2. TOXIC EEFECTS IN ANIMALS

2.1 Acute and Chronic Toxicity

2.1.1. Acute Toxicity

Tables 2.1.1 and 2.1.2 summarize the acute toxicity as detailed in earlier reviews (Hayes 1959). Table 2.1.3 summarizes the range of toxic doses in man and in male and female rats. Table 2.1.4 gives the oral LD50 values of some DDT metabolites.

The acute action of DDT is almost exclusively on the central nervous system (CNS). The first signs, which are generally similar in different species, are abnormally susceptible to alarm stimuli, motor unrest, and increased frequency of spontaneous movements. These are followed by tremors that become constant. As severity increases, attacks of epileptiform tonic-clonic convulsions occur. DDT poisoning may ultimately result in death from ventricular fibrillation. These symptoms may be caused by single large doses of DDT as well as repeated exposure to the pesticide (USDHEW 1969).

2.1.2 Factors Modifying Toxicity

Harbison (1975) found that newborn rats are less sensitive to the toxic effects of DDT than adults (Table 2.1.5). Pretreatment with phenobarbital increased the neonates' susceptibility to DDT poisoning. Harbison hypothesized that phenobarbital may stimulate production of a metabolite inherently more toxic than the parent compound in newborn rats.

TABLE 2.1.1

ACUTE ORAL AND DERMAL LD_{50} OF DDT IN ANIMALS

Species	Formulation	Oral (mg/kg)	Dermal (mg/kg)
Rat	Water Suspension or Powder	500-2,500	1,000,000
	Oil Solution	113-450	250-3,000
Mouse	Water Suspension or Powder	300-1,600	375,000
	Oil Solution	100-800	250-500
Guinea Pig	Water Suspension or Powder	2,000	1,500,000
	Oil Solution	250-560	1,000
Rabbit	Water Suspension or Powder	275	375,000
	Oil Solution	300-1,770	300-2,820
Cat	Water Suspension or Powder		
	Oil Solution	100-410	
Dog	Water Suspension or Powder		
	Oil Solution	> 300	

Adapted from Hayes 1959

TABLE 2.1.2

ACUTE SUBCUTANEOUS, INTRAVENOUS, AND INTRAPERITONEAL LD_{50} OF DDT IN COMMON LABORATORY ANIMALS

Species	Formulation	Subcutaneous (mg/kg)	IV (mg/kg)	IP (mg/kg)
Rat	Water Suspension or Powder	>2,000		
	Oil Solution	200- 1,500	47	80-200
Mouse	Water Suspension or Powder	1,000- 1,500		
	Oil Solution	300		
Guinea Pig	Water Suspension or Powder			
	Oil Solution	900		150
Rabbit	Water Suspension or Powder			
	Oil Solution	250->3,200	30-41	<2,100
Cat	Water Suspension or Powder			
	Oil Solution	<650	32	
Dog	Water Suspension or Powder			
	Oil Solution		68	
Monkey	Water Suspension or Powder			
	Oil Solution		55	

Adapted from Hayes 1959

TABLE 2.1.3

COMPARISON OF THE SUSCEPTIBILITY OF MAN AND OTHER ANIMALS TO DDT

	Dosage* (mg/kg)							
Species	Largest without Clinical Effect	Smallest with Clinical Effect	Median Clinical CD50	Smallest with Serious Effect	Largest Nonfatal	Smallest Fatal	LD50	Uniformly Fatal
Man	-	6	10	16**	285***	-	-	-
Rat, F	-	-	-	75	150	100	118	200
Rat, M	25	-	-	50	175	50	113	200

*Single oral dose unless otherwise noted
**Convulsions
***Part of dose vomited

Adapted from Hayes 1975

TABLE 2.1.4

ORAL LD50 VALUES FOR METABOLITES OF DDT

Compound	Species (sex)	LD50 (mg/kg)
DDE	Rat (M)	880
"	Rat (F)	1,240
"	Mouse	700
"	"	1,000
DDD	Rat (M)	>4,000
DDA	Rat	1,900
"	Rat (M)	740
"	Rat (F)	600
"	Mouse	720
"	"	590

Adapted from WHO 1977

TABLE 2.1.5

EFFECT OF AGE ON THE TOXICITY OF DDT TO RATS

Number of Doses	Age	LD50* (mg/kg)
1	Newborn	4,000
1	"	2,356
1	10 days	728
1	14-16 days	437.8
1	Weanling	355.2
1	2 months	250
1	3-4 months	194.5
1	Middle aged	235.8
1	Adult	225
4	Preweaning	279.2
4	Adult	285.6

*Total intake

Adapted from WHO 1977

Nutrition appears to affect DDT toxicity in that well-fed mammals, especially fat ones, are more resistant to DDT poisoning than are poorly fed animals. Increased dietary fat increases the toxicity of dietary DDT, while increased protein in the diet decreases it. These effects may be due to enhanced absorption and increased activity of degradative enzymes, respectively. In starvation tests, the mobilization of fat increases the concentration of DDT in the blood and, consequently, augments the chemical's toxic effects (USDHEW 1969). DDT has also been reported to be more toxic to rats at 36 C than at lower temperatures (4-8 C) (USDHEW 1969).

2.1.3 Mode of Action

The acute toxicity of DDT is believed to result from effects on the central and peripheral nervous system (Ecobichon 1970), but the precise mechanisms of action remain unknown. Narahashi and Yamasaki (1960) studied the effects of DDT on the giant axons of the cockroach. They found that DDT prolongs the recovery phase of the action potential indicating the pesticide's influence on potassium (K^+) efflux. They supported this finding with evidence that this effect is accentuated by a lower concentration of potassium ions in fluid surrounding the nerve. Narahashi and Haas (1967) showed that DDT also prolongs the flow of sodium ions into the giant axons of the lobster. Thus, DDT delays shutting of the Na^+ gate and prevents full opening of the K^+ gate.

In addition, it has been found that small concentrations of DDT inhibit Na^+-, K^+-, and Mg^{2+}--stimulated adenosine triphosphatase (ATPase) derived from a nerve ending fraction of the rabbit brain (Matsumara and Patil 1969). Schneider (1975) found that inhibition of ATPase by DDT

in the rat brain is not caused by the binding of the pesticide to a specific site on the enzyme. Rather, it is the result of indirect alterations of the membrane that interfere with allosteric transitions of the ATPases mediated by Na^+ and K^+.

Byczkowski (1976) examined the effects of single, sublethal doses of p,p'-DDT on liver and brain mitochondia of the rat and found a time- and dose-dependent decrease in oxidative phosphorylation efficiency. A time-dependent suppression of respiratory activity was noted, as well as a simulation of mitochondrial ATPase activity 24 hours after DDT activity. The author suggested that the uncoupling of oxidative phosphorylation in brain mitochondria may be responsible for some phenomena of DDT intoxication in mammals.

2.1.4 Long-Term Feeding Experiments

A number of experiments involving long-term dietary exposure of rats, mice, hamsters, dogs, and other mammals to DDT are summarized in Table 4.2.1. The most pronounced effects of exposure to DDT at high dietary levels are on the liver and the CNS. Dietary levels associated with reduced lifespan are 400 ppm in rats, 250 ppm in mice, and 1,000 ppm in hamsters. At these dietary levels, many animals suffered from tremors and convulsions, and most showed liver injury upon autopsy, although some survived for the normal lifespan (Fitzhugh and Nelson 1947, Tomatis et al 1972, Agthe et al 1970). At lower dietary exposure levels several adverse effects have been reported, including increased liver weight, histopathologic changes in the liver, impaired reproduction, and increased incidence of tumors of the liver, lungs, and lympathic system. These effects are summarized in the following sections.

2.2 Organ-Specific Toxicity

2.2.1 Liver and Kidney Effects

In addtion to the effects on the CNS and reproductive organs, chlorinated hydrocarbon insecticides have been shown to exert prominent pharmacologic and morphologic actions on hepatic and renal tissues. DDT treatment results in tubular degradation and vascular congestion in the kidney (Smith 1948). Histologic changes occur in the livers of rats with even low levels of DDT in their diet.

Nelson et al (1944) and Kunze et al (1949) reported that histopathology could be detected in the livers of rats maintained for 4-6 months on a diet containing DDT at 5 ppm. However, Cameron and Cheng (1951) were unable to demonstrate any pathology in rats killed after being exposed for more than a year at levels corresponding to food concentrations of up to approximately 350 ppm. Other investigators, including Treon and Cleveland (1955), reported liver changes induced by DDT at relatively low dosages (12.5 and 25 ppm). Ortega et al (1956a, b) reported that liver cell necrosis occurred with dosages in excess of 1,000 ppm but not at lower levels. Histologic changes restricted to the liver occurred at levels as low as 5 ppm, but liver function, as measured by bromsulphthalein excretion, was not affected in rats fed 400 ppm or less. The histologic changes in the parenchymal cells of the liver consisted of increased fat deposition, margination of cytoplasmic granules, and hypertrophy of the cells. The most characteristic change was the formation of complex, lipoid cytoplasmic inclusion bodies termed "liposphères." More recently, Ortega (1962) reported additional details

of these cytoplasmic alterations as noted in light and electron microscopy studies.

