# Response of Different-age Mallards to DDT

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Despite the fact that more data have been gathered for DDT than any other pesticide known, basic toxicity data for different age classes of specific wildlife species are scanty. This paper supplements existing knowledge on the response of mallards, Anas platyrhynchos, to DDT. Dose response curves for three age classes of mallards are given along with data for DDT brain residues, mortality patterns, and effects on body weights.

## Materials and Methods

Mallard ducklings were obtained on their day of hatch and raised to the desired experimental age. A duck starter ration was fed to ducklings under 30 days of age and a duck grower ration was fed to older ducks. These rations were formulated by the University of Wisconsin Poultry Department and were designed to provide adequate nutrition, but minimize the formation of body fat. Feed and water were provided ad libitum throughout these studies.

The purified (99+ percent) p,p'-isomer of DDT was used (ESA pesticide reference standard, City Chemical Co., New York) and formulation of premixes and mixing of rations to achieve the desired concentrations of DDT was done by the Wisconsin Alumni Research Foundation (WARF) of Madison, Wisconsin.

<u>DDT Exposure</u>: All birds were weighed, randomly assigned to treatment pens and allowed a 1- or 2-day acclimatization period, during which time control rations were fed. Pesticide treatments were then randomly assigned to treatment pens and experimental rations fed for 10 days, followed by control rations for an additional 10 days. Birds were inspected 2 or 3 times daily, at which time signs of toxicity were noted and dead birds removed, weighed, and brains collected for pesticide residue analysis. All survivors were weighed at the end of the 20-day experimental period and two birds from each group killed for brain pesticide residues.

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DDT test doses are given in Table 1. Preliminary feeding trials with adult mallards suggested a difference in sex susceptibility to DDT, therefore, only adult males were selected for use in subsequent studies except for a small number of adult female mallards to substantiate differences in DDT toxicity between sexes in the adult age class.

TABLE 1

Experimental protocol for DDT toxicity experiments in different-age mallards

Age of birds	DDT level (ppm)	Replicates per dosage level	Birds per replicate
Adult			
Male	250-2000 <u>a</u> /, <u>b</u> /	1	10
Female	250-2000 <u>a</u> /, <u>b</u> /	1	10
5-day-old	500-2000 <sup><u>c</u>/</sup>	2	30
30-day-old	500-2000 <sup><u>c</u>/</sup>	4	10
Adult			
Male	1000-1500 <sup><u>a</u>/</sup>	2-3	7-13
Female	1250-1750 <u>a</u> /	1-2	5-10

 $<sup>\</sup>underline{\underline{a}}^{\prime}$  Dosage levels increased by increments of 250 ppm.

Residue analyses: DDT residue analyses were carried out at the WARF using accepted methods for gas chromatography (UNITED STATES FOOD AND DRUG ADMINISTRATION 1968). These values are reported on a wet-weight basis.

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m LC}_{50}$  values: Experimental LC $_{50}$  values for the purpose of these studies are defined as the amount of pesticide (ppm on a dryweight basis) fed in the diet for 10 consecutive days that will kill 50 percent of the birds during a 20-day experimental period. These values were determined by probit analysis (FINNEY 1952).

### Results

Age susceptibility: Experimental LC<sub>50</sub> values for the different age classes of mallards ranged from 1200 to 1600 ppm of DDT in the diet (Table 2). Mortality from DDT was greatest among ducklings in the 5-day-old age class and adult males were more

 $<sup>\</sup>frac{b}{P}$  Preliminary experiment.

 $<sup>\</sup>underline{c}$  Dosage levels increased by increments of 500 ppm.

susceptible to DDT than 30-day-old ducklings. No overlap occurred at one standard error between any of the  $LC_{50}$  values, but the 95 percent confidence limits for adults and 30-day-old ducklings overlap with each other and the 5-day-old age class.

TABLE 2: Experimental LC  $_{\overline{\bf 50}}$  values for DDT in differentage mallards.

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Age of birds <u>a</u> /	LC <sub>50</sub> (ppm)	95% C. L lower limit	. (ppm) upper limit
5-day-old	1202	1050	1377
30-day-old	1622	1374	19 <b>1</b> 4
Adult b/	1419	1294	1556

 $<sup>\</sup>underline{\underline{a}}^{\prime}$ Age of birds at start of DDT feeding.

Probit regressions for these data when tested for homogeneity indicated that the regression coefficients for both the 30-day-old ducklings and adult males could be considered estimates of a common regression coefficient, as could those for adult male and 5-day-old ducklings. The difference between slopes of the 5- and 30-day-old duckling regression lines was clearly significant and indicates little probability (p < .001) that these two regression coefficients are estimates of a common dose-mortality response (Figure 1).

