Effect of Stress on Dieldrin Toxicity to Male Redwinged Blackbirds (*Agelaius Phoeniceus*)

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STICKEL et al. (1969) pointed out that field-collected dead birds which are believed to have died from dieldrin poisoning often have lower residue levels of dieldrin in their brains than do birds poisoned experimentally. One hypothesis by these authors is that uncaged birds living under field conditions experience greater stress* and/or exertion and this somehow increases the lethality of the residues. The experiment described below was undertaken to test this hypothesis.

MATERIALS AND METHODS

Experimental procedures. Four groups of 30 male redwinged blackbirds were each separately confined in large, outdoor cages (inside dimensions 2 m high, 1.8 m wide, 3.5 m long) that provided shelter from rainfall. Each cage contained 6 perches 3 m in length. From a row of cages, I chose 2 pairs of adjacent cages separated by 6 empty cages. Birds were assigned to cages by a random number procedure. Birds of one cage in each adjacent pair were fed a diet containing 10 parts per million (ppm) aldrin (95% pure 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene). Birds of both cages of one pair were stressed, whereas those of the other pair were not. The 4 treatments will be referred to as: control-no stress, aldrin-no stress, control-stress, aldrin-stress.

Birds were weighed and placed into cages on 15-16 May 1973; treatment (i.e., dosing with aldrin and stressing) began 29 May 1973. Stressing consisted of the investigator or an assistant entering the cage and disturbing the birds so that they flew and hopped about continuously for a period of 4 minutes. On 4 June one bird died, apparently from overheating, in each stress cage. Thereafter, stress periods were shortened to 3 minutes. There were 5 stress periods daily at approximately 7:30 a.m., 9:45 a.m., 12 noon, 2:15 p.m., and 4:30 p.m.

* In the present paper, the term "stress" is used in a general sense in referring to both the harassment that some birds received and to their presumed, but unmeasured, physiological reactions to harassment.

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On 7 July 1973 the last aldrin-stress bird died, whereupon the remaining 27 control-stress birds were weighed and 10 were randomly selected, killed, and frozen. Two control-stress and 2 control-no stress birds died of unknown causes.

Dosage of the aldrin-no stress birds continued until 13 August when 3 remained alive but none had died since 20 July. At this time the final 3 were killed and the 28 control-no stress birds were weighed and 10 were randomly selected, killed, and frozen.

All birds which died were weighed, frozen, and dissected later.

Feed preparation. Aldrin was dissolved in corn oil, which then was mixed into the feed (commercial medicated turkey starter crumbles) with a large mechanical mixer to produce a dry feed containing 10 ppm aldrin. Feed and water were provided *ad libitum.*

Source of birds. All birds were captured near Laurel, Maryland. They were acclimated to captivity and appeared healthy at the beginning of the experiment.

Sample preparation. Birds to be analyzed had feathers, skin, beak, feet, and gut removed and discarded. The brain was removed and analyzed separately from the remaining carcass. Samples were placed in clean glass jars, weighed, frozen, and shipped to WARF, Inc., Madison, Wisconsin for determination of dieldrin (1,2,3,4, 10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) and fat content.

Residue analyses. Analyzed were brains and carcasses of all 60 aldrin-dosed birds and brains of 7 control-no stress and 8 control-stress birds.

Samples were weighed, homogenized, mixed with sodium sulfate, and air dried for 36 to 48 hours. Samples were then extracted with petroleum ether:ethyl ether (170:70) in Soxhlet apparatus for 8 hours. Aliquots were placed on a Florisil column (petroleum ether:ethyl ether 150:5 followed by 250:15). Analysis was by electron-capture gas chromatography on a Barber-Coleman Pesticide Analyzer model 5360. The column was glass, 1219 mm by 4 mm, packed with 5% DC-200 on 80/100 mesh Gas Chrom Q. Injector temperature was 230°C; column was 200°C; and detector was 240°C. The carrier gas was nitrogen at a flow rate such that dieldrin had a retention time of 3 to 4 minutes. Lipid weight was determined from an aliquot of the extract which was dried on a steam bath and placed in a 40°C oven 2 to 4 hours before weighing.

Limit of sensitivity (ppm wet weight) was 0.005. Recovery from spiked samples was 77%; analytical readings were not corrected for recovery. Confirmation consisted of running

duplicate samples for 18 carcasses. All residues are expressed as ppm of wet sample weight.