Monkeys develop liver histopathology only with exposure at relatively high dosage levels of DDT (Durham et al 1963). No liver histopathology occurred in monkeys fed DDT at dietary levels of 200 ppm or less for periods of up to 7.5 years. One of six monkeys fed DDT at 5,000 ppm did develop the cytoplasmic inclusions that have been characteristically associated with chlorinated hydrocarbon poisoning in the rat.

Kimbrough et al (1971) made detailed studies of changes in liver ultrastructure. Rats were exposed to technical DDT at 250 and 500 ppm and to DDT in combination with 50 ppm and 100 ppm, respectively, of technical grade dieldrin at 50 ppm and 100 ppm, respectively. After rats had been exposed for 8 weeks, their livers were examined by light and electron microscopy. Liver weights of all exposed groups were significantly higher than those of the controls, and the livers of rats fed the combination of DDT and dieldrin weighed significantly more than those of rats given DDT alone. Morphologic changes (outlined in Table 2.2.1), including an increase in SER and atypical mitochondria, were observed in all exposed groups and were more pronounced in rats given both DDT and dieldrin.

2.2.2 Liver Enzyme and Other Biochemical Effects

Numerous studies have been conducted in which DDT has been identified as an inducer of hepatic microsomal enzymes and hence as an effector of the metabolism of drugs, pesticides, and other foreign chemicals (Conney 1967; Hart and Fouts 1963, 1965; Balazs and Kupfer, 1966b). As discussed in Section 2.3.4, the induction of hepatic microsomal enzymes can lead to

TABLE 2.2.1

LIGHT AND ELECTRON MICROSOPIC FINDINGS IN THE LIVERS OF RATS EXPOSED TO DIELDRIN AND DDT IN THE DIET

Dietary Concentration	Light Microscopic Findings	Electron Microscopic Findings
DDT, 250 ppm	Cells around central veins sightly enlarged; smooth-looking cytoplasm; lipid inclusions, mild to moderate	Mild to moderate increase in SER, occasional large myelin figures; atypical mitochondria seen in 1 of 5 animals
DDT, 500 ppm	Enlarged cells except in the periphery of the lobules; moderate number of vacuolated cells with inclusions in 2 of 5 rats; margination; lipid inclusions, moderate in 2 of 5	Less glycogen in 2 of 5 livers; marked increase in SER, swollen in some areas, occasional myelin figures, occasional atypical mitochondria in 3 of 5 livers
Dieldrin, 50 ppm, plus DDT, 250 ppm	All cells enlarged, smooth cytoplasm, margination, inclusions moderate to many; lipid inclusions, mild to moderate	Moderate to marked increase in SER, swollen in some areas, less glycogen, slight to moderate number of atypical mitochondria
Dieldrin, 100 ppm, plus DDT, 500 ppm	All cells enlarged, many cells with exceptionally large nuclei; margination in cytoplasm and inclusions in almost all cells; lipid inclusions, not studied	Marked increase in SER, which was swollen; indistinct cell borders; atypical mitochondria; vacuolated areas in cytoplasm surrounded by layers of dense lamellated material
None	Liver cells normal; lipid inclusions, none to moderate	Occasional small myelin figures

Adapted from Kimbrough et al 1971

enhanced metabolism of steroid hormones, with consequent effects on reproduction.

The lowest reported dosage of DDT for induction of various microsomal enzymes in the rat has been estimated at about 0.05 mg/kg/day, ie, a dietary level of 1 ppm (Kinoshita et al 1966) or 0.5 mg/kg/day (Schwabe and Wendling 1967). Gillett et al (1966) estimated that the threshold dose for enzyme induction was 0.125 mg/kg/day. Street et al (1969) estimated the threshold at 0.05 mg/kg/day. The different estimates are not necessarily inconsistent, since they depend on different test systems. In any event, the lowest estimate (0.05 mg/kg/day) is only 0.2 times that known to be effective in man (Laws et al 1967, Poland et al 1970).

A 27 percent reduction in pentabarbital sleeping time occurred in rats injected intraperitoneally with a single dose of DDT at 1 mg/kgs, which was associated with an average concentration of DDT in the fat of only 9.5 ppm (Conney et al 1967).

Hoffman et al (1970) administered DDT to weanling male rats for 14 days. No increase in the rate of p-nitroanisole (p-NA) metabolism was observed at dietary concentrations of DDT of 0.5 or 2.0 ppm. Concentrations of 4-750 ppm in the diet for 14 days produced increases proportional to the log dose. Extrapolation of this portion of the dose-response curve to the abscissa indicated a no-effect concentration of DDT in the diet of 3.27 ppm.

In addition to having effects on microsomal hydroxylating enzymes, DDT has been shown to influence some enzymes of intermediary metabolism and other miscellaneous enzymes and biochemical functions in vitro and sometimes in vivo (Table 2.2.2). Hrdina et al (1975) have discussed

TABLE 2.2.2

SUMMARY OF BIOCHEMICAL PARAMETERS AFFECTED BY DDT IN MAMMALS IN VIVO

Insecticide	Parameter	Effect
DDT	Serum asparatate aminotransferase	Increase
	Serum alanine aminotransferase	"
DDT; Dieldrin	Blood lactic and pyruvic acid	"
	Plasma free fatty acids	"
	Plasma corticosterone	"
DDT	Hexose shunt pathway	Decrease
	Total liver protein	Increase
	Hepatic NAD, NADP, NADH, NADPH	Decrease
	Hepatic NAD hydrolase	Increase
	Hepatic choline esterase	"
DDT; Dieldrin	Hepatic RNA	"
	Incorporation of leucine ^{14}C into protein	"
	Hepatic triglycerides	"
	Hepatic succinic acid dehydrogenase	Decrease
	Hepatic gluconeogenic enzymes	Increase
	Hepatic mixed function oxidases	"
DDT	Uterine weight	"
	Uterine glycogen	"
	Uterine RNA	"
DDT	Brain cytochrome oxidase	Decrease
	Brain acetyl choline	"
	Brain 5-hydroxy indole-acetic acid	Increase
DDT	Brain glutamine	"

Adapted from Kohli 1975

the importance of glucose metabolism in the toxic effects of DDT on mammals and reviewed extensive data suggesting that the effects of DDT involve interactions with cyclic AMP. They proposed that the changes in carbohydrate metabolism induced by DDT might involve alterations in the cyclic AMP-adenyl cyclase system of kidney cortex and liver.

2.2.3 Effects on the Cardiovascular System

Most dogs killed by a single dose of DDT die of ventricular fibrillation, and the same is true of some cats, monkeys, and rabbits. Monkeys differ from dogs in that the DDT-sensitized heart is able to recover from fibrillation and resume a normal rhythm (Philips and Gilman 1946). DDT not only sensitizes the myocardium in a way similar to that of halogenated hydrocarbon solvents but, through its action on the central nervous system, produces the stimulus that increases the likelihood of fibrillation.

There is no evidence that repeated, tolerated doses of DDT sensitize the heart. Rats were fed DDT at a dietary level of 200 ppm (about 10 mg/kg/day) for 8 months, during which they received weekly, intraperitoneal doses of vasopressin, a compound that causes a temporary myocardial ischemia. Electrocardiograms showed no significant increase in cardiac arrhythmias in the DDT-fed rats as compared with controls. The same results were obtained in rabbits treated in essentially the same way (Jeyaratnam and Forshaw 1974). Male mice exposed to p,p'-DDE at dietary levels of 125 or 250 ppm throughout life suffered a high incidence of myocardial necrosis (Tomatis et al 1974a).

2.2.4 Adrenal Effects

DDD has been used as a drug to control different forms of adrenal overproduction of corticoids in man. This therapy originally was based on

the demonstration that DDD (Nelson and Woodard 1948), especially o,p-DDD (Cueto and Brown 1958), caused gross atrophy of the adrenals and degeneration of the cells of the inner adrenal cortex in dogs. However, a dosage of approximately 100 mg/kg/day for many weeks was necessary to produce any benefit in man (Bledsoe et al 1964, Southren et al 1966a, b). In contrast, only 4 mg/kg/day produced marked atrophy of the adrenal in the dog. Kupfer (1967) reviewed the extensive literature indicating that the effect in man and most other species, except the dog, is caused by stimulation of corticoid metabolism by massive doses of o,p'-DDD and not by any direct effect on the adrenal. Southren et al (1966a,b) agreed that the effect was predominantly extra-adrenal in man when the drug was first given but offered evidence that adrenal secretion of cortisol was eventually reduced.

2.2.5 Other Endocrine Effects

Besides the pronounced effects of DDT and some of its metabolites on the adrenal cortex in various species, the pesticide appears to influence other mammalian endocrine organs. Nelson et al (1944) were the first to report mild changes in the thyroids of mice, rats, guinea pigs, rabbits, and dogs fed sublethal doses of DDT. Fregly et al (1968) noted changes in indicators of metabolic rate, such as food intake and oxygen consumption, of the rats they dosed with o,p'-, p,p'-, and m,p'-DDD. At 1,000 ppm, although there was an increase in thyroid weight, there were no overt symptoms of either hyperthyroidism or hypothyroidism. At 3,000 ppm, on the other hand, the increased thyroid weight was accompanied by reduced food intake, body weight gain, and oxygen

consumption, and an increased rate of cooling upon exposure to cold air. These changes are indicative of a hypometabolic state, and the authors concluded that DDD resulted in hypothyroidism in the rat.

