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Reproduction and Residue Accumulation in Black Ducks Fed Toxaphene[1]

Susan D. Haseltine, Mack T. Finley, and Eugene Cromartie

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20811

Abstract. Three sets of 15 pairs of black ducks (*Anas rubripes*) were given 0, 10, or 50 ppm toxaphene in a dry mash diet for a period of 19 months, which included two breeding seasons. Survival of adults was not affected, but the weights of treated males were depressed during the summer months. Egg production, fertility, hatchability, eggshell thickness, growth, and survival of young did not vary with toxaphene ingestion in either breeding season. However, the mean number of days required to complete a clutch was lower in birds fed toxaphene than in birds on the control diet. Clutches of hens fed 50 ppm toxaphene showed improved hatching success in the second year of the study.

Carcass wet-weight (70% moisture) residues in adults and the young birds averaged from 50 to 100% of the dietary concentration (7% moisture); egg residues showed a similar trend. Carcass residues did not reflect those found in the livers or brains of the adults, which seldom exceeded 0.5 ppm. Toxaphene residues were found in the brain of only one 10 ppm bird, but were present in nearly all of the 50 ppm birds. Toxaphene residues were present in the liver all all birds ingesting toxaphene.

›xaphene, a complex mixture of chlorinated camphenes, is the most widely ed chlorinated hydrocarbon insecticide in the United States (Andrelenas '74). It has been used in the southern regions of the Unitd States on cotton and ybeans for more than 30 years, and, with the phasing out of DDT, toxhene's use as a synergist with other chemicals on such crops as corn and its is increasing (von Rumker *et al.* 1975). In addition to agricultural applicans, toxaphene has been used in lakes as a fish poison (Hemphill 1954, yhew 1959, Hooper and Fukano 1960), and Johnson *et al.* (1966) reported its esence in lake water nine years after treatment. Thus, vertebrates are exsed to toxaphene in both aquatic and terrestrial habitats and their response to ubiquitous chemical must be considered.

dress correspondence to Susan D. Haseltine, Patuxent Wildlife Research Center, Laurel, MD

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Aquatic invertebrates and fish accumulate toxaphene from the water (Terriere *et al.* 1966) and tend to be sensitive to small amounts (Mayer *et al.* 1975; Mayer and Mehrle 1977; Schimmel *et al.* 1977). Ferguson *et al.* (1964) demonstrated the capacity of several species of fish for building up resistance to toxaphene contamination if use of the pesticide was heavy in a watershed area; in certain populations, sizeable residues of toxaphene may accumulate and become available to birds and mammals higher on the aquatic food chain. Mammals (Lehman 1952; Claborn *et al.* 1962) and birds (Genelly and Rudd 1956a; Bush *et al.* 1977a, 1978) are able to metabolize toxaphene adequately and tolerate steady ingestion of the chemical for periods of months without serious effects except for altered cartilaginous structures (Page *et al.* 1978). One reported exception to this tolerance is the white pelican (*Pelecanus erythrorhynchos*); 50 ppm toxaphene in the diet for four to five weeks has been associated with mortality in young birds of this species (Keith 1966b). Another exception is the reported death of young waterfowl in a marsh following toxaphene application (Hanson 1952).

Because of these reports of mortality in young aquatic birds, because there is an indication of reproductive effects at 100 ppm toxaphene and higher in the diet of galliform birds (Genelly and Rudd 1956b), later supported by Arscott *et al.* (1976), and because toxaphene is a widespread pesticide in wintering waterfowl habitat, a two-year study was initiated to assess the effects of dietary concentrations of 10 and 50 ppm toxaphene on survival and reproduction of adult black ducks, on growth and survival of young black ducks, and on residue accumulation in both young and adult birds.

Methods and Materials

Young-of-the-year black ducks from a captive colony were paired and placed in 4.6 × 9.1 m outdoor pens in November 1975. Each pen was equipped with a water trough, a cylindrical nest box, and a feeder. In December 1975, 15 pairs were placed on each of three experimental diets. The diet consisted of a commercial duck breeder mash containing 0, 10, or 50 ppm technical grade toxaphene (w/w) carried in propylene glycol (1% of the feed by weight). Breeder mash contained 2.8 to 3.2% Ca, 0.5 to 0.6% inorganic P and a minimum of 750 I.U. vitamin D_3. No additional organochlorines were present at levels above 0.1 ppm. At 2, 7, 12, and 19 months after the experiment began, all adults were weighed and a five ml blood sample was taken from each bird. One control female died in January 1976 and was replaced with a hen of the same age. When adults died later in the study they were not replaced, but their mates were continued on treatment in order to obtain weight and residue accumulation data.

