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Short Communication

REPRODUCTION AND HEALTH OF MALLARDS FED ENDRIN

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Abstract—Concentrations of 0, 1 and 3 ppm endrin in dry duck mash were fed to mallards (*Anas platyrhynchos*) starting in December, and health and reproduction were measured the following spring and summer. One male fed 3 ppm endrin died with 2.0 ppm endrin (wet weight), a diagnostically lethal level, in its brain. Birds fed 1 ppm endrin reproduced as well as, if not better than, controls. Birds fed 1 ppm endrin had significantly greater hatching success of fertile eggs than did those fed 0 or 3 ppm, and their clutches hatched significantly earlier than did those of birds fed 3 ppm. Mallards fed 3 ppm endrin appeared to reproduce more poorly than controls, but this finding must be regarded with caution because the results of statistical tests often were not significant. Endrin accumulated in eggs to a mean of 1.1 and 2.9 ppm (wet weight) when fed to hens at 1 and 3 ppm. The concentration of endrin in the carcasses of adults was similar to that in eggs, but the concentration in the fat of adults was about 4 to 7 times higher than in eggs.

Keywords—Endrin Mallard Reproductive effect

INTRODUCTION

Endrin is an organochlorine pesticide that is highly toxic to birds. Hill et al. [1] reported that median lethal dietary concentrations in 5-d tests with young northern bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), ring-necked pheasants (*Phasianus colchicus*) and mallards (*Anas platyrhynchos*) were 14, 18, 14 and 22 ppm, respectively. In the wild, endrin has been reported at lethal levels in the brains of dead bald eagles (*Haliaeetus leucocephalus*) and American white pelicans (*Pelecanus erythrorhynchos*) [2]. Blus et al. [3] reported mortality in many species of birds when orchards were sprayed with endrin to control rodents.

Less is known about the effects of endrin on reproduction in birds. In 1981 and 1982, endrin was used as an insecticide in western states and there was concern over possible reproductive effects in waterfowl [4].

The purpose of our study was to determine whether endrin in the diet of penned adult mallards adversely affects reproduction and health.

METHODS

On 7 December, one pair of 1-year-old game farm mallards was randomized to each of 81 1-m^2 outdoor pens. Each pen was equipped with a feeder, water pan and nest box. After an 8-d acclimation period, the 81 pairs were randomly divided into three groups of 27. These three groups were randomly assigned to diets containing 0, 1 or 3 ppm endrin in a dry duck mash. Endrin was dissolved in propylene glycol and mixed into a commercial duck mash so that the mixed feed contained 1% propylene glycol. Control birds received 1% uncontaminated propylene glycol in their diet. Chemical analyses of three feed samples from each treatment were used to verify the dietary concentrations. Endrin was not detected in any of the control samples. The diet mixed to contain 1 ppm endrin contained from 0.74 to 0.95 ppm endrin, and samples from the 3 ppm diet contained from 2.6 to 3.2 ppm endrin.

Hens were allowed to incubate their eggs. However, if a hen laid more than 20 eggs in her nest, if none of her eggs were fertile or if she deserted her nest, we allowed her to nest again. For three females fed 1 ppm endrin and three fed 3 ppm endrin, we examined endrin residues in the sixth

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egg of each clutch laid. There were no consistent trends in endrin levels from early to late clutches of the same female. Males were removed from the pens after 7 d of incubation but were kept on treated diets. Males were returned to the breeding pens in cases in which hens were allowed to begin egg laying again. Ducklings were fed a commercial duck starter mash containing the same concentration of endrin as that of their parents. Ducklings and their parents were killed when the young were 21 d old. The study was terminated on 21 July.

We collected data on (a) adult weights at the start of treatment and at death, (b) the number of hens completing incubation, (c) hatching dates, (d) clutch size, (e) fertility of eggs, (f) hatching success of fertile eggs, (g) survival of ducklings to 21 d of age and (h) weights of ducklings at hatching and 21 d of age. The proportions of hens in each treatment completing incubation were compared using a chi-square test. Other measurements were compared using analysis of variance; when there was a significant difference at $\alpha = 0.05$, means were separated by the Bonferroni multiple comparison test [5]. Percentage data were subjected to angular transformation before statistical analysis.

