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Acute and Sublethal Effects of 1080 on Starlings

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Sodium monofluoroacetate (Compound 1080) is highly toxic to mammals and has been used widely for control of rodents and mammalian predators (ATZERT 1971). In 1972 many uses of 1080 were cancelled due, in part, to mortalities that were reported among non-target animals (RUCKELSHAUS 1972). Though 1080 baits and dead poisoned animals may be available to birds during avian breeding periods, we are unaware of any investigation of long-term exposure in birds or possible effects on avian reproduction. Such studies are of particular interest as testing with rats (MAZZANTI et al. 1965, SULLIVAN et al. 1979) has shown testicular lesions and atrophy resulting from sublethal 1080 exposure. Our study was undertaken to investigate the short-term acute and long-term sublethal effects of 1080 on European starlings (Sturnus vulgaris) with emphasis on testicular morphology.

MATERIALS AND METHODS

Adult starlings were trapped on the grounds of the Agricultural Research Center (U. S. Department of Agriculture), Beltsville, Maryland, during January and February of 1981. The birds were maintained in a sheltered, unheated aviary (3 x 4 x 3 m) on a 12L:12D light regime. Birds had continuous access to water and a nutritionally balanced commercial bird feed (Beacon's Turkey Crumble).

Test diets were prepared by dissolving technical 1080 [90% active ingredient (a.i.)] in 10 ml of water and emulsifying this solution in a corn oil/lecithin (50:50) carrier. The amount of oil/lecithin was 5% of the total desired diet weight (e.g., 50 g in a 1 kg diet mixture). The carrier was thoroughly mixed with the bird feed in a commercial food mixer.

The experiment consisted of a short-term acute tox-

icity feeding study (5-day dietary LC_{50} test) and a 4-week sublethal feeding study. The acute study was run first to permit estimation of a sublethal dietary pesticide concentration for the long-term exposure. For the short-term study 36 starlings were selected at random from the aviary and transferred to wire cages (1.2 x 1 x 1 m) without regard to sex. Six birds were placed in each cage and constituted one treatment level. They were acclimated to the new cages for 5 days prior to testing. Five geometrically spaced treatment levels (27, 54, 108, 216 and 432 ppm a.i.) and a control diet were used. Birds were observed daily for signs of intoxication and mortality.

For the 4-week study 27 male starlings were weighed, banded with numbered plastic leg bands, and distributed randomly to 5 test cages (1.2 x 1 x 1 m). Three additional males were sacrificed to determine pre-exposure testis development. The birds had food and water continuously available for a 5-day acclimation period prior to testing. A test diet of 15 ppm technical 1080 (13.5 ppm a.i.) was prepared as described previously. Fifteen starlings received the test diet and 12 were used as controls. Two 1080 feed samples were submitted to the Denver Wildlife Research Center (U.S. Fish and Wildlife Service) for analysis and found to contain 13.5 $\pm$ 1.5 ppm of the pesticide each (I. Okuno pers. comm.). Food consumption was measured during the first two weeks of exposure.

In addition to the extensive window light available in our laboratory the ambient photoperiod was extended during the 28-day study to hasten gonad development. Lighting was provided by standard fluorescent bulbs which were positioned approximately 10 cm above the cages and were connected to automatic timers. Birds received 12 hours of light daily during the acclimation period, 14 hours during the first treatment week and 17 hours the remainder of the study. At the conclusion of the exposure period the birds were sacrificed and weighed. Testes were removed and immediately placed in a 10% buffered formalin solution. Approximately 1 hour after sacrifice testes were temporarily removed from the formalin solution and weighed. Testes were imbedded in paraffin, sectioned by standard histological techniques and stained with hematoxylin and eosin.