Egg-laying began in March 1976; after the first egg was laid, all nests and females were checked daily. Each egg laid by a hen was numbered, examined for cracks, and placed back in the nest box; the third egg in each clutch was collected for residue analysis and eggshell thickness measurements. Any hen which abandoned her nest or had her brood die in the first week after hatching was allowed to renest, and data from the second brood were utilized if the young survived. All clutches were candled at two weeks of incubation, and infertility and dead embryos were noted. Males were removed from the pen at 21 days of incubation so they would not disturb the young, but they were maintained on the treatment diet. After hatching, ducklings were brooded by the hens and fed a commercial starter mash of the same toxaphene content as their parents. Starter mash contained 1 to 1.2% Ca, 0.5 to 0.6% inorganic P, and a minimum of 5,000 I.U. vitamin D_3. No organochlorine chemicals except toxaphene were present at levels above 0.1 ppm. For eight weeks, a supplement of amprolium-ethopabate (0.013%) was added to the starter mash to prevent coccidiosis.

Ducklings remained with the hen until 12 weeks of age. At five days to one week of age, at two

weeks, and every two weeks thereafter, broods were weighed. At two weeks, one duckling from each brood was sacrified for residue analysis; at 12 weeks all ducklings in the treatment broods and one bird from each control brood were sacrified and prepared for analysis.

After the first reproductive season, drakes were again placed with hens, control broods removed, and the pairs maintained on treatment diets throughout the following winter. Reproduction began again in March 1977, and the same procedures were followed as in 1976. At the end of July, after all broods were at least seven weeks old, adult black ducks were sacrificed and their brains and livers excised, weighed, and frozen for residue analysis. For toxaphene analysis all carcasses were plucked, and beaks, extremities of wings and legs, and digestive tracts were removed. Carcasses were weighed, wrapped in aluminum foil, and frozen. Eggshell thickness was measured at the equator with a Starett 1010 M micrometer.

Entire brains, livers, and blood-samples, and 10-g portions of homogenized carcasses and eggs were analyzed individually for toxaphene. Extraction, sample cleanup with Florisil®, and silicic acid separation procedures, similar to those described by Cromartie *et al.* (1975), were made on adult tissue and eggs. Blood samples and young carcasses were cleaned up by gel permeation chromatography by the method of Johnson *et al.* (1976), except that column length was increased to 500 mm and solvent volume for lipid elution was increased to 175 ml. Toxaphene was quantified with a gas-liquid chromatograph (GC) with a ^{63}Ni detector, automatic sampler, computing integrator, and a 1.83 m × 6.35 mm glass column packed with 1.5% OV-17/1.95% QF-1 at 200°C. The detection limit was set at 0.1 ppm. The average recovery from tissues spiked with 10 or 20 ppm of toxaphene was 113%. The data were not corrected for recovery.

Because toxaphene is such a complex mixture of chlorinated camphenes, the components of which may be metabolized at different rates, and because many other chemicals may elute in the same fraction, it is difficult to accurately quantify toxaphene residues. Therefore, two methods were used for estimating toxaphene residues. The first method measured the total area under the GC curve and compared this value to the total area under the standard toxaphene curve; the second method, commonly used with samples from the field, measured only the area under two characteristic toxaphene peaks whose retention times were 1.4 and 1.8 in relation to 1.0 for *p,p'*DDT and compared this area to the area of the same peaks in the toxaphene standard. In both cases the peak areas were measured from valley to valley.

One-way analysis of variance with a Scheffe's test for mean separation was used when possible for reproductive, weight, and residue data. Arcsin transformations were used on percentage data before analysis. Difference of k proportions and Kruskal-Wallis tests were run on discrete data when appropriate.

Results

Adult Survival and Reproduction

Adult survival was not affected by toxaphene ingestion. Four females died during the treatment period: a 50 ppm bird in May 1976, a 10 ppm bird in July 1976, and two control hens in July 1977. Causes of death included ingestion of wire, head injury, and raccoon predation. No males died during the study, but summer weights of males fed 50 ppm toxaphene were depressed in both 1976 and 1977 (Table 1). This was not true of male weights during the winter months, nor did females ingesting toxaphene exhibit depressed weights during the study. At the end of the second breeding season, however, females eating either 10 or 50 ppm toxaphene had larger livers than control birds (Table 2). Liver enlargement was not apparent in the drakes who underwent only the stress of toxaphene metabolism and not the additional liver stress of yolk lipoprotein synthesis. Drakes fed 50 ppm toxaphene did exhibit an elevated brain weight which was not apparent in 50 ppm females (Table 2).

