THE EFFECT OF DDT ON REPRODUCTION IN MICE[1]

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ABSTRACT

Adult laboratory mice (*Mus musculus*) were fed diets of chick starter mash containing 200 and 300 ppm of DDT. Some tolerated DDT feeding quite well; others died early in the experiment, shortly after feeding had begun. Some mice were able to survive for extended periods.

The average number of young born to mice exposed to levels of 200 and 300 ppm of DDT was similar to that of the control animals. However, the number of female mice dying during the gestation period was much higher among the test animals. The number of young surviving from the test animals was much smaller as compared to that of the control litters.

No changes in the gross histology of tissues in treated animals were noted. While both kidney and ovaries in test females decreased in weight, the adrenal glands increased in weight. The kidneys and testes of test males decreased in weight as the adrenal glands increased in weight, and they lost body weight appreciably before death.

A two-sided t-test showed a significant difference between the mean weights of adrenals from control and from test male animals treated with 200 and 300 ppm of DDT.

Conception was nearly identical in the control and treated groups. Better reproductive results were obtained in the control animals because many treated females died before giving birth. Starving DDT-fed mice led to almost immediate death, accompanied by symptoms of DDT intoxication.

It is concluded that reproduction in mice can be affected by exposure to 200 and 300 ppm of DDT, resulting in death of females during the gestation period, death of male animals, and/or death of young. Further indiscriminate use of insecticides may increase reproductive failures among all wildlife, which could be of more importance than direct mortality.

INTRODUCTION

Tolerance to DDT has been achieved in the white laboratory mouse (*Mus musculus*) (Osburn and Morrison, 1962, 1964). In these studies, the DDT was dissolved in sesame oil and administered by intraperitoneal injection. Dale, Gaines, and Hayes (1963) studied the clinical signs of DDT poisoning and its concentration in the brain of the rat, and found that the action of DDT was manifested almost entirely through the nervous system. Laug and Fitzhugh (1946) studied the accumulation of DDT in the tissues of the rat following oral ingestion for periods of six months to two years. DDT was found in all tissues of animals exposed to DDT. The concentration of DDT was highest in adipose tissue, due apparently to its preferential solubility in fat.

Field studies have been conducted to evaluate the effects of DDT on vertebrates. Population studies of the deer mouse (*Peromyscus leucopus*) were conducted by Stickel (1946) at the Patuxent Research Refuge at Laurel, Maryland. No adverse change was found in the population as a result of DDT application there. House sparrows (*Passer domesticus*), which weigh approximately the same as the mice, all died when fed DDT at levels ranging from 120 to 300 ppm (Bernard, 1963).

Bernard and Gaertner (1964) studied the effects of various concentrations of DDT upon reproduction in the laboratory mouse. Their results suggest that exposure to DDT can result in lower productivity in mice. The tests also indicate that mice show variable effects of DDT exposure in their behavior.

The objective of the present experiment was to test the effect of DDT (2, 2 Bis-

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(Parachlorophenyl)–1, 1, 1–Trichloroethane) upon reproduction in mice.   Experimental and control animals were used, with results being based upon: 1) physical behavior of the animals, 2) number of young born to each female, 3) survival and behavior of the young, 4) appearance at autopsy of the adult animals and certain tissues, 5) appearance of some of the young.

PROCEDURE
Part I of the Experiment

The animals used in this study were a white strain of the laboratory mouse, whose origin was the Institute for Cancer Research (Hal/wf/lcr) and which were obtained from Oshkosh (E. G. Steinhilber, Oshkosh, Wisconsin).   All mice were four to five months old when the experiment began.   The animals were kept at 72–76°F., and given continuous lighting.

Bernard and Gaertner (1964) maintained their mice on a diet of chick starter mash; this same food was used in the following experiment.   The mash contained 20 percent protein, 4 percent fat, and 5 percent fiber.   The weight of the male mice was determined approximately two and one-half weeks after commencing an experiment, except for those which had died earlier, and again upon death of the animal.   An approximation of food consumed by three pairs of mice from each group was determined at 24-hour intervals for a period of six days.

Throughout the experiment, food and water were allowed "ad libitum." Thirty-nine pairs of virgin mice were obtained and fed chick starter mash (CSM) containing no DDT for one week while housed separately.   The mice were then divided into three groups of 13 randomly selected pairs.   For this study a pair signifies a male and female, rather than statistically paired animals.   Groups 1 and 2 were given diets containing 200 and 300 parts per million (ppm) of DDT, respectively.   Thirteen pairs of mice in Group 3 were fed mash containing no DDT, thus serving as controls.

One pair of control animals was sacrificed after the first week and periodically during the experiment.   Their tissues and any tissues from animals having died in Groups 1 and 2 were examined, weighed, and preserved in 10 percent formalin prior to histological processing.

Each pair of animals in Groups 1, 2, or 3 was given a number, making observations easier to record.   Pairs 1–13 were fed CSM containing 200 ppm DDT. Pairs 14–26 were fed CSM containing 300 ppm DDT, and pairs 27–38 served as control animals receiving only CSM.