Mortality: The onset of mortality and mean time of death at each dosage level of DDT was related to the age of the mallards. Deaths among 5-day-old ducklings began 1 to 3 days earlier than those in 30-day-old ducklings and 3 to 5 days earlier than mortality among adult mallards (Figure 2). These data indicate a dose-dependent mortality relationship. For 5-day-old ducklings the distribution of mortality with time was relatively uniform (but of different magnitude) at all DDT levels, but for 30-day-old ducklings and adults the greatest proportion of deaths occurred during a relatively short period of time.

Mean death times for 5-day-old ducklings were less than 30-day-old ducklings and adults at all dosage levels, but no similar relationship occurred between the two older age classes (Table 3). Variation in mortality among replicates was minimal for 5-day-old ducklings and increased substantially in older birds. No adult females died, confirming the observations of preliminary feeding trials of a sex differential in susceptibility of adult mallards to DDT.

Body weights: The mean body weights of 5- and 30-day-old ducklings surviving DDT feeding were less than those of controls; these differences were greatest for the 2000-ppm DDT treatment

 $<sup>\</sup>frac{b}{Males}$  only; sexes mixed in undetermined ratio for ducklings.

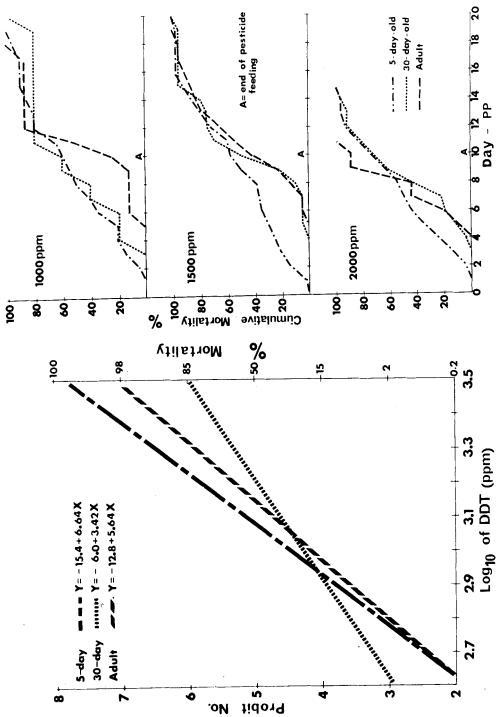


Figure 2. Comparative DDT mortality curves for Figure 1. Probit regression lines expressing toxicity of p,p'-DDT to different-age mallards.

different-age mallards.

TABLE 3: Mortality in different age classes of mallards fed DDT in their rations.

A 1	DDT level (ppm)					
Age class	0	500	1000	1250	1500	2000
5-day-old	0/30 <u>a</u> /	0/60	20/60	_	43/60	56/60
Mean day of death	-	-	9.2±4.9 <sup>b</sup> /	′ –	9.0±4.9	7.6±3.6
30-day-old	0/20	3/40	5/40	-	20/40	26/40
Mean day of death	-	11.7±6.8	10.2±6.1	_	11.2±3.2	9.0±2.7
Adult males	0/15	-	8/26	8/20	16/33	9/10
Mean day of death	-	-	11.5±3.3	9.1±2.7	9.3±1.2	8.0±1.9

 $<sup>\</sup>underline{\underline{a}}^{\prime}$ Number dead/total sample; - indicates no values.

group, but none were significant (Table 4). Changes in body weights among surviving adults were relatively constant and not correlated with sex or treatment level of DDT. Adults surviving DDT feeding lost approximately 9 percent of their starting body weight by the end of the feeding trials while adults that died during this period suffered weight losses that ranged from 25 to 28 percent. These weight losses in dead birds undoubtedly occurred as a result of anorexia which accompanied signs of DDT toxicity including prostration 1 to 2 days before death.

Residues: Comparisons of DDT residues between birds that died from DDT and those that survived DDT feeding were made only for adults. Residues of DDT and its metabolites (DDD and DDE) in the brains of mallards that died 5 to 8 days after the completion of DDT feeding were approximately 6 to 17 times greater than residue levels in the brains of mallards surviving comparable feeding levels of DDT and killed for analysis 10 days after completion of DDT feeding (Figure 3). Generally, the ratio of DDE:DDD:DDT in these samples was approximately 2:5:3. This ratio was shifted slightly to the right in birds killed as opposed to values for birds that succumbed to DDT, but these differences were not significant (Chi square = 1.51, df = 2).

Pools of 2 to 4 brains each from birds dying within each age class were analyzed at various time intervals following the start of DDT feeding. There was no correlation between the level of DDT residues detected in the brain at the time of death and the amount of DDT fed. Mean DDT brain residues, irrespective of time of death, also failed to indicate any consistent relationship with DDT levels in the diet. For 5-day-old ducklings mean brain residues of DDT and its metabolites ranged from 69.8 to 82.8 ppm; for

 $<sup>\</sup>frac{b}{Mean} \pm standard error.$ 

TABLE 4. Mean body weights of different-age mallards surviving DDT feeding trials.