The sixth egg from the incubated clutch or the last clutch laid before the hen was killed was saved. Egg weight and eggshell thickness were compared among groups by analysis of variance. Egg contents were analyzed for endrin for all treated hens plus three controls. A two-tailed t test was used to compare endrin residues in the eggs of females that succeeded in producing at least one 21-d-old duckling with residues in the eggs of unsuccessful hens; a separate t test was run for the 1 and 3 ppm endrin treatments.

Samples of abdominal fat and the carcasses from three pairs of controls and seven pairs of birds fed 1 or 3 ppm endrin were analyzed for endrin. Carcasses were prepared by removing the feathers, wing tips, feet, bill and gastrointestinal tract. A two-tailed t test was used within each treatment to compare endrin residues in the fat or carcass of males with residues in females. The brains of two males that died in the 3 ppm endrin treatment were analyzed for endrin to determine whether a lethal level of endrin was present.

Sample preparation, extraction and cleanup on Florisil have been described in detail by Cromartie et al. [6]. Feed samples were extracted by adding a 1.0-g aliquot to 25 ml methylene chloride (pesticide-grade; Fisher) and shaking for 1 h on a Precision Equipoise shaker. The methylene chloride was then filtered, concentrated to approximately 4.0 ml and eluted through a Florisil column.

For all samples, the Florisil column eluent was quantified by electron-capture gas-liquid chromatography using a 1.83 m × 4 mm i.d. glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport at 210°C. Endrin was quantified to 0.05 ppm.

RESULTS

One pen of controls and one of birds fed 3 ppm were eliminated from the analysis of reproductive success because of mortality unrelated to treatment. Two other males fed 3 ppm endrin died on 28 April. Their brains contained 0.47 and 2.0 ppm endrin on a wet-weight basis. The female in the same pen as one of these males had already started to incubate her clutch and later hatched her eggs, so data on her eggs and ducklings were included in the statistical analyses.

There were no differences among the three groups in the weights of males or females at the start of endrin treatment or at the time of sacrifice (Table 1). Egg weight and eggshell thickness also were unaffected by endrin (Table 2).

Hens fed 1 ppm endrin hatched their clutches significantly earlier than did birds fed 3 ppm endrin, but not significantly earlier than controls (Table 3). Birds fed 1 ppm endrin also had significantly greater hatching success of fertile eggs than did controls or birds fed 3 ppm endrin. In addition, fewer hens fed 3 ppm endrin completed incubation, their young had the lowest survival rate and they produced the fewest 21-d-old ducklings of any group, but these differences were not significant. There were no differences among the three treatment groups in duckling weights at hatching or at 21 d of age (Table 4).

The eggs of 17 hens fed 1 ppm endrin that successfully produced at least one 21-d-old duckling contained 1.0 ± 0.08 ppm endrin (mean ± SE), which was not significantly different from the 1.1 ± 0.08 ppm in the eggs of 10 unsuccessful hens. The eggs of 12 successful hens fed 3 ppm endrin contained 2.6 ± 0.20 ppm endrin, which was nearly significantly different ($p = 0.052$) from the eggs containing 3.2 ± 0.22 ppm endrin of 13 unsuccessful hens.

Endrin was detected in only one control sample; the fat of one female contained 0.26 ppm on a wet-weight basis (Table 5). Levels of endrin in the eggs of birds fed 1 or 3 ppm endrin were similar to the levels in the carcasses of adults, but the fat of adults contained about 4 to 7 times more

Table 1. Weights (mean ± SE) of adult mallards fed diets containing 0, 1 or 3 ppm endrin

Endrin added to diet (ppm)	Body weight[a] (g)			
	Males		Females	
	At onset of treatment	At sacrifice	At onset of treatment	At sacrifice
0	1149 ± 16.7 a (26)	1167 ± 31.3 a (25)	1063 ± 20.7 a (26)	1127 ± 31.8 a (26)
1	1181 ± 16.0 a (27)	1252 ± 26.7 a (27)	1073 ± 17.4 a (27)	1171 ± 26.2 a (27)
3	1172 ± 19.2 a (26)	1232 ± 18.5 a (24)	1069 ± 16.1 a (27)	1104 ± 26.2 a (26)

[a]Means in the same column that share a letter in common were not significantly different at $\alpha = 0.05$ by analysis of variance. Sample size in parentheses.