Male mice remained with the females for two weeks.   They were then separated for a total of 58 days, at which time the following adult mice were still alive: seven of 26 animals fed DDT at 200 ppm, eight of the 26 animals fed DDT at 300 ppm, and fourteen of 26 control animals.

The young were left with the female until weaned.   They were then removed, and placed together or in separate cages, depending upon their size.   By the 55th day from the start of the experiment, all remaining young from the first litter had been separated from their mothers and retained on their respective diets.

Part II of the Experiment

In Part II of the experiment, some of the adult animals were bred for a second time and the numbers of young surviving in the second litters were recorded. To accomplish this, selected adults from each group were placed together in pairs for another two-week period.   The animals were then separated for the remainder of the experiment, an additional 22 days.

The remaining young from the first litters were subjected to varied conditions to test further tolerance or susceptibility to DDT intoxication.   A male and female mouse remained from F19 (300 ppm).   Each was kept in a separate cage and placed on a starvation diet, all food being removed but water being allowed

"ad libitum." A male mouse was also left from F25 (300 ppm). It continued to receive DDT at 300 ppm.

The control animals had a number of surviving young. Some were maintained on plain CSM and others were fed CSM containing DDT at 300 ppm. Four male and three female young remained from F29 (control). These four males were placed in the same cage and maintained on their diet containing no DDT. The females were placed together and fed CSM containing DDT at 300 ppm.

One male survived from F28 (control) and was continued on plain CSM. Two males remained in F31 (control); these were housed in separate cages, one given its previous diet, the other fed DDT at 300 ppm. From F35 (control) four animals survived. The two males were placed together and fed plain CSM; the two females were placed together and fed DDT at 300 ppm. Of the two surviving mice in F36 (control), the male was given 300 ppm DDT; the female, plain CSM. The two surviving males from F38 (control) were put together and given 300 ppm DDT; the third remaining animal, a female, continued as a control.

RESULTS
Part I of the Experiment
Pairs 1–13—200 ppm DDT

An average pair of mice consumed approximately 10 grams of food in a 24-hour period. At the conclusion of Part I of the experiment, three females and four males were alive.

The female animals were first to react to DDT, showing hyperactivity and tremors earlier than the males. On the average, female mice first showed tremors on the 20th day after the DDT feeding was started and males on the 41st day. The time from the first sign of tremors until death was seldom more than five hours.

Upon autopsy in Group 1, each mouse, except F5, revealed hemorrhage in the gastrointestinal tract, but showed no abnormalities in the gross histology of testes, ovaries, oviducts, kidneys, or adrenals.

Pairs 14–26—300 ppm DDT

At the conclusion of Part I of the experiment, five females and three males remained alive. Upon dissection, two females and three males showed no hemorrhage within the gastrointestinal tract. The gross histology of the various organs appeared normal.

F25 gave birth to five young, three of which were still alive on the 45th day from the start of the test. The one male and each of the two female young had been kept in separate cages. The two females were put together on the 45th day. Two days later they died, demonstrating typical DDT symptoms. The single male young animal remained alive until termination of the experiment, 22 days later.

The time interval between tremors and death in animals fed DDT at 300 ppm was similar to mice fed DDT at 200 ppm. Food consumption had been similar to that in Group 1.

Pairs 27–38—controls

Food consumption per pair in Group 3 was similar to that in Groups 1 and 2, although weight loss among the male animals was much less (table 2). Hemorrhage was not found within the gastrointestinal tract of any control animal. Fecundity was highest in this group of mice (table 1).

There was an obvious decline in body weight and in most tissue weights, except for the adrenals. Because the adrenals appeared larger in the treated animals, a two-sided Student's t-test was used to discover if there was a significant difference ($P < .05$ considered significant). A significant difference was observed between the mean weights of adrenals of control males and males treated with 200 and 300 ppm

of DDT, but no difference was noted between the two treated groups (table 2). The mean adrenal weight of females treated with 300 ppm was different from that of the control females. The mean adrenal weight of those females treated with 200 ppm was not different from that of the control group or of those treated with 300 ppm DDT (table 1).

Conception

Nine of the 13 females given DDT at 200 ppm became pregnant during the first two-week interval that pairs were together. Only two females gave birth; the others died during the gestation period. Two non-pregnant female mice fed DDT at 200 ppm also died during this time (table 1).

Eight of the 13 females fed DDT at 300 ppm became pregnant during the first two-week interval. Three of these females died during gestation; five gave birth (table 1).

Eight of 12 female controls became pregnant during the first two-week interval. Only two of these animals (F27 and F33) had no surviving young by the 55th day after the start of the experiment. The young from F27 died because the female died 11 days after giving birth (table 1).

Part II of the Experiment

Three pairs of mice in the groups receiving 200 and 300 ppm of DDT, and six pairs of control animals were employed in Part II of the experiment. Litters were produced by one pair of unproven breeders receiving 200 ppm, by one pair of proven breeders receiving 300 ppm, and by four pairs of control mice. Two of four pairs of control mice were considered unproven breeders, since they had been members of a pair not producing young in Part I of the experiment. Five days after the adult mice were paired, males 33 and 34 died. On the seventh day male 35 died. These control males had shown no symptoms of illness, so death was probably attributable to natural causes. The control animals had better breeding results in both parts of the experiment.