Bastomsky (1974) found that DDT did not affect the biliary excretion of thyroxine in rats but did increase bile flow and the biliary clearance rate of plasma thyroxine. It also caused a marginal elevation of bile: plasma ratios and slightly increased the proportion of biliary iodine present as glucuronide. DDT did not affect uptake of iodine by the thyroid.

In a study by Seidler et al (1976), administration of DDT at 30-75 mg/kg produced marked increases in the thyroid mass and levels of triiodothyronine and thyroxin in the thyroid of rats. Other simultaneous effects included a decrease in the thyroid iodine level, a reduction of serum iodine and protein-bound iodine, a slight increase in serum thyroxin, and a marked increase of serum triiodothyronine and the iodine fraction in the liver.

Jefferies (1975) reviewed the effects of DDT and other organochlorine compounds on the thyroid in birds and mammals and suggested that a number of sublethal effects of DDT may be associated with primary effects on the thyroid. These effects include changes in metabolic rate, body temperature, respiratory rate, behavior, reproduction, Vitamin A storage and circulation, lipid metabolism, carbohydrate metabolism, and calcium metabolism.

From examination of isolated pancreatic islets of DDT-exposed mice, Yau and Mennear (1977) determined that oral exposure to DDT at 50 mg/kg reduced insulin secretion to 32% of the control value. Similarly, islets from DDT-treated mice were significantly less responsive to tolbutamide, indicating that the pancreatic inhibitory effect of DDT is not specific for glucose stimulation.

2.2.6 Effects on the Immune System

Street and Sharma (1974) tested the immune response of rabbits fed graded concentrations of p,p'-DDT in their diets. The animals were challenged with sheep red blood cells and Freund's adjuvant after 4 weeks of feeding and then continued on the same diet during a subsequent 4-week evaluation of the status of their immune systems. DDT was found to reduce the count of plasma cells in popliteal lymph nodes, to reduce the number of germinal centers in the spleen, and to induce atrophy of the cortex of the thymus. These responses were generally scaled to increasing levels of the pesticide in the diet and were significant even at a dosage of 0.92 mg/kg/day for 28 days. Hemolysin and hemagglutinin titers were not significantly affected by treatment with DDT and no consistent trends observed. DDT decreased, though not significantly, the antigen-induced increase in serum gamma-globulin and significantly increased the preantigen gamma-globulin values. Skin sensitivity to tuberculin was decreased, but only at the high doses of DDT.

Hamid et al (1974) exposed rats to o,p'-DDD and found a decreased body weight, as well as decreases in the weights of the thymus, spleen, and adrenals. In well-nourished rats the numbers of plaque-forming cells (PFC) and rosette-forming cells (RFC) in the spleen and thymus were lowered by treatment with o,p'-DDD. Similarly treated rats on a protein-deficient diet had numbers of PFC and RFC in their spleen almost equal to those of controls. Atrophy of both the adrenal cortex and the thymolymphatic organs was found in the groups exposed to DDD, regardless of nutritional condition.

In another study, rats immunized with diptheria toxoid and fed diets containing DDT at 20 and 200 ppm levels for 31 days showed no effects on their serum antitoxin titers. However, the numbers of metachromatic, histamine-containing mast cells in mesenteries were reduced, by 46% in the 20 ppm group and by 61% in the 200 ppm group. The severity of anaphylactic shock was also reduced in the rats exposed to DDT (Gabliks et al 1975).

2.2.7 Central Nervous System Effects

The clinical signs and pathologic changes seen in mammals exposed to chlorinated hydrocarbon insecticides have led to attempts to establish whether any correlation exists between the observed neurotoxic effects of these compounds and changes in the function of the cerebral motor cortex and the cerebellum. Electroencephalographic (EEG) studies showed significant alterations in spontaneous electrical activity of the brain in cats, monkeys (Crescitelli and Gilman 1946), and rats (Woolley and Barron 1968, Henderson and Woolley 1970) after acute exposure to DDT. Administration of a single dose (50-75 mg/kg iv) of this insecticide to cats and monkeys caused the normal pattern of irregular bursts of waves to be shifted to a persistent type of rhythm in the cerebral motor cortex, whereas in the cerebellum there was a progressive increase in amplitude of waves followed by a constant peak at the time the tremors appeared (Crescitelli and Gilman 1946). Pollock and Wang (1953) showed that cats ingesting DDT in the diet manifested EEG changes associated with ataxia and tremors. These were observed initially in the cerebellum and, as the intensity of insecticide poisoning increased, seizure activity was noted

in the cerebral cortex. Dēsi et al (1966) and Farkas et al (1968) reported that when rats were given daily doses of DDT at 20 mg/kg, marked changes in the EEG pattern (increase in both frequency and amplitude) were observed after 4 weeks and slight ataxia was noted after 5 weeks. Woolley and Barron (1968) found that DDT-induced changes in cerebellar electrical activity occurred sooner and were greater in magnitude than those observed in other brain areas such as the motor cortex and the reticular formation. Further the changes in cerebellar activity occurred before signs of toxicity such as tremors became evident, suggesting that the cerebellum is particularly sensitive to the effects of DDT. Haymaker et al (1946) pointed out that the tremors and ataxia seen in acute DDT poisoning were similar to the signs of cerebellar dysfunction. The preceding EEG studies offered evidence to support the view that the cerebellum and the cerebral motor cortex may be two important target areas for the action of DDT on the central nervous system.

Scudder and Richardson (1970) found that chronic administration of technical DDT at low concentrations (0.1 or 1.0 µg/liter in drinking water) to pregnant mice and their offspring resulted in a significant decrease in the aggressiveness of male offspring. The mean latent period for attack behavior in paired encounters was 4-10 times greater in the exposed mice. No effects were observed in the offspring of mice exposed at 0.01 µg/liter.

Another effect of prenatal exposure to low levels of DDT was reported by Al-Hachim and Fink (1968). The offspring of female mice exposed to DDT at 2.5 mg/kg in the 2nd or 3rd week of pregnancy showed a delayed acquisition of conditioned avoidance responses when tested at age 32-37 days. Craig

and Ogilvie (1974) exposed female mice to technical DDT at 200 ppm in their diets throughout pregnancy and lactation. Their offspring subsequentl made significantly more errors and took significantly longer than controls in running a T-maze.

Peterle and Peterle (1971) studied the effects of feeding technical DDT at a dietary concentration of 7 ppm on the aggressive behavior of male mice. Treated mice "lost" more bouts, as determined by posturing and avoidance behavior, and controls made more biting attacks. Thus, the DDT-fed mice were significantly less agressive than control mice and were more likely to submit to territorial fights.

Medved et al (1968) reported that administration of DDT to cats (by an unspecified route) affected conditioned reflexes. A single dose of 100 mg/kg caused a total extinction of conditioned reflexes, which did not return to normal for 8-12 days. Doses of 50-75 mg/kg caused a small increase in latency of conditioned reflexes, which returned to normal after 3-4 days. No effects were observed after doses of 10 or 25 mg/kg.

Sobotka (1971) reported alterations in several behavioral and neurophysiologic parameters in mice given single low doses of DDT. The open-field exploratory activity was significantly enhanced 24 hours after a single oral dose of DDT at 25 mg/kg. At the same time, the ability of animals to adapt to the open-field situation was attenuated. In a multi-generation study with mice exposed to technical DDT at 2.8-3.0 ppm in the diet, no effects were observed on spontaneous or caffeine-induced motility (Tarján and Kemény 1969).

2.3 Effects on Reproduction

2.3.1 In Rats

Ottoboni (1969) reported that female rats reproduced normally when fed technical DDT at dietary concentrations as high as 200 ppm for two generations. At a dietary concentration of 20 ppm, female rats had a significantly longer reproductive lifespan (14.55 months) than littermate controls (8.91 months). The number of treated females conceiving and the number of successful pregnancies after the age of 17 months was significantly greater than in controls.

Jonsson et al (1975) administered technical DDT to female rats at concentrations of 75 and 150 ppm in the diet, for periods of 8 and 36 weeks. At the higher concentration of DDT, only one of seven females mated and no pups were born. At the lower concentration, the number of rats producing litters was reduced, but the size of litters was not altered significantly. Adverse effects on reproduction were noted at plasma DDT concentrations above 800 ppb, while concentrations below 500 ppb were associated with nearly normal reproduction.

Male and female Wistar rats given diets containing o,p'-DDT at 0, 20, 200, or 1,000 ppp or p,p'-DDT at 0, 20, 200, or 500 ppm were studied throughout a 6-month breeding period. Growth was severely depressed in pups nursing from dams fed p,p'-DDT at 200 or 500 ppm, and all pups in the 500 ppm group died within 10 days of birth. The growth of pups from the group fed o,p'-DDT at 1,000 ppm was reduced below that of controls, and surviving females from this group showed significantly reduced fertility

and fecundity when mated at age 80 days. Two sterile females from this group had polycystic ovaries. Offspring of rats fed o,p'-DDT at 200 ppm were reported to have reproduced normally (Clement and Okey 1974). In a study by Wrenn et al (1970), feeding o,p'-DDT at dietary concentrations of 1 or 2.5 ppm had no significant adverse effects on reproduction.

Treon and Cleveland (1955) reported the results of a three-generation reproductive study in Carworth Farms rats fed recrystallized DDT at 2.5, 12.5, and 25 ppm in the diet. The number of pregnancies and the size of litters were unaffected by DDT treatment, but all dose regimens caused a "slight" increase in mortality in offspring during the first 21 days of life.