Statistical analyses were done with SAS (Statistical Analysis System, SAS Institute, Raleigh, NC 27605)

procedures GLM (general linear models), CORR (Pearson product-moment correlation) and PROBIT on an IBM 370 computer. As the probit method of LC_{50} calculation can only be used when 2 or more dose levels produce partial mortality, the binomial method (STEPHAN 1977) was used for LC_{50} determinations at 72, 96 and 120 hours (Table 1).

RESULTS AND DISCUSSION

The short-term dietary study demonstrated that 1080 is highly toxic to starlings. The 5-day LC_{50} was 47 ppm. All birds (6/6) were killed in the 432 ppm level at 48 hours and by 72 hours mortality was complete in the 216 and 108 ppm levels as well (Table 1). Two mortalities occurred in the 54 ppm group on test day 2 and by day 5 had increased to 4 (4/6). No mortalities occurred in the 27 ppm group nor were toxic symptoms observed at this level thoughout the 5-day test period. All controls survived and appeared healthy.

Table 1. Starling mortality and associated statistics during a 5-day acute toxicity feeding trial with Compound 1080. The number of dead birds are shown relative to the number exposed at each concentration.

	0	24h	48h	72h	96h	120h
Control	0/6	0/6	0/6	0/6	0/6	0/6
27 ppm	0/6	0/6	0/6	0/6	0/6	0/6
54 ppm	0/6	0/6	2/6	3/6	3/6	4/6
108 ppm	0/6	2/6	4/6	6/6	-	-
216 ppm	0/6	3/6	4/6	6/6	-	-
432 ppm	0/6	5/6	6/6	-	-	-
LC_{50}		198[a]	97[a]	54[b]	54[b]	47[b]
95% C.L.		119-400	53-170	27-108	27-108	27-108
Slope		3.0	2.7	10.5[c]	10.5[c]	11.0[c]

[a] Probit analysis
[b] Binomial Method (STEPHAN 1977)
[c] Graphical estimate

Toxic symptoms appeared in tne 432 ppm group within 4 hours of exposure to the test diet and in other groups on the second day (>24h). Symptoms were similar in affected birds at each test concentration and included reduced activity, prostration, tremors and convulsions.

Typically, (HILL et al. 1975) short-term avian dietary studies consist of 5 days of toxicant exposure followed by 3 days of basal diet. As this study was largely directed toward providing information for the 4-week study to follow, we deviated from the standard protocol and after the fifth exposure day combined the birds surviving the 54 ppm test level (2/6) with those in the 27 ppm group and continued the test exposure (27 ppm) for an additional 5 days. During this period no mortalities occurred nor were toxic symptoms observed.

The test concentration for the 4-week study was 13.5 ppm: 1/2 the 'no-effect' level in the acute study. Throughout the 28-day exposure no behavioral toxic symptoms were observed, however, a starling was found dead after 9 days of exposure to the 1080 diet. On day 19 of the study the thermostatically controlled windows failed to open and the temperature in the test chamber temporarily reached 43° C. When the problem was discovered 1 treatment and 1 control bird were dead. All remaining birds survived the duration of the study without incident.

Scattering of food by starlings made estimations of consumption approximate. Monitoring during the first two weeks of exposure did not indicate differences between treatment and control groups (control = treatment = 24 g/bird/day). These data suggest a daily dosage of 0.32 mg 1080/bird.

All birds lost weight during the exposure period (3/3/81 - 3/30/81). A weight loss in starlings occurs normally in spring (R.G. SCHWAB pers. comm.). Control birds, with a mean body weight of 85.9 g (SD=5.5) at the initiation of the study, lost an average of 7.6 g (SD=3.9) by the conclusion of the test. Treatment birds began the study with an average weight of 84.8 g (SD=3.9) and over the 4-week exposure lost slightly more than controls ($\bar{x}$=8.9 g, SD=2.3). Analysis of variance indicated that the body weights of the control and treatment birds were not different at the beginning of the study (P>0.3). In addition, the difference in weight loss between groups was not significant (P>0.35). However, the starlings receiving 1080 weighed ($\bar{x}$=75.9

g, SD=3.1) significantly less than controls ($\bar{x}$=78.9 g, SD=3.7) at the time of sacrifice (one-tailed F test, P<0.05). As the treatment birds had a mean weight 1.1 g less than controls at the beginning of the test and weight loss was significantly different, the terminal body-weight difference is not clearly treatment related.