		Age of birds			
DDT level (ppm)	5	-day-old <sup>a</sup> /	3	30-day-o1d <sup>b/</sup>	
	N	Mean body <sup>c</sup> / weight (g)	N	Mean body <u>c</u> / weight (g)	
0	30	484.8±12.6	20	877.3±35.5	
500	58	429.2±16.5	37	799.9±29.7	
1000	38	432.1±11.6	36	789.8±24.4	
1500	17	422.0±17.0	19	805.7±29.9	
2000	4	368.5±38.4	14	765.4±43.3	

 $\frac{a}{F} = 2.55$ , df = 4,142; no significant differences.

 $\frac{b}{F}$  = 1.34, df = 4,121; no significant differences.  $\frac{c}{Mean}$  ± standard error.

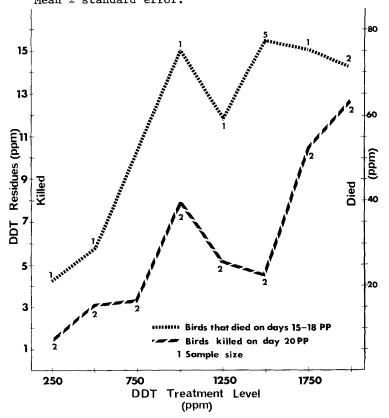


Figure 3. DDT brain residues in adult mallards that died or were killed during feeding trials.

30-day-old ducklings these values ranged from 52.8 to 67.9 ppm; for adults, brain DDT residues of 21.6 to 111.2 ppm were detected. By contrast, mean brain DDT residues among adults killed for residue analysis ranged from 1.4 to 12.7 ppm.

### Discussion

The greater resistance of adult female than adult male mallards to DDT toxicity is probably in part a result of egg laying by females providing an excretory mechanism for DDT and its metabolites. This difference in susceptibility may also reflect physiological differences between the sexes and account for the greater resistance of 30-day-old ducklings than adults to DDT toxicity. Only data for males were used to calculate the adult toxicity curve while the 30-day-old age class contained both sexes in an undetermined ratio (these birds were 50 days old at the completion of the experiment). A greater resistance of females than males in this age class would increase the  $LC_{50}$  value over that for males only and could possibly be manifest by different storage rates of DDT by males and females. Female rats store more DDT in their fat than males (HAYES 1959), and most pesticides tested by the oral route were more toxic to female than to male rats (GAINES 1969). To the best of our knowledge, a sex differential response among avian species to organochlorine pesticides has not been reported.

An alternative hypothesis is that differences in susceptibility to DDT between 30-day-old ducklings and adult male mallards may reflect physiological differences in these age classes. Young adult rats were found to be more susceptible to DDT than weanling or pre-weanling-age rats (LU et al. 1965) even though young animals (but not recent-born) are generally more susceptible to toxicants than older members of the same species (DURHAM 1967).

Weight losses among adult mallards that died of DDT in the present study are similar to those for DDT-exposed cowbirds, Molothrus ater (STICKEL and STICKEL 1969). Body weight data for live mallards were restricted to experimental starting and ending weights because it was felt that the stress of weighing birds immediately after completion of DDT feeding would adversely influence survival. Only at 2000 ppm of DDT in the diet was there any suggestion that DDT suppressed growth. This level of DDT is sufficiently toxic that mortality rather than growth suppression is the primary effect in the mallard.

The brain was selected as the tissue of choice for DDT residue assay because of the relative independence of levels of DDT in this tissue to dosage or length of time on dosage (BERNARD 1963, DALE et al. 1963, HAYES 1965, WURSTER et al. 1965). Results obtained support the use of the brain as a suitable index tissue for DDT mortality determinations, however, greater DDT levels were observed in the brains of surviving mallards than have been reported in other species (STICKEL and STICKEL 1969, STICKEL et al. 1966).

### Summary

Experimental LC<sub>50</sub> values for DDT in mallards of different ages ranged from 1200 to 1600 ppm. Mortality from DDT was greatest among 5-day-old ducklings and the lowest in 30-day-old ducklings. Adult female mallards were more resistant to DDT intoxication than adult males. The onset of mortality and mean time of death at each dosage level of DDT was related to the age of the mallards and was indicative of a dose-dependent relationship. Deaths among the youngest, and therefore lightest birds, began 1 to 5 days prior to mortality among adults.

Body weights of ducklings surviving DDT feeding were less than those of control ducklings, but not significantly so. Among adult mallards, DDT survivors lost approximately 9 percent of their starting body weight while adults that died from DDT lost 25 to 28 percent of their body weights.

Residues of DDT and its metabolites in the brains of adult mallards dying from DDT intoxication were approximately 6 to 17 times greater than DDT residue levels detected in the brains of adults fed comparable levels of DDT and killed for residue analysis.

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