Duby et al (1971) exposed rats to p,p'-DDT, o,p'-DDT, or technical DDT at dietary concentrations of 1 and 15 ppm through two generations. They found no effects by the three compounds on reproductive performance, as measured by litter size at birth, litter weight at day 21, growth patterns of offspring, time of vaginal opening, fertility, or fecundity.

Green (1969) exposed Sprague Dawley rats to DDT at a dietary concentration of 7 ppm and observed marked reductions in fertility and survival of offspring in the first generation of the exposed rats. No rats in the second generation exposed to DDT conceived. Simultaneous exposure to aldrin (5 ppm), endrin (5 ppm), or heptachlor (5 ppm) increased the adverse effects on conception rate and pup survival, but the effects appeared less than additive. Green (1969) also cited unpublished data by C. Agthe indicating impaired reproduction in rats exposed to DDT at 10 ppm.

2.3.2 In Dogs

Deichmann et al (1971) and Deichman and MacDonald (1971) reported a study in which four male and three female beagle dogs were exposed to p,p'-DDT at 12 mg/kg and four males and four females were exposed to a mixture of p,p'-DDT at 6 mg/kg and aldrin at 0.15 mg/kg. The dogs were exposed five times a week for 14 months, and attempts were made to breed them between the 2nd and 70th weeks after cessation of exposure. By then the concentrations of DDT and metabolites had fallen below 3 ppb in the blood and below 32 ppm in the fat in most of the dogs. Exposed dogs showed a moderate increase in serum alkaline phosphatase activity but no change in other measured biochemical parameters. Reproduction was severely affected, as evidenced by diminished libido in the males and delayed estrus in females. At the time of partuition, infertility, reduced mammary development and milk production, and increased infant and maternal mortality were apparent.

In contrast to these results of Deichmann et al (1971), Ottoboni et al (1977) reported little effect on reproduction in beagle dogs exposed to technical DDT at 1, 5, and 10 mg/kg/day for three generations. The only statistically significant effect they reported was an earlier occurence of first estrus (by 2-3 months) in treated females. They noted no adverse effects on gestation period, fertility, success of pregnancy, litter size, and lactation or on the viability, survival, sex ratio, or growth of pups. There was a consistent increase in liver weight in pups littered from exposed dogs.

2.3.3 In Mice

In a six-generation study in mice, DDT at 25 ppm was reported to have no effects on fertility, gestation, viability, lactation, and survival. At 100 ppm, lactation and survival were slightly reduced. Severe effects on reproduction were reported at 250 ppm (Keplinger et al 1968). Some adverse effects on reproduction, primarily decreased lactation indices and viability indices, were also reported in groups of mice fed combinations of pesticides, including aldrin at 10 ppm plus DDT at 100 ppm, chlordane at 100 ppm plus DDT at 100 ppm, and dieldrin at 10 ppm plus DDT at 100 ppm (Deichmann and Keplinger 1966, Keplinger et al 1968, Deichmann and MacDonald 1971).

Two large-scale feeding tests were conducted with BALB/c and CFW mice to investigate effects of techinical DDT on reproduction. In these studies DDT at 7 ppm in the diet did not affect adult mortality, fertility, or fecundity, and offspring were not adversely affected (Ware and Good 1967).

In a five-generation study, no effects were observed on the reproductive performance of BALB/c mice exposed to DDT at a dietary concentration of 2.8-3.0 ppm. Reproductive parameters studied included the numbers of pregnancies and births, litter size, survival to weaning, average weight at weaning, and average lifespan (Tárjan and Keměny 1969).

Lundberg and Kihlstrom (1973) investigated the effects on the number of implanted embryos in mice given injections of p,p'-DDT (containing "small amounts" of o,p'-DDT and p,p-DDD) at high doses (20, 50, and 100 mg/kg). Depending upon the dose regimen of DDT and the particular days injections were given during gestation, the number of implantation sites in the DDT-treated mice was decreased. Subsequent studies by Lundberg (1974) confirmed that DDT caused a decreased in the frequency of implanted embryos.

2.3.4 Interactions with Steroid Hormones

A number of investigators have shown that DDT and its metabolites are capable of inducing hepatic mixed function hydroxylating enzymes with consequent secondary effects on the circulating concentrations of steroid hormones. Kupfer (1975) and Kupfer and Bulger (1976a) have reviewed the literature on these effects, tracing the studies to the original discovery by Hart and Fouts (1963, 1965) that chlorinated hydrocarbons are potent inducers of mixed function oxidases (MFO). In the case of DDT, effects have been measured at dietary levels as low as 1 ppm (Hart and Fouts 1965, Kinoshita et al 1966).

Balazs and Kupfer (1966a,b) first established the link between induction of MFO by chlorinated hydrocarbons and enhanced steroid metabolism in vivo. Conney (1967), Conney et al (1967, 1973), and Welch et al (1971) demonstrated that exposing rats to chlorinated hydrocarbons and other inducers of MFO stimulates androgen and estrogen metabolism (measured in vitro) and diminishes the hormonal activity of administered steroids. Both o,p'-DDD and technical DDT have been shown to stimulate the hydroxylation of cortisol in vivo (Balazs and Kupfer 1966a, Kupfer et al 1964, Southren et al 1966a). The action of technical DDT is thought to be attributable to the o,p' isomer, because p,p'-DDT had little effect (Kupfer and Bulger 1976a).

Other interactions of DDT with steroid hormones and the male and female reproductive systems have been reviewed by Thomas (1975). DDT can interfere with male sex accessory gland metabolism as evidenced by a decrease in the affinity of the mouse prostate gland for radioactive

testosterone (Smith et al 1972). Doses of DDT that exerted this inhibitory effect on the assimilation of androgen by the mouse prostate gland failed to exert any uterotrophic effect in female mice. Such findings tend to exclude the possibility of inherent estrogenicity accounting for this inhibition of androgen uptake by the prostate gland. Studies by Wakeling and Visek (1973) revealed that o,p'-DDT can inhibit the binding of dihydrotestosterone to specific receptor proteins in the cytoplasmic fraction of the rat prostate gland.

Administration of DDT to male mice had no effect on testicular weights and produced no changes in sex accessory organ weights (Thomas and Lloyd 1973). Neither the seminal vesticles nor the prostate glands were affected by the oral administration of technical grade DDT. Prostate gland fructose, a chemical indicator of androgenic activity, was not altered by a 10-day administration of DDT in large doses.

Administration of a single oral dose of radiolabeled DDT resulted in the localization of considerable amounts of radioactivity in several organs of the male reproductive system (Smith et al 1972). The prostrate gland and gonads contained sizeable concentrations of radioactivity as early as 1 and 2 hours after ingestion of labeled DDT. Epididymal fat pads retained some DDT or its metabolites as long as 12 days after an oral dose. DDT or its metabolites were also detected in the seminal vesicles and in the seminal plasma of mice (Smith et al 1972). DDT has been reported to be present in high concentrations in the fat tissues and gonads of exposed female mice (Bäckström et al 1965). Tomatis et al (1971)

reported that, while fat tissues of exposed mice contained the highest amounts of DDT and its metabolites, appreciable levels were also found in the reproductive organs. In mice exposed to technical DDT at 50 ppm in the diet for 13-30 weeks mean concentrations were 48 ppm DDT, 16 ppm DDD, and 7.2 ppm DDE in ovaries and 15 ppm DDT, 1.7 ppm DDD, and 0.7 ppm DDE in testes.

Studies by Kuntzman et al (1966) showed that DDT exposure caused a marked increase in hepatic 16-alpha-androgen hydroxylase activity in immature male rats. Smaller increases reportedly occurred in the 6-beta and the 7-alpha hydroxylation of testosterone. These authors suggested that the 16-alpha hydroxylation of testosterone was catalyzed by a different enzyme system from that required for the 6-beta or the 7-alpha hydroxylations.

In the male mouse, DDT exposure can actually inhibit hepatic androgen hydroxylase activity (Thomas and Lloyd 1973). Both the 7-alpha- and 16-alpha-testosterone hydroxylases were profoundly inhibited by DDT in mouse hepatic microsomes. DDT exerted little effect on the 6-beta-testosterone hydroxylase enzyme.

It is noteworthy that o,p'-DDD exerts a remarkably consistent effect on the metabolism of androgens in humans (Hellman et al 1973). This compound can profoundly decrease the conversion of testosterone and androsteredione to androsterone and etiocholanolone. DDD can also increase the rate of conversion to uncharacterized polar metabolites. Thus the chemicals in the DDT complex can affect changes not only in the metabolism of steroids in the hepatic microsomes but also in the periphery (Thomas 1975).

Lloyd et al (1974) administered technical DDT at 25 or 50 mg/kg orally for 10 days to male mice. There was a significant reduction in the accumulation of testosterone and its principal metabolite 5-alpha-dihydrotestosterone by the anterior prostate gland. Hepatic formation of polar metabolites of testosterone was also reduced by DDT-pretreatment. No significant changes were observed in accessory sex organ weights or prostate gland fructose concentration. The authors suggested that DDT may alter the accumulation of prostatic androgens as a result of altered hepatic steroid hydroxylation.

