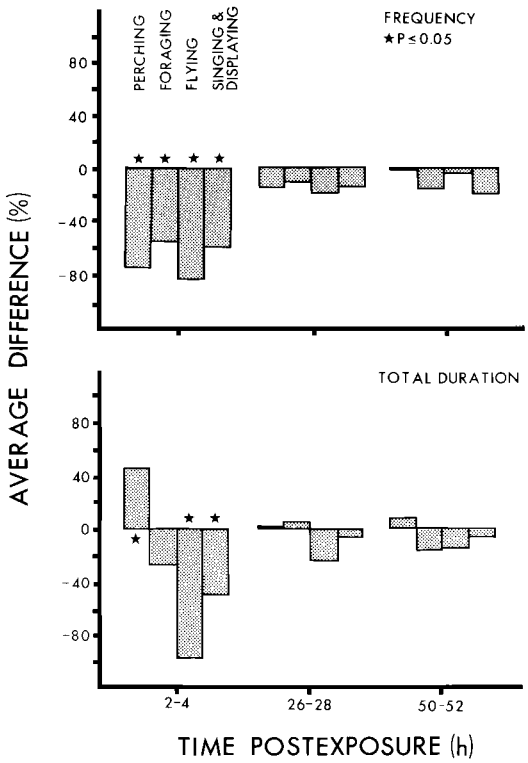

FIGURE 1. Average difference (%) in changes in total duration (seconds per hour) and frequency (per hour) of perching, foraging, flying, and singing and displaying of OP-dosed (2.5 mg dicrotophos per kg of body weight) male Starlings relative to controls.

DISCUSSION

Organophosphates and carbamates act by inhibiting ChE with subsequent disruption of nerve activity caused by accumulation of acetylcholine at nerve endings (O'Brien 1967:55, 88). Since behavior is dependent on nerve function, alterations in neural transmission may be expected to result in changes in behavior. Though no comparable data exist for birds, results of most studies with laboratory rats (for review, see Banks and Russell 1967) indicate a close correspondence between ChE activity and behavioral change with a critical level of ca. 40–60% inhibition (for exception, see Kurtz 1977). The level of brain ChE inhibition (ca. 50%) in the male Starlings we dosed with dicrotophos was within this critical range and was comparable to that observed in wild birds following applications of organophosphates and carbamates (Elder and Henderson 1969; Seabloom et al. 1973; Richmond et al. 1979; Zinkl et al. 1979, 1980). Though a reduction in brain ChE activity of 50% may be considered severe (diagnostic of poisoning by ChE inhibitors in dead birds, Ludke et al. 1975), OP-dosed males suffered no apparent muscular incoordination or impairment of flight.

Physiological and behavioral effects of ChE inhibitors reported in captive birds and other vertebrates may account for the changes in activity we observed in OP-dosed male Starlings. Intoxication of passerines following sublethal OP exposure under laboratory conditions is usually characterized by a reduction in activity and anorexia followed by a state of lethargy (Hill 1971, Pope and Ward 1972). Studies with laboratory rats (Adams 1977) suggest that inhibition of brain ChE is associated with a reduction in food seeking behavior. Sublethal OP exposure has also been associated with reduced visual acuity (Oba and Oto 1976), information processing, and psychomotor speed (Levin and Rodnitzky 1976) in man, and auditory detection in squirrel monkeys (*Saimiri sciureus*) (Reischl et al. 1975).

Why we did not observe a statistically significant reduction in the amount of time OP-dosed males spent foraging within the 2–4 h after treatment is not clear. OP exposure in birds has been

associated with decreased food intake (Keith and Mulla 1966; Mehrotra et al. 1967; Hill 1971; Pope and Ward 1972; and this study). The majority ($\bar{X}$ = 88.2%) of all foraging activity by our male Starlings during pretreatment observations occurred on the ground. Difficulty in differentiating active searching for food from movement on the ground not associated with foraging may account for this discrepancy.

Reductions in activity, particularly song production, similar to that we observed in OP-dosed male Starlings have been reported in wild birds during censuses conducted after applications of organophosphates or carbamates (Finley 1965, McLeod 1967, Edwards and Graber 1968, Giles 1970, Doane and Schaefer 1971, Fowle 1972, Pearce et al. 1976, Bart 1979). With the exception of Edwards and Graber, these investigators and others have considered differences between pre- and postspray census results to be indicative of pesticide-induced changes in population density due to emigration or mortality. However, our data suggest that changes in behavior related to pesticide exposure may reduce detectability and make interpretation of census results difficult. Though changes in behavior associated with OP poisoning appear to be short-lived after exposure ceases (Keith and Mulla 1966; Mendelssohn and Paz 1977; and this study), effects may be present weeks after field applications. Zinkl et al. (1979) reported brain ChE inhibition of greater than 40% in birds 33 days after an aerial application of the OP, acephate. Probably these birds were still being exposed to the OP several weeks after treatment as brain ChE activity in birds exposed to ChE inhibitors may be expected to reach 20% of normal within ca. 26 days after exposure ceases (Fleming and Grue, MS).