Table 1. Body weights (g) of adult black ducks fed 0, 10, or 50 ppm toxaphene for 19 months (mean ± se)

	Months on treated diet				
Group	0	2	7	12	19
Females					
Control	1123 ± 21.5	1035 ± 18.8	959 ± 11.5	1093 ± 29.4	954 ± 11.9
10 ppm	1110 ± 21.1	1023 ± 17.1	947 ± 8.8	1099 ± 28.3	960 ± 17.8
50 ppm	1093 ± 20.7	1006 ± 17.4	952 ± 21.4	1059 ± 25.5	939 ± 22.8
Males					
Control	1255 ± 25.6	1184 ± 23.5	1134 ± 18.4	1234 ± 36.4	1080 ± 28.1
10 ppm	1268 ± 32.2	1167 ± 24.6	1082 ± 23.8	1250 ± 43.4	1096 ± 22.8
50 ppm	1264 ± 29.9	1168 ± 21.0	1027 ± 18.1[a]	1230 ± 36.6	1006 ± 23.1[b]

[a] Mean differs from other males, $p < 0.01$, One-way Analysis of variance, Scheffe's test
[b] Mean differs from other males, $p < 0.05$, One-way Analysis of variance, Scheffe's test

Although in both years of the study, breeding pairs on the 50 ppm toxaphene diet appeared hyperactive, caging facilities did not allow the testing of this possibility. Table 3 reports reproductive success for 1976 and 1977. Initiation of egg-laying occurred simultaneously in all groups, but several 50 ppm hens laid very late in the season and caused the mean date of the first egg in that group to occur somewhat later than in the others. Once laying commenced, however, treated hens were faster than control hens in initiating incubation. This was not due to a smaller clutch size, since in neither year of the study did this parameter differ among control and toxaphene groups. In fact, no reproductive parameter measured in 1976 or 1977 differed in relation to clutch or treatment except the rapidity with which the clutch was laid. Almost all hens laid eggs, but in 1976 only about one-half incubated their eggs; in 1977 the number of incubating hens increased slightly in all groups. Completely infertile clutches were non-existent. Three nests (1 control, 2 treated) were abandoned in 1976; none were abandoned in 1977. One nest was destroyed in 1977 by raccoons; eggshell thickness did not vary with toxaphene treatment of hens in either reproductive season.

Table 2. Liver and brain weights (g) of black ducks after 19 months on 0, 10, or 50 ppm toxaphene (mean ± se)

	Liver	Brain
Females		
Control	19.21 ± 1.32[a]	5.52 ± 0.12
10 ppm	23.10 ± 1.49	5.69 ± 0.10
50 ppm	25.36 ± 1.62	5.66 ± 0.17
Males		
Control	23.16 ± 1.92	5.90 ± 0.10
10 ppm	25.76 ± 1.29	5.83 ± 0.16
50 ppm	25.93 ± 1.78	6.16 ± 0.09[b]

[a] Mean differs from other groups of the same sex, $p < 0.01$, One-way Analysis of variance, Scheffe's test
[b] Mean differs from other groups of the same sex, $p < 0.05$, One-way Analysis of variance, Scheffe's test

Table 3. Reproductive success of black ducks fed 0, 10, or 50 ppm toxaphene through two reproductive seasons, 1976 and 1977

	1976			1977		
	Control	10 ppm	50 ppm	Control	10 ppm	50 ppm
No. breeding pairs	15	15	15	15	14	14
No. hens laying	14	14	14	14	14	13
No. hens incubating	9	7	7	10	8	9
Mean date of 1st egg (Days after 1/31)	57	58	67	51	50	64
Mean no. days of laying (before incubation)	25	21	15[a]	26	16	14[b]
No. nests hatching	8	5	6	9	8	9
Mean clutch size	7.7	6.9	7.7	9.9	8.0	8.9
Mean no. infertile/clutch	1.4	0.3	1.6	1.1	0.5	1.0
Mean embryo mortality/clutch						
0-2 weeks	0.0	0.2	0.7	0.3	0.5	0.3
2 weeks–hatch	0.7	0.4	0.6	1.5	0.4	0.6
No. hatched	5.6	6.0	4.8	7.0	6.6	7.0
Eggshell thickness (mm)	0.30	0.28	0.28	0.32	0.32	0.31