2.3.5 Estrogenic Effects

In addition to DDT having the effects summarized above, o,p'-DDT has been reported by a number of authors to be estrogenically active and to have significant effects on development when administered to neonates. The estrogenic activity of o,p'-DDT (and of technical DDT which contains this isomer) was first reported by Bitman et al (1968) and Levin et al (1968). Singhal et al (1970) demonstrated that exposure to o,p'-DDT elevated certain uterine enzymes in female rats. Forster et al (1974) tested several homologs of DDT for their ability to inhibit specific binding of estradiol to uterine cytosol and nuclear fractions. Both o,p'-DDT and o,p'-DDE inhibited the in vitro binding of estrogen, but o,p'-DDD, m,p'-DDD, p,p'-DDE, p,p'-DDT, and DDA were inactive. Nelson et al (1976) found that o,p'-DDT required no metabolic activation to exert estrogenic effects, unlike analogs such as methoxychlor. The effects of various isomers and an analog are summarized in Table 2.3.1.

TABLE 2.3.1

ORDER OF ESTROGENIC POTENCIES OF VARIOUS DDT HOMOLOGS IN THE RAT

Uterotropic	Glycogen Elevation	Ornithine Decarboxylase Induction	Receptor Inhibition of E_2 Binding
o,p'-DDT	o,p'-DDT	o,p'-DDT	o,p'-DDT
Methoxychlor	o,p'-DDE (e)	o,p'-DDD	o,p'-DDD
p,p'-DDT	Methoxyclor (e)	p,p'-DDT	o,p'-DDE
o,p'-DDD	p,p'-DDT (e)	p,p'-DDE (e)	Methoxychlor
m,p'-DDD	o,p'-DDD (I)	p,p'-DDD (e)	p,p'-DDT
p,p'-DDE (I)	m,p'-DDD (I)		p,p'-DDD (I)
p,p'-DDD (I)	p,p'-DDD (I)		p,p'-DDE (I)
	p,p'-DDE (I)		

e = Equally active; I = Inactive

Adapted from Kupfer and Bulger 1976a

Duby et al (1971) reported that injection of o,p'-DDT into 21-day-old female rats (1,2,or 4 mg/rat) led to increased uterine weight at maturity. Similar exposure to p,p'-DDT had only slight effects, while treatment with technical DDT led to effects corresponding to the content of the o,p' isomer. Similar estrogenic effects were observed in mink exposed to o,p'-DDT at age 120-150 days.

Gellert et al (1974) found that neonatal rats exposed to o,p'-DDT had permanently altered neuroendocrine differentiation. Female rats given 0.1 mg of o,p'-DDT on the 2nd, 3rd, and 4th days of life showed precocious puberty, persistent vaginal estrus, and anovulation. Administration of o,p'-DDT to neonatal female rats also led to the development of polycystic ovaries and uterine histopathology, including patches of stratified squamous epithelium in the endometrium, after puberty. Male neonates were unaffected by similar treatment. However, Campbell (1976) reported precocious development of the adrenal cortex in male rats exposed to up to 400 µg of o,p'-DDT on days 1-5 after birth.

Wrenn et al (1970) found that o,p'-DDT innduced precacious puberty in rats fed doses as low as 50 µg daily on days 18-33. Lee and Visek (1975) reported that male rats injected with 3 mg of o,p'-DDT at 1-3 hours after birth subsequently showed an abnormal pattern of sexual brain differentiation. This was attributed to inhibition of normal action of testosterone on the developing brain.

Preweaning exposure of neonatal males to milk from dams injected with 50 mg o,p'-DDT daily during postnatal days 1-25 caused statistically significant alterations in body weight and in the weights of the testes and ventral prostate (Campbell and Mason 1975).

Krause et al (1975) reported that male rats exposed to technical DDT at 500 mg/kg on the 4th and 5th days of life or at 200 mg/kg/day on days 4-23 showed lower fertility than controls. This was associated with degeneration of spermatogenic cells and a decrease in the number of Leydig's cells. Damage to the seminiferous epithelium was attributed to reduction in testosterone.

Most of these studies indicating lasting effects of neonatal exposure to o,p'-DDT or technical DDT have involved direct administration of the compounds to the infant rats or mice. Kihlström et al (1975) found that the reproductive capacity of mice was also impaired by exposure to DDT in maternal milk, as a result of exposure of the mothers to four weekly doses of 50 mg/kg during lactation. Jonsson et al (1975) found that ingestion by female rats of diets containing technical DDT at 75 or 150 ppm, with consequent exposure of the embryos and neonates, did not lead to polycystic ovaries in the offspring. Wrenn et al (1970) observed that rats given o,p'-DDT at dietary levels of 1 and 2.5 ppm showed no measurable effects on reproduction, except an increase in the birth weight of pups in the 2.5 ppm group.

Kupfer and Bulger (1976a,b, 1977) have reported detailed studies of the mechanisms by which o,p'-DDT exerts estrogenic and other effects in mammals. They attributed at least some of the effects to the binding of o,p'-DDT to the estrogen binding receptor, a binding that has been demonstrated in rat and human tissues in vitro.

2.4 Teratogenesis

p,p'-DDT administered to pregnant mice at a rate of 1 mg/kg on days 10, 12, and 17 of gestation caused morphologic changes in the gonads and

reduced the fertility of offspring, especially females (McLachland and Dixon 1972).

Schmidt (1973) reported blastotoxic, embryotoxic, and fetotoxic effects of DDT given in single or repeated doses to pregnant mice. The minimum single embryotoxic dose was 25 mg/kg administered 10 days postcopulation; daily doses of 2.5 mg/kg were significantly embryotoxic. No teratogenic effects were reported.

In a study by Ottoboni (1969), the offspring of female rats exposed to technical DDT at dietary levels of 200 ppm throughout pregnancy and lactation showed a significant increase of "ringtail," a constriction of the tail followed by spontaneous amputation.

Hart et al (1971) reported that exposure of rabbits to DDT at 50 mg/kg on days 7,8, and 9 of gestation caused premature delivery, increased resorption, and decreased intrauterine growth, but no congenital defects were produced.

Green (1969) administered DDT at a dietary concentration of 7 ppm to Sprague-Dawley rats for 60 days before breeding and throughout pregnancy. There was a marked decrease in fertility and a small increase in the frequency of resorptions, but the incidence of abnormal embryos in the exposed rats (2/131) was no larger than that in the controls (9/396).

In other studies of reproduction of mammals exposed to DDT, congenital defects have been reported to be within the normal frequency range (Ottoboni 1969) or have not been mentioned. However, it is not clear that the offspring have been adequately examined in any study except those of Green (1969), Hart et al (1971), and Schmidt (1973).

2.5 Carcinogenesis

Data on the carcinogenicity of DDT and metabolites published through 1973 have been reviewed and summarized by the International Agency for Research on Cancer (IARC 1974). Extensive studies conducted by IARC, itself, have been reviewed by Tomatis and Turusov (1975).

2.5.1 In Mice

Innes et al (1969) reported the results of a study in which 18 male and 18 female (C57-BL/6 x C3H/Anf) F1 mice and a similar number of (C57BL/6 x AKR) F1 mice were given single doses of 46.4 mg/kg p,p'-DDT by stomach tube at 7 days of age, and the same absolute amount was then given daily until the animals were 28 days of age, when they were transferred to a diet containing p,p'-DDT at 140 ppm. Mice were killed at 81 weeks. In both strains, about 30% of the females died during treatment. Hepatomas were found in 11/18 male and 4/18 female exposed (C57BL/6 x C3H/Anf)F1 mice, compared with 8/79 in male and 0/87 in female controls, and in 7/18 male and 1/18 female exposed (C57BL/6 x AKR)F1 mice, compared with 5/90 in male and 1/82 in female controls. In addition, 6/18 exposed (C57BL/6 x AKR) F1 females died with malignant lymphomas, compared to 4/82 female controls. Both o,p'-DDD and p,p'-DDD were tested in parallel experiments in the same study, but results were not given in sufficient detail for evaluation.

A five-generation experiment, originally set up to investigate the effects of DDT on behavior, was used to provide animals for a carcinogenicity study. The tumor incidence in one test group and one control group of BALB/c mice from each of the five generations was studied. A total of 683 mice received a diet containing p,p'-DDT at 3 ppm, and 406 were given

the control diet. Lung carcinomas were observed in 16.9% of the treated mice and 1.2% of the controls. (The incidence of lung adenomas was not reported, although the authors noted an average incidence of 5% in their colony of mice.) The incidences of lymphomas were 4.8% in exposed mice and 1.0% in controls. Incidences of leukemias were 12.4% and 2.6% in exposed and control mice, respectively. Other tumors occurred in 5.8% and 1.0%, respectively (Tárjan and Kemény 1969). (Table 2.5.1).