Data excluded. Results from 6 aldrin-dosed birds have been omitted from all statistical analyses (summarized in Figs. 1-3), but they are given separately in appropriate sections of the text. These 6 include 3 aldrin-no stress birds which had not died after 76 days on dosage and 3 aldrin-stress birds (the first and the last 2 to die) which are believed to have died of overheating caused by excessive stressing. The first aldrin-stress bird to die was excluded because its death occurred during the late afternoon stress period when air temperature was high and because a control-stress bird also died at the same time; both apparently died of overheating. Furthermore, dieldrin residue in the brain of the aldrin-stress bird was very low (0.43 ppm). The last 2 aldrin-stress birds had brain levels of dieldrin (6.87, 12.3 ppm) below those of all other 27 birds in this group even though those dying previously showed a significant rise in brain dieldrin level with days on dosage. Also, the assistant present when these last 2 died noted that their behavior indicated overheating rather than dieldrin as the cause of death.

Statistics. Means are given with 1 standard error. Statistical significance levels: 1 asterisk $0.05>P>0.01$; 2 asterisks $P<0.01$. Regression lines were fitted by least squares method.

RESULTS

Brain levels of dieldrin. Figure 1 summarizes dieldrin residues in brains relative to days to death on dosage. The plot for unstressed birds was curvilinear and asymptotic at about 24 to 25 ppm but was made linear by log transformation of days. Analyzed by covariance, the amount of dieldrin in the brain required to kill is greater in stressed than in unstressed birds (i.e., elevations of regression lines are different $F = 13.85^{**}$; slopes are not $F = 1.25$). Means of days to death are 29.9 ± 2.7 days for unstressed and 22.0 ± 1.1 days for stressed birds. Means of residue levels are 19.8 ± 1.7 ppm for unstressed birds and 22.2 ± 0.8 ppm for stressed birds. The 3 unstressed birds killed at the end of the experiment had brain dieldrin levels of only 6.70, 7.28, and 7.40 ppm.

Although the data for redwinged blackbirds (Fig. 1) show highly significant correlations between concentration of dieldrin in the brain and days to death, ROBINSON et al. (1967) found no such correlations after feeding dieldrin to Japanese quail (*Coturnix coturnix japonica*), and domestic pigeons (*Columba livia*). STICKEL et al. (1969) also found no correlation after feeding dieldrin to Japanese quail, but these authors noted that "The first bird to die in each sex and treatment group had the

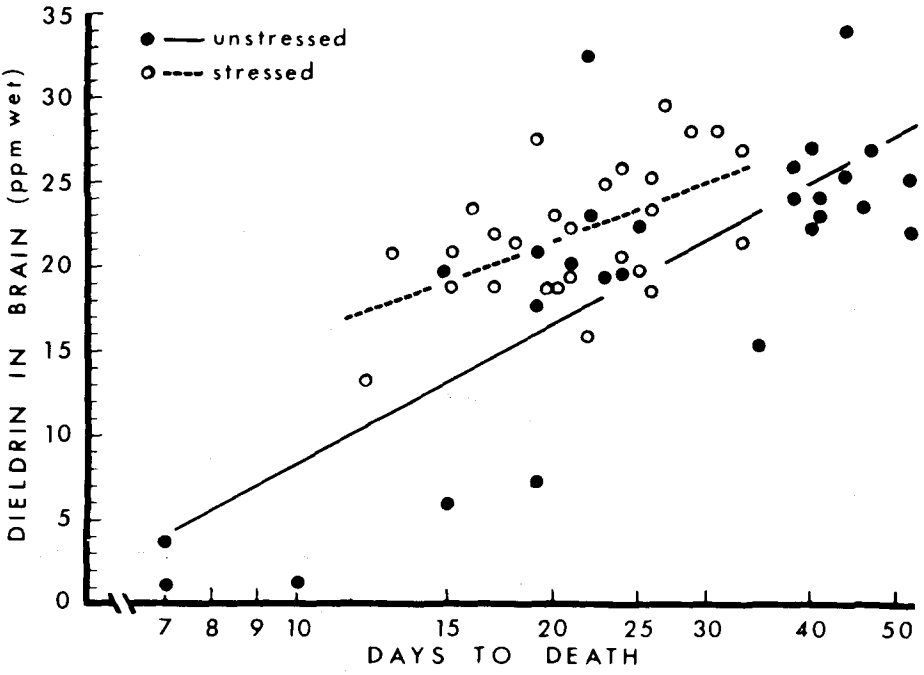

Figure 1. Dieldrin residues in brain versus days to death (log scale) for male redwinged blackbirds dosed with aldrin. Unstressed: Y = 27.6 log X - 19.3; r = 0.797 ($P<0.001$); $r^2(100) = 63.5\%$. Stressed: Y = 18.4 log X - 2.18; r = 0.545 ($0.01>P>0.001$); $r^2(100) = 29.7\%$.

lowest brain residue for that group." This discrepancy may have occurred because poisoning of redwinged blackbirds continued until mortality was essentially complete whereas the other studies ended prior to this point.