[a] Groups differ significantly, $p < 0.05$, Kruskal-Wallis
[b] Groups differ significantly, $p < 0.005$, Kruskal-Wallis

Duckling Survival and Growth

Toxaphene ingestion did not affect duckling survival to two weeks of age, after which no mortality occurred in either breeding season of the study (Table 4). Although 50 ppm toxaphene was associated with lower survival in both years of the study, the differences were not statistically significant ($p > 0.05$). Likewise, weight gain of 50 ppm-broods up to two weeks of age appeared slower than weight gain in broods fed control or 10 ppm toxaphene diets, but the difference was not statistically significant when analyzed on a brood-mean basis (Table 5). In 1976, brood growth curves were similar within treatments; therefore, weight

Table 4. Number of young surviving in black duck broods fed 0, 10, or 50 ppm toxaphene from hatch (mean ± se)[a]

		Days of age		
	N[b]	1 Day	7 Days	14 Days
1976				
Control	8	5.6 ± 1.4	4.6 ± 1.5	4.6 ± 1.5
10 ppm	5	6.0 ± 1.1	5.2 ± 0.9	5.0 ± 0.8
50 ppm	6	4.8 ± 0.9[c]	3.5 ± 1.1	3.3 ± 1.0
1977				
Control	9	7.0 ± 1.0	6.1 ± 1.3	6.1 ± 1.3
10 ppm	8	6.6 ± 1.0	5.5 ± 1.1	5.4 ± 1.1
50 ppm	9	7.0 ± 0.8	4.8 ± 1.2	4.6 ± 1.1

[a] No treatment variations were observed; numbers were transformed to percentage of eggs hatched, angularly transformed, and one-way Analysis of variance performed
[b] N = number of broods considered
[c] Different from same treatment group in the 1977 breeding season, $p < 0.05$

Table 5. Weight (g) of black duck young fed 0, 10, or 50 ppm toxaphene from hatch (mean ± se)[a]

Group	5 days	2 wks.	4 wks.	6 wks.	8 wks.	10 wks.	12 wks.
1976							
Control	51.45 ± 3.14	188.74 ± 12.64	542.40 ± 31.14	787.29 ± 23.00	901.88 ± 29.24	944.31 ± 27.86	964.11 36.80
10 ppm	51.20 ± 4.16	178.62 ± 5.32	542.73 ± 21.55	814.83 ± 41.79	884.27 ± 49.46	941.03 ± 74.10	899.38 ± 61.00
50 ppm	41.14 ± 2.37	145.67 ± 15.32	481.75 ± 11.52	799.81 ± 8.29	888.04 ± 27.39	964.38 ± 38.33	964.12 ± 32.92
1977	1 wk.	2 wks.	4 wks.	6 wks.	8 wks.	10 wks.	12 wks.
Control	69.88 ± 5.10	186.04 ± 13.94	494.58 ± 42.56	822.32 ± 17.32	944.69 ± 29.28	975.12 ± 14.86	987.06 ± 17.12
10 ppm	73.25 ± 4.24	184.27 ± 10.74	526.38 ± 20.94	862.06 ± 18.34	941.55 ± 21.92	1020.33 ± 26.98	945.69 ± 22.43
50 ppm	58.63 ± 5.55	153.68 ± 10.04	482.47 ± 24.86	801.65 ± 25.25	895.45 ± 21.37	956.06 ± 34.57	944.76 ± 22.89

[a] Each value is the mean of all brood means in a treatment group. No significant differences were observed in weights at any age in 1976 or 1977

gain could be analyzed using individual ducklings and the 50 ppm group lagged significantly behind in weight until two weeks of age. In 1977, brood growth curves were different within treatments; the data could not be pooled and no significant growth differences were observed with toxaphene ingestion. Survival and weight gain of young birds did not increase from 1976 to 1977, but total production was increased significantly in the 50 ppm group due to increased hatching success. The control and 10 ppm treatment group did not increase hatching success from 1976 to 1977.

Toxaphene Residues

Of the two methods used to quantify toxaphene, method I, which measured the total area under all toxaphene peaks, was consistently less variable than method II, which used only two peaks characteristic of field samples (Table 6). Quantification by method I also gave a lower residue level than method II, except in all liver samples and in the brains of females on the 50 ppm toxaphene diet (Table 6), which suggested a differential metabolism, absorption, or translocation of toxaphene peaks in different organs.