Tomatis et al (1972) reported a two-generation dose-response study on the CF1 mice fed DDT. A total of 881 exposed and 224 control mice were included. Technical DDT at dietary concentrations of 2, 10, 50, and 250 ppm were administered for the animals' lifespans. In both the parent (P) and offspring (F1) generations there was an increased number of deaths from week 60 onwards in mice receiving DDT at 250 ppm. The only tumor incidence affected by exposure to DDT was that of liver-cell tumors, and in the two sexes, it ranged as follows:

Exposure Group	Male*	Female*
0 ppm	25/113	4/111
2 ppm	57/124	4/105
10 ppm	52/104	11/124
50 ppm	67/127	13/104
250 ppm	82/103	69/90

(*Number of animals surviving at the time the first tumor appeared at any site in each group)

The excess incidence of liver-cell tumors in mice of both sexes fed DDT at 250 ppm, relative to that of controls, was significant at the 1% level. The

TABLE 2.5.1

INCIDENCE OF TUMORS AND LEUKEMIAS IN THE F1-F5 GENERATIONS FED DDT AT 2.8-3.0 PPM IN THE DIET FOR 6 MONTHS

Group and Generation	No. of Mice	Tumors		Leukemia	
		Male	Female	Male	Female
DDT-Exposed					
F1	10	1	2	1	3
F2	35	9	8	2	-
F3	69	6	15	1	10
F4	264	30	34	7	28
F5	305	31	60	10	23
Total no.		77	119	21	64
Percentage		11.27	17.42	3.07	9.37
Control					
F1	3	-	1	1	1
F2	39	-	2	1	-
F3	51	1	3	-	-
F4	144	-	3	-	3
F5	169	1	2	1	3
Total no.		2	11	3	7
Percentage		0.49	2.71	0.74	1.72

Adapted from Tarján and Kemény 1969

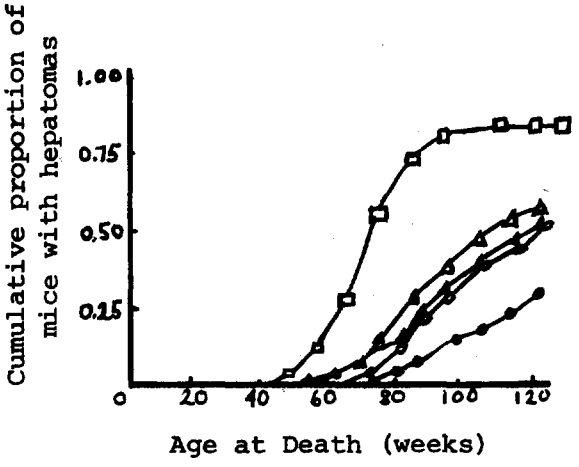
excess incidences of liver-cell tumors in males fed 2, 10, or 50 ppm were significant at the 1% level in animals surviving more than 60 weeks. In females, the excess incidence of liver-cell tumors was significant only at the 250 ppm level. In both sexes, liver-cell tumors was significant only at the 250 ppm level. In both sexes, liver-cell tumors appeared earlier in exposed mice than in controls (Figure 2.5.1). Four liver-cell tumors, all occurring in DDT-exposed mice, had metastasized. No remarkable differences between P and F1 mice were observed in this study.

These results were confirmed in a later study of the effects of technical DDT on six consecutive generations of CF1 mice (Turusov et al 1973). Terracini et al (1973a) reported a two-generation study in which 515 female and 431 male BALB/c mice were given technical DDT at dietary concentrations of 0, 2, 20, or 250 ppm for the animals' lifespans. The only tumors found in excess in the treated animals were liver-cell tumors. In females, the survival rates were comparable in all groups, and liver-cell tumors were found in 0/131 control mice, 0/135 mice fed DDT at 2 ppm, 1/128 mice at 20 ppm, and 71/121 mice at 250 ppm. In males, early deaths occurred in all groups as a consequence of fighting and in the group fed at 250 ppm because of the toxicity of DDT. In males that survived beyond 60 weeks of age, liver-cell tumors were found in 1/62 control mice, in 3/58 receiving DDT at 2 ppm, in 0/48 at 20 ppm, and in 15/31 at 250 ppm. The distribution of liver-cell tumors was unrelated to the litter of origin. No metastases were found. The tumors grew after transplantation into animals from the same strain (Terracini et al 1973a).

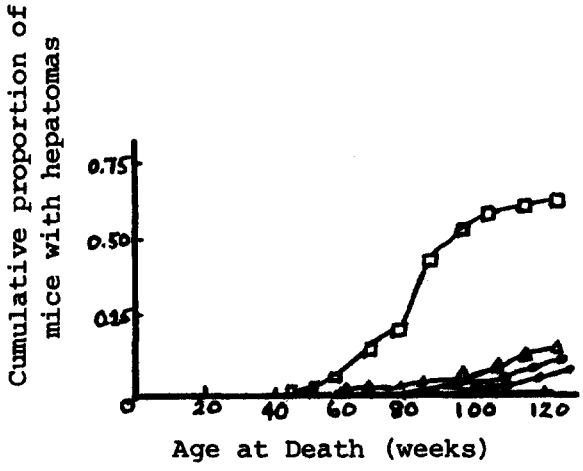
Confirmatory results were obtained in two subsequent generations of BALB/c mice fed technical DDT. In this experiment, however, F1-F3 mice,

FIGURE 2.5.1 (Tomatis and Turusov 1975)

LIVER CELL TUMORS IN MICE FED p,p'-DDT
AT VARIOUS DOSAGE LEVELS



a. Male Mice with Hepatomas
Dying at Different Time Periods



b. Female Mice with Hepatomas
Dying at Different Time Periods

• control; ▲ DDT 2 ppm; O 10 ppm; Δ 50 ppm; □ 250 ppm

which were exposed to DDT both in utero and throughout life, developed more liver tumors than did P mice, which were exposed to DDT only after weaning (Terracini et al 1973b).

Shabad et al (1973) reported a multigeneration study in A strain mice. DDT in sunflower-seed oil was administered to 234 mice at a concentration of 10 ppm. Data in the paper are ambiguous about the exact dose received by the mice. In two control groups, a total of 206 mice received either no treatment or sunflower-seed oil alone. Mice in the F0, F1, F2, F3, F4, and F5 generations were treated similarly. Thirty other mice were given 0.1 ml of a 50 ppm solution, **ie**, 5 mg of DDT, which adversely affected pregnancies, so no subsequent generations were obtained at this level. Approximately 30-50% of the animals in the exposed groups died before 6 months; all animals were killed after 12 months. The only tumors found were lung adenomas. The incidences of pulmonary adenomas in the F0-F5 generations exposed to DDT at 100 ppm were as follows:

F0, 8/42 (19%); F1, 4/26 (15%); F2, 6/25 (24%); F3, 19/41 (46%); F4, 16/37 (43%); F5, 8/63 (13%); controls (F0-F5), 15/206 (7%). Of the 30 mice receiving 50 ppm doses, 14 died before 6 months, and 3 of these (21.5%) had lung adenomas; of the 16 dying after this time, 8 (50%) had lung adenomas. The average number of lung nodules/mouse, about 7.2, was similar in both sexes, whereas there were 1.0-4.7 nodules/mouse in the six generations receiving 10 ppm doses and 1.0 nodule/mouse in controls. Because the animals were sacrificed after only 12 months of exposure, the absence of liver-cell tumors in the treated groups is of no significance.

Walker et al (1972) administered diets containing p,p'-DDT at 50 or 100 ppm to groups of 30-32 CF1 mice of each sex for 2 years.

The control groups included 47 mice of each sex. At 0,50, and 100 ppm, liver tumors occurred in 13%, 37%, and 53% of the males, respectively. In females, the corresponding incidences were 17%, 50%, and 76%. Liver tumors were characterized morphologically into two types. Type "a" tumors were characterized by simple nodular growths of solid cords of parenchymal cells. Type "b" tumors were those growing with papillary or adenoid growths, the cells proliferating in confluent sheets with necrosis and increased mitosis. The ratio of type "a" to type "b" tumors was greater than 3:1 in the treated group; no type "b" tumors occurred in controls. The incidences of other tumors were comparable in control and DDT-exposed mice. Metastases were found in one treated female.

In a subsequent study in which p,p'-DDT at 100 ppm was fed in the diet to 30 male and 30 female CF1 mice for 110 weeks, the animals were not sent for autopsy until the intra-abdominal masses were large enough to cause the animals to become anorexic or clinically affected. In this experiment, 79% of the males and 96% of the females developed liver tumors within 26 months, compared with 24% and 23% in the controls. The ratio of type "a" to type "b" tumors was about 1:1 in the DDT-treated mice (Thorpe and Walker 1973).

Subsequent to the original publications, additional information on the studies by Walker et al (1972) and Thorpe and Walker (1973) has been provided by Stevenson (1974), Thorpe (1974), Reuber (1974, 1976), Hunt (1974), and Epstein (1975). In the study by Walker et al (1972) 10 mice of each sex were fed p,p'-DDT at 200 ppm and 32 mice of each sex were fed

a mixture of p,p'-DDT at 50 ppm and HEOD (dieldrin) at 5 ppm. The incidence of liver tumors was significantly increased above that in controls in both of these exposure groups, as well as in the groups exposed to DDT at 50 ppm and 100 ppm, as reported in the original publication. Stained liver sections from six groups of animals in this study were evaluated by Reuber (1974), with the results shown in Table 2.5.2. According to Reuber (1974, 1976), type "b" tumors as defined by Walker et al (1972) consist primarily of hepatocellular carcinomas, whereas type "a" tumors include both hyperplastic nodules and well-differentiated hepatocellular carcinomas. Reuber's diagnoses of liver tumors as listed in Table 2.5.2 show a significant increase in hepatocellular carcinomas in females exposed to DDT at 50 ppm and a marked additive or synergistic effect of dieldrin. Metastases were found in one female exposed to DDT at 100 ppm and in one female exposed to DDT plus dieldrin (Walker et al 1972). The incidence of other tumors was comparable in control and DDT-exposed mice, but the age-adjusted incidence of lung tumors was increased in both sexes exposed to the DDT-dieldrin mixture (Hunt 1974).

