The Effect of DDT on Aggression in Laboratory Mice

by ANN F. PETERLE and TONY J. PETERLE
Department of Zoology
The Ohio State University
Columbus, Ohio 43210

The ubiquitous distribution of persistent pesticides in the global environment results in low-level contamination and chronic exposure of many organisms. The resultant accumulation in food-chains can terminate in death, lowered reproductive success, alteration in behavior or no observable effect (1). Salmon (Salmo salar) selected different temperature preferenda following exposure to DDT, while other species (Cyprinus carpia) took longer to learn a light shock reaction (2). Laboratory studies of discriminatory ability in quail (Colinus virginianus) showed they are influenced by exposure to DDT (3). Rats fed DDT following a learning experience took longer to relearn the maze than did animals not so exposed (4). Tolerance to cold exposure was lower in rats fed DDT (5). Function of the avian thyroid is altered by dietary intake of DDT, and breakdown of steriods is also changed in birds following exposure (6). Reproduction in laboratory mice was influenced by feeding 7.0 ppm DDT (7). Estrogenic effects of DDT in rats has been suggested (8). Scott concluded that fighting in male mice would persist without male hormone (9).

We studied the effect of dietary intake of 7.0 ppm technical grade DDT (Diamond Shamrock Chemical Company, Houston, Texas) in a group of 16 male laboratory mice. The colony, (all males, no liter-mates, approximately 10 weeks old) of Swiss-Webster strain laboratory mice (Lab Supply, Indianapolis, Indiana) were obtained in late December, 1970, and housed in individual cages in a rack of three tiers. No other mice were in the building. Cages were kept approximately the same distance apart and rotated daily throughout the experiment to minimize the possible effect of position in the rack and any phermones. Laboratory chow and water were supplied ad lib. The mice were held under normal daylight at a temperature of approximately 39°C. Following a 10-day period of acclimation, the mice were weighed, individually marked and matched in a neutral cage for 3 min each day with another mouse in the colony. The neutral cage was the same as those in which the mice were being held (stainless steel, 30.5 x 13.5 x 13 cm) with the exception that no cedar shavings were provided. A rubber mat was placed in the bottom of the neutral cage and between each bout, the cage and mat were thoroughly washed with soap and water. Bouts were timed following the simultaneous introduction of the two mice. Records were kept of the behavior patterns and at the end of each bout one of the mice was declared the most aggressive. The outcome of some of these bouts was determined by posturing, anal sniffing or avoidance behavior in the cage, while others were determined by aggressive fighting and biting (10). Bouts were observed at approximately the same time each day and took about 45 min to 1 hr to complete.

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Following the completion of two cycles of paired bouts, (30 days) the mice were ranked according to the number of "wins" or the number of bouts in which a mouse was considered to be the most aggressive. We selected every other mouse from the ranked list and placed the 8 mice on a diet of 7.0 ppm DDT. The normal laboratory chow was ground to a fine meal, and the DDT was dissolved in double-distilled benzene and added to the food. A similar quantity of benzene without the DDT was added to the control diet. The mice were fed the DDT and control diets for 20 days before we began to match them again. We weighed each mouse at the termination of each round of bouts (every 15 days) prior to feeding DDT, after 20 days on DDT, and at the beginning of the second set of bouts.

Following the termination of the feeding experiment and the behavior studies (after 80 days) the mice were sacrificed, both testes were weighed and the quantity of DDT and metabolites in the brain was determined by electron capture gas chromatography (11).

The results of the aggression tests before DDT are shown in Table 1.

TABLE 1

Win-Loss Ratio -- Before DDT

	Number	Wins	Losses	Total Bouts
Treatment Group	8	122	118	240
Control Group	8	118	122	240

$\chi^2 = 0.132$ 1 dF P=0.750

Differences in ratios of wins to losses between the two groups was not significant (P = 0.750) for the 30 days prior to addition of 7.0 ppm DDT to the diet. Following 20 days on the diet, the wins and losses significantly changed in the two groups (P = 0.028) during the next 30 days.

TABLE 2

Win-Loss Ratio -- After DDT

	Number	Wins	Losses	Total Bouts
Treatment Group	8	108	132	240
Control Group	8	132	108	240

$$\chi^2 = 4.8 \text{ 1 dF } P = 0.028$$

In addition to determining the wins and losses during each trial period, we recorded the number of instances of actual biting when one mouse attacked the other. The number of instances where biting occurred in both groups prior to the addition of DDT to the diet was equal.

TABLE 3

Number of Biting Attacks

	Treatment Group	Control Group	Total Biting Attacks
Before DDT	43	43	86
After DDT	44	85	129

$$\chi^2 = 5.39 \quad 1 \text{ dF} \quad P=0.02$$

Following the treatment period, nearly twice as many instances of biting was initiated by the control group. The change in ratio was significant ($P = 0.02$). The weights of individual mice increased from the initiation of the experiment, but when the weight of each individual was tested against the number of wins, both before and after DDT treatment, there was no significant correlation ($r = 0.23$ before DDT, and $r = 0.03$ after DDT). We assume the difference in aggressiveness was the result of feeding 7.0 ppm DDT to the mice in the treated group.

Testes weight/body weight ratios were not correlated with the number of wins, either in the treated or in the control groups (treated group $r = -0.26$; control group $r = 0.04$). We assume that while 7.0 ppm DDT was sufficient to alter behavioral patterns, it was not sufficient to alter testes weights for the 50 days the mice were on the treated diet.

Residue levels in the brains of the 8 treated mice ranged from 0.79 ppm to 1.107 ppm p,p' DDT. The mean and standard error was 0.584 $\pm$ 0.139 ppm. Residue levels in the brains of control mice were below our minimum detection level of 0.2 ppb. There was no apparent correlation between residues in the brain and our measure of aggressiveness among the treated mice.

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11. Brain samples were prepared for analysis in our laboratory using a mixture of sea sand and sodium sulphate, fluorsil and silica-gel columns, extracted in glass distilled hexane. The extracts were quantified in a Barber-Column Series 5000 gas chromatograph equipped with a tritium foil electron capture detector, 4 ft. glass column, 1:1 mixture of 1.95% QF-1 and 1.7% OV-17 on Gas Chrom Q 100/120 mesh, injector temperature 212°C, column 190°C, detector 208°C, pre-purified nitrogen gas flow 200cc per min. Recovery from spiked samples for o,p'-DDT was 78%. We wish to acknowledge the assistance of J. W. Lehman in the pesticide residue analyses.