Carcass levels of dieldrin. Dieldrin in carcasses increased linearly with time (Fig. 2) and showed no tendency to level off as was seen in brains of unstressed birds. In fact, the 3 unstressed birds killed at the end of the experiment contained 70.3, 82.8, and 147 ppm. Elevations of the regression lines are significantly different ($F = 15.49^{**}$; slopes are not $F = 0.02$). Thus stressed birds at death contained significantly less dieldrin in their carcasses and more in their brains than did unstressed birds.

Weight loss. Birds fed aldrin experienced pronounced loss of weight. Mean weight loss at death was 30.7 ± 1.2% for unstressed birds (n = 27) and 30.1 ± 0.9% for stressed birds (n = 27). Twenty-eight control-no stress birds had a mean weight loss of 13.7 ± 1.1% on 13 August, and 27 control-stress birds had lost an average 15.3 ± 0.8% on 9 July.

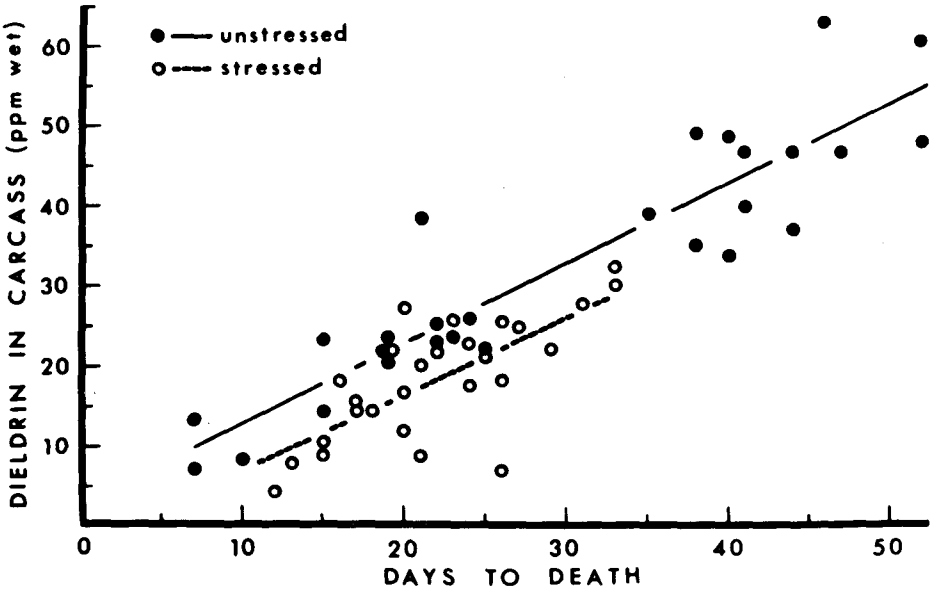

Figure 2. Dieldrin residues in carcass versus days to death for male redwinged blackbirds dosed with aldrin. Unstressed: $Y = 0.997 X + 3.14$; $r = 0.915$ ($P<0.001$); $r^2(100) = 83.8\%$. Stressed: $Y = 0.966 X - 2.66$; $r = 0.737$ ($P<0.001$); $r^2(100) = 54.3\%$.

Per cent loss of weight by aldrin-dosed redwings was not significantly correlated with days to death as ROBINSON et al. (1967) found in Japanese quail. However, the correlation coefficient for aldrin-no stress birds was 0.373; $0.1>P>0.05$.

Lipid levels. The amount of carcass fat was significantly greater in birds that survived longer in both stressed and unstressed groups once an initial period of rapid fat-loss was passed (Fig. 3). These data suggest that dieldrin alone caused marked fat-loss within 16 days and that stress hastened this loss. Presumably, the birds that survived longest did so partly because they were able to retain more fat during the fat-loss period. However, the 4 unstressed birds with high fat levels which died early indicate that high fat content alone does not insure survival. These 4 appear to represent a more sensitive component of the population, since they are the same 4 which had the lowest brain levels of dieldrin at death. The 3 unstressed birds killed at the end of the experiment contained 10.7, 15.3, and 19.3% fat. The final 2 stress birds to die (apparently by overheating due to excessive stressing) contained 3.2 and 6.2% fat.

Stressed birds had significantly less fat than did unstressed birds (Fig. 3; elevations are different $F = 11.09^{**}$, but slopes are not $F = 0.04$).