After 19 months on a mash diet (7% moisture) containing 10 ppm toxaphene, adult carcasses (70% moisture) averaged approximately 3 and 8 ppm toxaphene, on a wet-weight basis, when quantified by methods I and II, respectively. Only one male of the 12 adults analyzed from this 10 ppm group contained toxaphene in the brain. Liver residues averaged well below 0.5 ppm on this dietary treatment. In the 50 ppm group, male and female carcasses averaged 28 and 11 ppm toxaphene, respectively, when quantified by method I. Quantification by method II produced higher values, especially in male carcasses (217 ppm), but these high carcass residues were not accompanied by high residues in liver or brain tissues. Liver residues in the 50 ppm group were higher than those of the 10 ppm birds, but the highest values (those of the males

Table 6. Residues[a] in tissues, eggs, and carcasses of black ducks fed 10 or 50 ppm toxaphene for up to 19 months

		10 ppm		50 ppm	
	N[b]	I[c]	II[c]	I	II
Adult carcasses					
Male	6	3.1 ± 0.6	8.5 ± 2.5	28.0 ± 5.7[e]	217.0 ± 60.1[e]
Female	6	3.0 ± 0.04	8.0 ± 1.7	11.1 ± 1.4	74.8 ± 10.9
Adult livers					
Male	6	0.40 ± 0.08	0.26 ± 0.06	6.1 ± 1.3[e]	2.4 ± 0.36[e]
Female	6	0.40 ± 0.07	0.31 ± 0.06	2.2 ± 0.38	0.81 ± 0.08
Adult brains					
Male	6	—[d]	2.3 (1)	1.4 ± 0.6 (5)	2.7 ± 1.0[e]
Female	6	—	—	0.68 ± 0.45	0.51 ± 0.03 (5)
Blood					
Male-12 month	6	—	0.16 ± 0.04 (3)	0.19 ± 0.04 (5)[f]	0.22 ± 0.04 (3)[f]
Male-19 month	6	0.14 ± 0.01 (2)	0.14 (1)	0.50 ± 0.16 (5)	0.66 ± 0.24 (4)
Female-12 month	6	0.1 (1)	0.13 ± 0.01 (2)	0.22 ± 0.04 (4)	0.33 ± 0.06 (3)
Female-19 month	6	0.18 ± 0.02 (3)	0.35 ± 0.18 (2)	0.29 ± 0.03	0.34 ± 0.07
Eggs					
1976	6	4.4 ± 0.4	6.8 ± 0.7	23.5 ± 1.9[g]	40.5 ± 3.1[g]
1977	6	5.6 ± 0.6	8.6 ± 1.2	29.5 ± 2.0	50.2 ± 4.2
Young–1976					
2 wks old	5	6.3 ± 0.3[g]	7.5 ± 0.6	20.8 ± 3.0[g]	32.6 ± 5.3[g]
12 wks old	5	7.7 ± 0.9[g]	8.6 ± 1.6[g]	28.0 ± 3.5[g]	43.1 ± 7.0[g]
Young–1977					
2 wks old	6	3.1 ± 1.3	4.7 ± 1.9	7.0 ± 1.4	12.6 ± 2.6
12 wks old	6	3.1 ± 0.2	4.9 ± 0.3	11.6 ± 3.4	20.1 ± 6.3

[a] ppm toxaphene, wet weight, mean ± se

[b] N = the number of samples analyzed from each group; means are based on the number of samples analyzed which showed reportable toxaphene levels. If less than N, the number is placed in parentheses after the standard error

[c] Column I reports toxaphene by quantifying total area under the toxaphene profile; column II reports toxaphene by quantifying only two peaks with retention times of 1.4 and 1.8 compared to 1.0 for p,p'-DDT

[d] No samples contained above the reportable level (0.1 ppm) of toxaphene

[e] Different from the same treatment sample for the other sex, student t-test, $p < 0.05$

[f] Different from the same treatment sample after 19 months on toxaphene diet, Student t-test, $p < 0.05$

[g] Different from the same treatment sample in 1977, Student t-test, $p < 0.05$

In spite of levels of toxaphene in eggs and carcasses which might be suspect in field samples, black ducks survived and reproduced through two breeding seasons with few suggestions of detrimental toxaphene effects. Young, hatched during the study, showed no mortality on the toxaphene diets and little or no growth effect. In this pen situation, black ducks were able to tolerate the ingestion of 10 and 50 ppm toxaphene with few problems. However, additional stresses on birds in wild situations might serve to magnify the behavioral, reproductive, or growth effects suggested by this pen study into factors significant to population recruitment.

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