The day after the young from F19 (300 ppm) were placed on a starvation diet, the female young died. The next day the male had entered terminal DDT tremors. The male from F25 that continued to receive 300 ppm DDT was still alive at the termination of the experiment, having been maintained on this diet for approximately 82 days. One female from F29 (control), given DDT at 300 ppm in this part of the experiment, died 37 days after first being observed in tremors.

In general, the young from F31, F35, F36, and F38, given feed containing 300 ppm DDT, were more hyperactive and nervous. They demonstrated greater irritability and more tendency to bite, but were alive 49 days after they had been placed on this diet.

DISCUSSION

Laug and Fitzhugh (1946), after feeding rats DDT for periods of six months to two years, noted that the livers, and to a lesser extent the kidneys, of the "test" animals were larger than those of the litter mate controls. Our results indicate that kidneys were larger in the control mice; the control animals maintained original body weight. Possibly the difference in results can be attributed either to variations in methods of feeding, differences in animals, or individual responses of the animals to the experiment. Laug and Fitzhugh fed the test animals DDT at 800 and 1200 ppm; the powdered DDT was dissolved in corn oil. The test mice in the present study were fed powdered DDT incorporated in chick starter mash at 200 and 300 ppm.

There was no gross indication of hypertrophy of the adrenals in treated groups. Perhaps the observations of larger adrenals in treated groups may have been due

to exhaustion of adrenal secretions in hyperactive treated animals and subsequent fatty deposition in these tissues.

The female from F25 (DDT, 300 ppm) gave birth to five young, three of which were alive on the 45th day from the start of the test. The one male and each of the female young were kept in separate cages. The two females were put together on the 45th day and died two days later; the male remained alive throughout the experiment. Possibly the two females ate more feed when placed together, this, in turn, increasing the intake of DDT.

Hayes et al. (1958), working with rats, showed that, at any substantial level of intake, female rats store more DDT-derived material than do males. Bernard and Gaertner (1964) deprived DDT-fed mice (300 ppm) of food for three days, after they had received this diet for 60 additional days. Four of 10 mice died in tremors; the remaining mice, although tremoring, were able to recover when placed on a DDT-free diet.

Hayes (1955) has shown that DDT passes through the placenta into the fetus. It is likely that this affects litter size and the number of young born that will survive. The young on feed containing DDT in our study showed greater nervousness, irritability, hyperactivity, and tendency to bite as compared to young of control animals. Gaertner (personal communication) indicated having observed such hyperactivity among mice receiving DDT in his studies.

Dahlen and Haugen (1954), working with commonly used insecticides and their toxic effect upon bobwhite quail (*Colinus virginianus*) and mourning doves (*Zenaidura macroura*), noted the appearance of hemorrhages in the pleural cavity, trachea, and neck region of poisoned birds. This could indicate an increase in blood pressure before death, with increased capillary permeability, or it might indicate a weakening of the capillary wall structure.

Leedy and Cole (1950) fed corn treated with various fungicides to ring-necked pheasants (*Phasianus colchicus*). "Spergon" (tetrachloro-para-benzoquinone) used alone was harmless, but when mixed with DDT it was poisonous. Pheasants fed this mixture showed a catarrhal-hemorrhagic enteritis at autopsy.

In experimental animals the major effects of DDT are on the nervous system (Hayes et al., 1956). Dale et al. (1962) showed that rats of both sexes that had been fed DDT weighed slightly less than control animals. Perhaps the experimental animal being fed DDT dissipates more energy in its excitable state, as DDT is known to cause an increase in the metabolic rate. DDT-treated animals also eat less before death and the gastrointestinal tract of casualties is nearly always empty (Hayes, 1955). Despite the great amount that is known about the toxicology and pharmacology of DDT, its basic mode of action still remains unknown.

TABLE 1
Organ weights and productivity of female mice in DDT tests

Test	200 ppm	300 ppm	Controls
Number of mice	10	8	7 (sacrificed)
Mean weight (mg)			
Kidneys	234.0	217.6	251.6
Adrenals†	4.2	4.8	3.6
Ovaries	8.9	7.2	9.5
Oviducts	3.5	3.2	3.0
Average number of embryos	6 (7)*	6 (8)*	**
Average number of young in litter	11 (2)*	8 (5)*	8 (8)*

*Number of females in parentheses.
**None of the control females had embyros at death.
†Two sided t-test.

TABLE 2
Organ and weights of male mice in DDT tests

Test	200 ppm	300 ppm	Controls
Number of mice	9	10	8 (sacrificed)
Mean weight (mg)			
Kidneys	248.5	289.4	431.3
Adrenals†	4.2‡	3.7‡	2.8‡
Testes	127.0	120.5	138.2
Average weight of males at 2½			
weeks*	35.6 (12)**	33.3 (12)**	33.8 (12)**
Average weight of males at death*	25.1 (8)**	24.7 (9)**	32.3 (8)**

*Body weights are in grams.
**Number of males in parentheses.
†Two sided t-test.
‡Probability <.05 was significant.

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