The interpretation of results of censuses conducted after pesticide applications may be further complicated by the movement of birds in and out of treated areas. Several authors (McEwen et al. 1965, Giles 1970, Doane and Schaefer 1971, Moulding 1976, Bart 1979) have suggested out-of-area feeding as an avian response to insecticide-induced food shortages. As in the case of pesticide-induced reductions in activity, long sorties for food by adult birds with young would decrease the probability of detecting individuals still utilizing treated areas. Conversely, the immigration of birds into areas following pesticide applications may be rapid and mask treatment effects on population density. Within breeding bird populations, there appear to be "floaters," silent nonterritorial birds, and vacant territories are quickly reoccupied (Stewart and Aldrich 1951, Robbins 1964). These replacements may be more active and vocal than their predecessors (Stewart and Aldrich 1951). Conventional censusing techniques and population estimating procedures (e.g., Williams 1936, J. T. Emlen 1971), therefore, appear inadequate to assess changes in bird populations after pesticide applications. Pesticide-induced changes in behavior (e.g., reduced detectability) may result in overestimation of decreases in density due to mortality or emigration, whereas immigration of birds into treated areas may mask pesticide effects. The "disappearance" of birds after pesticide applications should not be considered synonymous with death (Heinz et al. 1979).

Use of mist nets to capture, mark, and recapture individual birds may be an effective way to more accurately determine the effects of pesticide applications on resident bird populations. Potential difficulties outlined by Heinz et al. (1979) and Richmond et al. (1979) should be considered. Handling of birds during the nesting season may adversely affect reproductive success. If pesticide effects are species specific, examination of only those species most trappable may lead to erroneous results. In addition, the number of man hours required to capture, mark, and recapture large numbers of birds may be prohibitive.

Several other techniques have been used in conjunction with census methods to assess the impact of pesticide applications on bird populations. These techniques also have their drawbacks. Brain ChE determinations appear to be an excellent means of monitoring exposure of birds to ChE inhibitors and diagnosing related mortality (Ludke et al. 1975). However, brain ChE assays have only recently been included in field investigations of the effects of applications of organophosphates and carbamates (Zinkl et al. 1977, 1979, 1980; DeWeese et al. 1979; Richmond et al. 1979) and relationships between sublethal ChE inhibition ($\geq$20%) and changes in bird behavior are poorly known.

Carcass searches may be a necessary tool (Heinz et al. 1979). If dead birds are observed after a pesticide application, it is essential to obtain samples so that the cause of the mortality can be confirmed and the magnitude of the kill estimated. Searching for carcasses is seldom easy and requires much time, skill, and motivation (Heinz et al. 1979). Considering the difficulty in locating carcasses and the rapidity with which they may disappear (Davis 1970), location of a small number of dead birds may be reason to suspect some unusual cause of mortality (Rosene and Lay 1963, Heinz et al. 1979).

Nesting studies are probably the most effective technique used in evaluating the impact of pesticide applications. None of the methods dis-

cussed previously is sufficiently sensitive to assess the potential subtle effects of pesticide exposure on bird behavior and reproduction. Though we are aware of only two studies which have examined the effects of ChE inhibitors on avian reproductive behavior in detail (Grue et al., MS; and this study), both have reported significant effects. In the former study, care of nestlings by OP-dosed wild female Starlings was significantly reduced. Under most field situations, however, it is difficult to obtain adequate reproductive data on enough nests of one or more species to permit statistical analysis. Time and manpower may be limited and nests may be scarce, hard to locate, or inaccessible. We believe the use of nest boxes may enhance the capability of investigators to collect reproductive data before and after pesticide applications. Though nest boxes have been used effectively in studies of avian ecology (e.g., Dahlsten and Copper 1979) and the effects of DDT on passerine reproduction (Mitchell et al. 1953, McCluskey et al. 1977), few investigators (Black and Zorb 1965, Bednarek and Davidson 1967, Powell and Gray 1980) have utilized them to study the effects of ChE inhibitors. In addition, reproductive data may be collected automatically from nest boxes using a variety of electronic devices (Royama 1959, Dahlsten and Copper 1979).

Considering the potential difficulties in interpreting the results of conventional censusing techniques following pesticide applications, we recommend, as have others (DeWeese et al. 1979, Richmond et al. 1979), that future studies concentrate on quantifying pesticide exposure and its effects on avian behavior and reproductive success. Carcass searches may provide valuable additional information if mortality of adult birds is suspected.

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