Pre-exposure testis weights averaged 0.0143 g (n=3, SD= 0.0036). Terminal mean testis weights (Table 2) indicated a 29- to 41-fold increase in organ weight by the end of the study. Testis weights were not well correlated with body weights (left testes vs body weights: r= 0.36, P>r=0.09; right testes vs body weights: r=0.27, P>r= 0.21) therefore analyses of treatment effects were based directly on organ weights, not organ/body weight ratios. The mean testis weights (right and left) of birds fed 1080 were lower than control means. Analysis of variance tests indicate that observed weight differences are not statistically significant (Table 2).

Examination of the testis sections by light microscope did not reveal lesions nor suggest significant differences in sperm abundance or development.

HILL et al. (1975) investigated the dietary toxicity of 131 environmental pollutants, mostly pesticides, to several bird species. Only twelve of the substances tested showed 5-day LC_{50} values lower than that determined here for starlings (47 ppm). These authors give probit dose/response slopes for most of their avian 5-day LC_{50} values. Using all the slopes reported (N= 251) we calculated a mean slope of 5.85 (SD=2.50).

Table 2. Average testis weights, standard deviations and F statistics for starlings receiving treated (13.5 ppm 1080) and control diets for 28 days.

Treatment	Side	No.	Mean Weight (g)	SD	F	P*
1080	Right	13	0.495	0.148	2.05	0.08
Control	Right	11	0.590	0.180		
1080	left	13	0.509	0.172	1.33	0.13
Control	left	11	0.578	0.112		

*Probability > F, One-tailed test

We find, by comparison, that 1080 has a relatively steep slope (>10) after 5 days of exposure. The response slope is so steep that a dietary concentration of 54 ppm was fatal to 2 of 6 birds after 2 days exposure, 3 of 6 after 3 days and 4 of 6 birds at 5 days, but a diet containing 27 ppm produced no mortality or overt toxic symptoms after 10 days. Similarly, in the long-term feeding trial conducted at 13.5 ppm, only 1 of 15 starlings was considered killed by 1080 after 28 days exposure.

ATZERT (1971), in his review of the toxic properties of 1080, indicates that some bird and mammal species accumulate sublethal doses to toxic levels while, conversely, repeated sublethal doses in other species have appeared to yield a tolerance for 1080 in subsequent 'lethal' doses. He reports that studies with rats have shown that over 30% of administered doses can be metabolized to non-toxic metabolites or excreted. Our testing indicates that starlings tolerate doses in the 13.5 to 27 ppm range for relatively long periods, but concentrations one geometric step higher (54 ppm) are quickly fatal. This suggests to us that starlings may be able to detoxify or effectively excrete 1080 below a certain threshold.

Bird testes undergo marked annual enlargement and regression. SCHWAB (1965) studied testis development in starlings exposed to natural light in California. Sacrificing birds monthly from February to June he found that starling testes developed from a mean of 0.032 g in February to a peak mean weight of 0.65 g in April. This peak weight is within 0.81 and 1.05 standard deviations of the 1080-testis means and within 0.33 and 0.64 standard deviations of the control means (Table 2) indicating that starlings in the current study approached full development.

Starlings fed 1080 showed roughly 14% less testis weight development than controls but the difference was not statistically significant. This result suggests a substantial difference in sensitivity between birds and mammals as testing with rats (SULLIVAN et al. 1981) at a lower doseage (0.14 mg/rat/day vs 0.35 mg/starling/day), for a shorter period (7 days vs 28 days), resulted in significant reductions in testis weights (>50%) and pronounced morphological lesions.

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