Bennison and Mostofi (1950) reported a study in which 14 BALB/c mice of both sexes were exposed to DDT by skin painting with a 5% solution of DDT in kerosene once weekly for 52 weeks. Sixteen controls received no treatment. No skin tumors were found.

Gargus et al (1969) gave 42 neonatal Swiss mice single subcutaneous injections of DDT (composition unspecified) at 15,000 mg/kg within 72 hours after birth. The mice were killed after 6 months and examined for lung

TABLE 2.5.2

DIAGNOSES BY REUBER (1974) OF LIVER LESIONS IN MICE FED p,p'-DDT AT 50 PPM IN THE DIET

Exposure Group	Sex	Number Examined	Incidence of Liver Lesions* (%)					
			NH	H	N	SC	LC	TC
Control	M	45	62	29	9	0	0	0
"	F	32	47	44	9	0	0	0
DDT, 50 ppm	M	31	31	34	28	6	0	6
"	F	31	32	16	35	13	3	16
DDT, 50 ppm, plus HEOD**	M	33	3	18	21	15	42	58
"	F	31	0	0	6	29	65	94

*NH = no hyperplasia, H = hyperplasia, N = nodules, SC = small carcinomas (less than 5 mm), LC = large carcinomas, TC = total carcinomas
**Diets containing p,p'-DDT at 50 ppm and recrystallized dieldrin at 5 ppm

Adapted from Epstein 1975

tumors, which were not found in excess. The incidence of other tumors was not reported.

In a study reported by Tomatis et al (1974b), eight groups of CF1 mice, each containing about 60 males and 60 females, were exposed to technical DDT at 250 ppm in the diet. Four groups were exposed for 15 weeks; the other four groups were exposed for 30 weeks. Groups of mice from each exposure regimen were sacrificed after exposure and at 65, 95, and 120 weeks after the start of the experiment. Five additional groups of mice served as negative controls. The incidence of liver tumors was increased in both sexes in all six groups of mice exposed to DDT and killed at 65, 95, or 120 weeks. The size and multiplicity of liver tumors was also increased in all exposed groups. In mice exposed for 30 weeks, a similar proportion of males with liver tumors was observed when they were killed at 65, 95, and 120 weeks. In females, the incidence of liver tumors increased from the 65th to the 95th week. A similar pattern was observed in mice exposed for 15 weeks, but the incidence of liver tumors was much lower than that after 30 weeks of exposure. The authors concluded that DDT exposure, even for a limited period early in life, results in an increased incidence of liver tumors and in a shortening of latent period for their appearance. The results also show that the hepatocarcinogenicity of DDT is dose related and indicate that DDT-induced tumors do not regress but continue to grow after cessation of treatment (Tomatis et al 1974b).

Four groups of 30 male and 30 female inbred Swiss mice were exposed to technical grade DDT as follows: (a) in the diet at 100 ppm, (b) by oral

intubation at 10 mg/kg daily, (c) by subcutaneous injection in olive oil at 10 mg/kg twice/month; (d) by skin painting in olive oil at 10 mg/kg twice/week. A fifth group of 30 males ad 30 females were controls. Exposure started at 6-8 weeks of age and continued for 80 weeks. Tremors and convulsions were frequent in groups (b) and (c), but mortality was similar in all groups. Oral and subcutaneous DDT administration resulted in a significant increase in the incidence of malignant lymphomas, lung adenomas, and hepatocellular carcinomas. The highest tumor incidence was observed in the mice receiving DDT by subcutaneous injection. No significant increase in tumor incidence was observed in the skin-painted group (Kashyap et al 1977).

Kuwabara and Takayama (1974) compared liver tumors induced in mice by dietary DDT at 250 ppm, hexachlorocyclohexane (BHC) at 600 ppm, and 2-fluorenylacetamide (2-FAA) at 250 ppm. Mice exposed to DDT and BHC developed focal hepatic hyperplasia leading to hepatic cellular adenomas. Liver tumors induced by 2-FAA showed more strongly malignant characteristics and, only 2-FAA stimulated the production of alpha-fetoprotein.

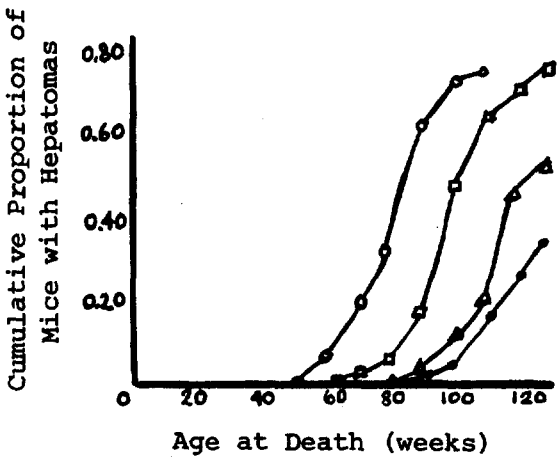
A group of 59 male and 59 female CF1 mice was fed a diet containing p,p'-DDD at 250 ppm for their lifespan, and tumor incidences were compared to those observed in a control group of 98 males and 90 females. Liver-cell tumors were found in 52% of the exposed males and 34% of the control males but only sporadically in females. Lung tumors were found in 86% of the males fed DDD, compared with 54% of male controls, and in 73% of the females given DDD, compared with 41% of female controls (Tomatis et al 1974a).

In the same experiment, 53 male and 55 female CF1 mice were fed a diet containing p,p'-DDE at 250 ppm for the animals' lifespans. Liver-cell tumors were found in 74% of the exposed males and in 98% of the exposed females, compared with 34% and 1% of the controls. The incidences of other tumors were not increased. In a third group fed a mixture of p,p'-DDE and p,p'-DDD, each at 125 ppm, the incidence of liver tumors was 76% in exposed males and 76% in exposed females. In the three exposed groups, tumors appeared earlier and in greater numbers than in the controls, both effects being greatest with p,p'-DDE and intermediate in the groups fed the mixture (Figure 2.5.2). In fact, this experiment shows that p,p'-DDE is more effective in inducing liver tumors than the parent compound p,p'-DDT (Tomatis and Turusov 1975).

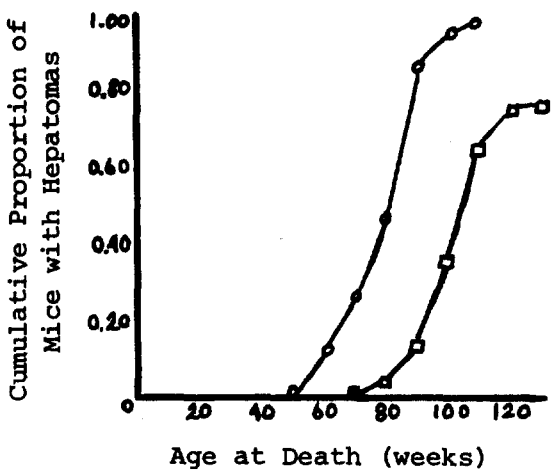
A bioassay of p,p'-DDT, p,p'-DDD, and p,p'-DDE for possible carcinogenicity in mice has recently been completed by Hazleton Laboratories America, Inc., for the National Cancer Institute, but the results are available only in preliminary form (NCI 1978). Groups of 50 male and 50 female B6C3F1 mice were exposed to p,p'-DDT, p,p'-DDD, or p,p'-DDE in the diet for 78 weeks, followed by 12 weeks on uncontaminated diets. The dietary concentrations used were as follows: DDT at 43 and 22 ppm for males and at 174 and 87 ppm for females, DDD at 822 and 411 ppm for males and females, and DDE at 253 and 147 ppm for males and females. The preliminary report indicated that DDT and DDD were not carcinogenic in the experiment, whereas DDE was carcinogenic, causing a statistically significant, dose-related increase in incidence of hepatocellular carcinomas in both sexes.

FIGURE 2.5.2 (Tomatis and Turusov 1975)

LIVER CELL TUMORS IN MICE FED p,p'-DDE, p,p'-DDD,
OR A MIXTURE OF THE TWO CHEMICALS



a. Male Mice with Hepatomas
Dying at Different Time Periods



b. Female Mice with Hepatomas
Dying at Different Time Periods

● control; △ DDD 250 ppm; ○ DDE 250 ppm; □ DDD 125 ppm plus DDE 125 ppm

2.5.2 In Rats

In two overlapping 2-year experiments, a total of 228 Osborne-Mendel rats received diets containing technical DDT (as a powder or as a solution in oil) at concentrations of 0 ppm (24 males and 12 females), 100 ppm (12 males), 200 ppm (24 males and 12 females), 400 ppm (24 males and 12 females), 600 ppm (24 males and 24 females), and 800 ppm (36 males and 24 females). Of the 192 rats exposed to DDT, 111 died before the 18 months of exposure when only 14 rats given 800 ppm, 23 rats given 600 ppm, 14 given 400 ppm, 24 given 200 ppm, 6 given 100 ppm, and 20 controls were alive. Tumor incidences for each dose level were not given. Of the 81 rats surviving for at least 18 months, 4 had "low-grade" hepatic-cell carcinomas (measuring 0.5-1.2 cm) and 11 had "nodular adenomatoid hyperplasia" (nodules measuring up to 0.3 cm). No liver lesions were found in the control rats. Hepatic-cell tumors were reported to occur spontaneously in 1% of the rats of this colony, and nodular adenomatous hyperplasia was reported to be rare (Fitzhugh and Nelson 1947).