Table 2. Whole-egg weights and eggshell thickness (mean ± SE) of the sixth eggs laid by mallards fed diets containing 0, 1 or 3 ppm endrin

Endrin added to diet (ppm)	n[a]	Egg weight[b] (g)	Eggshell thickness[b] (mm)
0	26	60.1 ± 1.76 a	0.37 ± 0.007 a
1	27	58.5 ± 1.12 a	0.37 ± 0.005 a
3	25	59.4 ± 1.04 a	0.37 ± 0.004 a

[a]Number of hens.
[b]Means in the same column that share a letter in common were not significantly different at $\alpha = 0.05$ by analysis of variance.

endrin than did the eggs. Males fed 3 ppm endrin had significantly more endrin in fat than did females. There were no other differences between males and females.

DISCUSSION

The early hatching dates and improved hatching success of eggs of birds fed 1 ppm endrin indicate that this level of the compound may have had a stimulatory effect on mallards. Stimulatory effects of endrin and other compounds have been described by Kreitzer [7].

Fleming et al. [8] reported a reduction in repro-

Table 3. Reproductive success of mallards fed diets containing 0, 1 or 3 ppm endrin

Measurement	Endrin added to diet[a] (ppm)		
	0	1	3
Pairs of breeders	26	27	26
Hens completing incubation	18 a	17 a	13 a
Hatching date[b] (number of days after 1 January)	146 ± 6.3 ab	129 ± 5.9 a	154 ± 8.8 b
Clutch size[b]	15.2 ± 0.92 a	12.9 ± 0.96 a	14.3 ± 1.21 a
Percent fertility of eggs[b]	98.6 a (94.4–100)	96.4 a (87.3–100)	97.9 a (91.9–100)
Percent hatch of fertile eggs[b]	65.9 a (50.1–80.0)	91.4 b (82.6–97.3)	64.0 a (42.9–82.5)
Percent survival of ducklings to 21 d of age[b]	91.6 a (73.6–99.8)	99.0 a (96.1–100)	87.9 a (65.0–99.4)
Number of ducklings surviving to 21 d of age[b]	8.2 ± 1.08 a	9.4 ± 2.27 a	6.8 ± 1.20 a

[a]Means in the same row that do not share a letter in common were significantly different at $\alpha = 0.05$ by analysis of variance followed by the Bonferroni multiple comparison test or, in the case of "Hens completing incubation," by a chi-square test. Means are shown with standard error of the mean or, for percentage data, 95% confidence interval. Means and 95% confidence intervals for percentage data were retransformed from angular-transformed values.
[b]Based on the number of hens completing incubation.

Table 4. Weights (mean ± SE) of ducklings fed diets containing 0, 1 or 3 ppm endrin

Endrin added to diet (ppm)	Duckling weight[a] (g)			
	n[b]	At hatch	n	21 d old
0	16	38 ± 0.9 a	16	367 ± 10.9 a
1	17	38 ± 0.8 a	17	366 ± 9.2 a
3	13	35 ± 1.0 a	12	373 ± 13.6 a

[a]Means in the same column that share a letter in common were not significantly different at $\alpha = 0.05$ by analysis of variance.
[b]The number of hens with ducklings represented. A mean weight for all ducklings from the same hen was calculated.

Table 5. Endrin (ppm, wet-weight basis; mean ± SE) in eggs, carcasses and fat samples from mallards fed diets containing 0, 1 or 3 ppm

		Endrin added to diet[a] (ppm)		
Sample	Sex	0	1	3
Egg		ND (3)	1.1 ± 0.06 (27)	2.9 ± 0.16 (25)
Carcass	Male	ND (3)	1.1 ± 0.38 a (7)	2.4 ± 0.33 a (7)
	Female	ND (3)	1.0 ± 0.13 a (7)	2.5 ± 0.49 a (7)
Fat	Male	ND (3)	6.4 ± 0.88 a (7)	16 ± 0.9 a (7)
	Female	ND–0.26[b] (3)	7.6 ± 0.40 a (7)	11 ± 1.5 b (7)