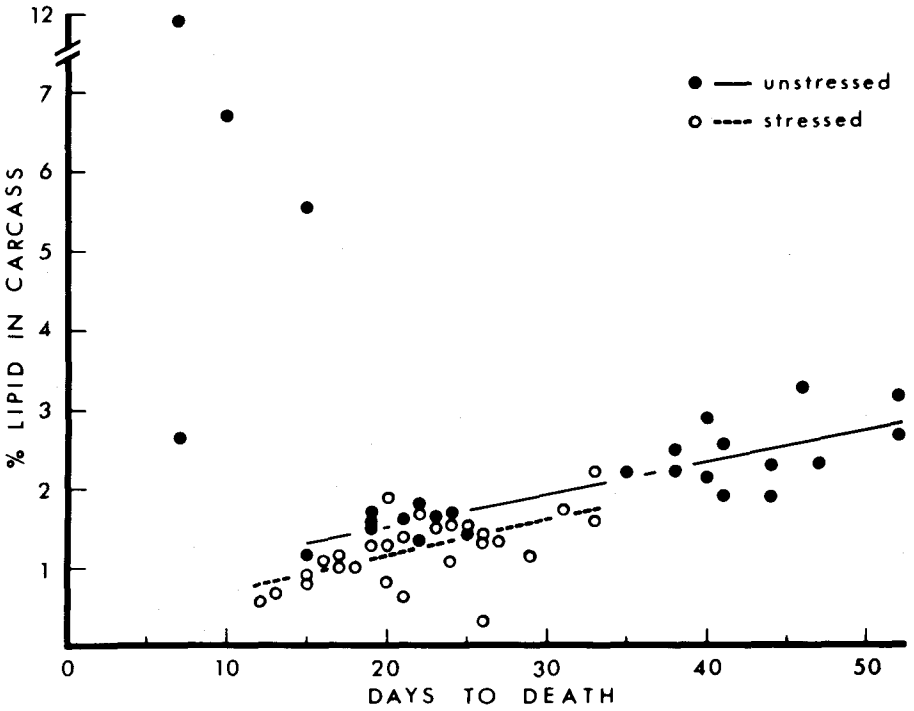

Figure 3. Per cent lipid in carcass versus days to death for male redwinged blackbirds dosed with aldrin. Unstressed (omitting 4 values at upper left): $Y = 0.041 X + 0.704$; $r = 0.848$ ($P<0.001$); $r^2(100) = 71.9\%$. Stressed: $Y = 0.044 X + 0.262$; $r = 0.585$ ($0.01>P>0.001$); $r^2(100) = 34.2\%$.

Fat content of brains was not correlated with days to death, and it was not significantly different between the groups: unstressed mean 4.37 ± 0.07% fat (n = 27); stressed mean 4.57 ± 0.08% fat (n = 27); $t = 1.97$, $0.1>P>0.05$.

DISCUSSION

Stressed redwinged blackbirds died in a shorter time and had higher brain levels of dieldrin than did unstressed redwings (Fig. 1). DRIEVER et al. (1966) demonstrated stress-induced increases (via the pituitary-adrenal axis) in liver metabolism of certain drugs in the laboratory rat; reduced blood serum levels of the drugs were measured. Perhaps a related phenomenon occurred in the stressed redwings with dieldrin metabolism being accelerated in such a manner that increased amounts occurred in the brain at death.

Clearly, the hypothesis that additional stress under field conditions causes lethal brain levels of dieldrin below those observed in caged experimental populations is not supported by this experiment. What, then, might account for these lower

lethal levels? STICKEL et al. (1969) offered another hypothesis, "...the individuals that will die with the lowest brain residues tend to be the ones that die first. Such individuals may be over-represented among animals found dead in the field unless mortality is sweeping." The strong correlations between days to death and brain levels (Fig. 1) favor this latter hypothesis.

Combining the fact that brain residues of dieldrin which appear to have been lethal in wild, free-ranging birds range down to 4 or 5 ppm (STICKEL et al. 1969) with the present conclusion that stress increases rather than decreases brain levels at death leads to the speculation that residues in unstressed redwings resemble more closely those of wild, free-ranging birds killed by dieldrin than do residues in stressed redwings. Furthermore, it may be that wild birds normally experience less stress than did the unstressed (but caged) redwings of this experiment. Thus it is conceivable that brain levels for these birds are somewhat elevated in relation to what they would be if the birds were free-ranging.

If the 5 unstressed redwings that died with low brain residues of dieldrin (i.e., 1.11 to 7.32 ppm) were diseased or otherwise weakened birds, rather than merely being especially dieldrin-sensitive individuals, it seems that a corresponding group of such weak birds should have died even earlier among the stressed redwings.

In conclusion, the hypothesis that the most sensitive individuals in a bird population (i.e., those which die first and with the smallest residue levels in their brains) are the most likely to be found (and analyzed) is better supported by present data than is the hypothesis that stress heightens the lethality of dieldrin residues in the brains of birds.

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