[a]Within the same sample and dietary concentration of endrin, the sexes were significantly different at $\alpha = 0.05$ by a t test if they do not have the same letter. ND, not detected at a reportable level of 0.05 ppm. Sample size in parentheses.
[b]One of the three samples contained 0.26 ppm endrin; in the other two endrin was not detected at 0.05 ppm.

ductive success in screech owls (*Otus asio*) fed a diet containing about 2.25 ppm endrin on a dry-weight basis. A level of 0.3 ppm or more endrin on a wet-weight basis in screech owl eggs appeared to be associated with reproductive impairment. Based on the interpretation of field data, Blus et al. [9] estimated that an endrin level of about 0.5 ppm or more (wet weight) in the eggs of brown pelicans (*Pelecanus occidentalis*) caused reproductive impairment. In our study, an average of 1.1 ppm endrin (wet weight) accumulated in the eggs of mallards fed 1 ppm endrin and caused no apparent reproductive problems. Mallards may not be as sensitive to the reproductive effects of endrin as some other birds.

A diet containing 3 ppm endrin seems to pose a hazard to mallards. The 2.0 ppm endrin (wet weight) in the brain of one male that died on this diet was above the diagnostic lethal level of 0.8 ppm endrin (wet weight) in the brain established for several species of birds, including mallards, by Stickel et al. [2]. In addition to the threat of adult mortality, 3 ppm endrin may reduce reproductive success in mallards. The only statistically significant effects of 3 ppm endrin were a later hatching date and poorer hatching success compared with those of birds fed 1 ppm. However, when compared with controls and mallards fed 1 ppm endrin, the pairs fed 3 ppm endrin also had the smallest proportion of hens that completed the incubation of a clutch, the lowest duckling survival and the fewest 21-d-old ducklings produced, although these differences were not significant. Also, the endrin level in the eggs of hens that failed to produce any 21-d-old ducklings was nearly significantly higher ($p = 0.052$) than in eggs of successful hens.

More effects of the 3 ppm endrin diet could have been statistically significant had the statistical power of the tests been what we had expected it to be in planning the study. Fewer hens in all three treatments incubated clutches than anticipated and the variability of results was greater than expected. As a result, we calculated that at a significance level of $\alpha = 0.05$ and a power of the test at 0.8, a 57% reduction from controls in mean number of 21-d-old ducklings produced per hen was required for statistical significance. In hens fed 3 ppm endrin, we observed a 17% depression in this effect from control levels, and a 28% depression compared with hens fed 1 ppm endrin. The observed statistical strength of the test dictates that caution and common sense be used in interpreting the effects of the 3 ppm endrin diet on mallard reproduction. Furthermore, there may be differences between our results and the effects of dietary levels of 1 or 3 ppm endrin on wild mallards exposed to various environmental stresses.

Our findings of no effect of endrin on adult weights, weight of the young and eggshell thickness agree with those of Fleming et al. [8] for screech owls. They found no differences in carcass residues of endrin between male and female screech owls, which agrees with our work. However, there were great differences in the accumulation of endrin in adults and eggs between our mallards and

screech owls. The screech owls were fed 0.75 ppm endrin on a wet-weight basis, which resulted in an average of 0.28 ppm endrin in eggs and 0.36 and 0.55 ppm in male and female carcasses (prepared in a manner similar to our mallards), all also on a wet-weight basis. Our dietary concentrations of 1 and 3 ppm endrin in duck mash containing only about 7% water translate into about 0.2 and 0.6 ppm on a wet-weight basis in natural duck foods, assuming a correction factor of 5 [10]. Screech owls accumulated less than half the dietary concentration in eggs and only slightly more in adult carcasses. However, residues of endrin in the eggs and carcasses of our mallards were about 5 times the dietary concentration on a wet-weight basis. Although mallards appear to be less sensitive to the reproductive effects of endrin, they accumulate much more endrin in eggs and tissues than do screech owls. This is an important finding because concern about the effect in humans of eating endrin-contaminated waterfowl was a major issue in the 1981-1982 endrin problem in Montana.

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