Two experiments with Osborne-Mendel rats have been reported from the University of Miami. Groups of 30 males and 30 females were exposed for at least 2 years to recrystallized DDT at either 80 or 200 ppm and compared to 2 control groups of 30 animals of each sex. Undifferentiated bronchogenic carcinomas were seen in 8/60 rats fed DDT at 80 ppm, in 2/60 controls, and in none of the animals given DDT at 200 ppm. Two liver tumors were found in the two experiments; one occurred in a control female and the other in a female given DDT at 200 ppm. Incidences of other tumors were similar in control and treated rats (Deichmann et al 1967, Radomski et al 1965).

Fifteen male and 15 female Fischer rats were each given 15 mg of DDT (unspecified composition) by stomach tube, 5 times/week, starting at weaning. Exposure lasted 1 year, and survivors were observed for a further 6 months, the average survival being 14 months. No liver tumors were found, and no data were provided on the occurrence of other tumors (Weisburger and Weisburger 1968).

Rossi et al (1977) reported that DDT induced liver tumors in Wistar rats. Technical DDT was administered at 500 ppm in the diet to groups of 45 male and 45 female rats. Survival was similar in exposed and control rats. The incidence of liver tumors was 15/28 (56%) in females and 13/38 (35%) in males, versus zero in controls. The number of tumors per rat and the average size of the tumors increased with age, and both were greater in females than in males. Histologically, the liver tumors were nodular growths, which compressed surrounding parenchyma but did not infiltrate it. None of the tumors had metastasized. The tumors were classified as neoplastic nodules according to the terminology of the Rat Liver Workshop (Squire and Levitt 1975). The incidences of tumors at other sites were not significantly different in exposed and control rats except in the adrenals, in which the incidence of tumors was reduced from 6/38 in controls to 0/38 in DDT-exposed males (Rossi et al 1977).

Ten adult male Wistar rats were fed a low-protein, low-riboflavin diet containing o,p'-DDT at 600 ppm and killed at intervals from 24-469 days. Testicular damage was observed from the 2nd month onwards. Of the three rats killed after 348 or more days, one had microscopic adenomatous nodules and two had tumors of the interstitial cells of the testes. These

lesions were considered to be related to specific degenerative changes induced by o,p'-DDD on the adrenal cortex (Lacassagne and Hurst 1965).

A bioassay of p,p'-DDT, p,p'-DDD, and p,p'-DDE for possible carcinogenicity in rats has recently been completed by Hazleton Laboratories America, Inc., for the National Cancer Institute, but the results are available only in preliminary form (NCI 1978). Groups of 50 male and 50 female Osborne rats were exposed to p,p'-DDT, p,p'-DDD, or p,p'-DDE in the diet for 78 weeks, followed by 32 weeks on uncontaminated diets. The dietary concentrations used were as follows: DDT at 642 and 321 ppm for males and 420 and 210 ppm for females; DDD at 3,294 and 1,647 ppm for males and 1,700 and 850 ppm for females; and DDE at 839 and 437 ppm for males and 457 and 242 ppm for females. The preliminary report indicated that DDT and DDE were not carinogenic in the experiment, whereas DDD was carinogenic in male rats, causing a combination of follicular-cell carcinomas and follicular-cell adenomas of the thyroid.

2.5.3 In Hamsters

Groups of 25-30 Syrian golden hamsters of each sex were fed a diet containing p,p'-DDT at either 500 or 1,000 ppm in olive oil for 44 out of 48 weeks. Survival of the exposed hamsters at 50 weeks was 61% versus 75% of controls. All exposed animals and 62/79 (78%) controls were dead by the 90th week. Eleven exposed animals developed tumors at different sites, including one liver tumor, as did eight controls (Agthe et al 1970).

Groups of Syrian golden hamsters were exposed to p,p'-DDT in the diet at 100 ppm, 250 ppm, and 500 ppm for their lifespans. Growth and survival rates were similar in experimental and control animals. The experiments has been reported only in an abstract, which stated that exposure to DDT did not increase significantly the percentage of tumor-bearing animals in the exposed groups. The average number of tumors/exposed hamster and the percentage of hamsters with more than one tumor were not influenced by the exposure (Cabral and Shubik 1977).

Craillot et al (1975) administered technical DDT at dietary concentrations of 250, 500, or 1,000 ppm to groups of 30 male and 30 female hamsters for 18 months. Growth rates in the exposed hamsters were similar to those in the controls, but the control animals survived less well (average lifespan 13.0 months in control males and 14.9 months in control females, versus 15.3-17.3 months in exposed groups). The incidences of lymphosarcomas were markedly lower in hamsters exposed to DDT than in control hamsters, as follows:

Males: controls, 50%; DDT at 250 ppm, 23%; DDT at 500 ppm, 13%; DDT at 1,000 ppm, 0;

Females: controls, 41%; DDT at 250 ppm, 17%; DDT at 500 ppm,0; DDT at 1,000 ppm, 0.

The occurence of other types of tumors was not reported.

2.5.4. In Dogs

A total of 22 animals, approximately equally divided by sex, were fed diets containing DDT at 0 (2 dogs), 400 ppm (2 dogs), 2,000 ppm

(4 dogs), or 3,200 ppm (14 dogs). Only the control dogs, the two dogs given 400 ppm, and two of the dogs receiving 2,000 ppm survived to the time of sacrifice (39-49 months). Liver damage was reported (Lehman 1965, IARC 1974), but the study was too short to serve as an adequate carinogenicity test.

2.5.5 Carcinogenic Interactions

As noted in the preceding sections, the actions of DDT and dieldrin appear to be additive or synergistic inducing liver tumors in mice. Several additional experiments have demonstrated interactions between exposure to DDT and the effects of other carcinogens.

Female Sprague-Dawley rats, 36 days old, were fed a diet containing p,p'-DDT at 100 ppm for 2 weeks. Starting on day 50 of life, they were given, via stomach tube, 21 consecutive daily doses of 0.714 mg of DMBA (7,12-dimethylbenz[a]anthracene), a known carcinogen. Rats exposed to DDT and DMBA had a significantly lower incidence of mammary tumors than rats given DMBA alone; they also had fewer tumors/rat, and longer latent periods for the development of tumors. Leukemia incidence in rats surviving to day 280 was 2/29 in DDT-treated animals, versus 11/20 in animals given DMBA alone. DDT may inhibit the induction of mammary tumors and leukemia by DMBA, by stimulating hepatic metabolism and excretion of DMBA (Silinskas and Okey 1975). In agreement with this hypothesis, DDT and DDE reduced the toxic and adrenolytic effects of DMBA when they were administered at 1-100 mg/kg 0-10 days before exposure to DMBA (Turusov and Chemeris 1976). In contrast to these results showing

inhibitory effects of DDT on the carcinogenic effects of DMBA, dietary exposure to DDT at 100 ppm accelerated the development of cervical carcinoma induced in mice by topical application of methylcholanthrene (Uchiyama et al 1974).

In an experiment reported by Peraino et al (1975), DDT enhanced the hepatocarcinogenicity of 2-acetylaminofluouene (2-AAF). Male Sprague-Dawley rats, 22 days of age, were fed a diet containing 2-AAF at 200 ppm for 18 days, a control diet for 7 days, and then a diet containing technical DDT at 500 ppm for 389 days. Neither control rats nor rats exposed to DDT alone for 389 days developed liver tumors. Among rats fed 2-AAF alone, 28% (31/108) developed liver tumors, with an average multiplicity of 2.2 tumors/rat. Post-treatment with DDT increased the incidence of liver tumors to 75% (77/103), with an average multiciplity of 2.5 tumors/rat. The enhancing effect of DDT was associated with an increase in liver weight and an increase in liver DNA synthesis, as measured by the uptake of radiolabeled thymidine.

Weisburger and Wesiburger (1968) also found that DDT enhanced the hepatocarcinogenicity of 2-AAF. Groups of 15 male and 15 female Fischer rats were given daily doses of either 1 mg of 2-AAF or 1 mg of 2-AAF plus 10 mg of DDT, 5 days/week, for 52 weeks, followed by an average of 60 days without exposure. Hepatomas were observed in 90% of the males and 33% of the females in the DDT-exposed groups, versus 67% and 7%, respectively, in the groups exposed to 2-AAF alone.

2.6 Mutagenicity and Related Cytotoxic Effects

Kelly-Garvert and Legator (1973) described cytogenetic and mutagenic effects of purified (99%) DDT and DDE in a Chinese hamster cell line. The index of mutagenic activity was the shift from sensitivity to 8-azaguanine to resistance to it. DDE consistently induced a significant increase in the mutation frequency at nominal levels of 25-35 mg/ml. DDT induced a nonsignificant increase in two of five experiments at nominal levels of 35 mg/ml. The variability in cell survival may have been due to binding of the test chemicals to the medium, which reduced the effective concentrations. Cytogenetic studies indicated that DDE-treated cells displayed a significant increase in chromosome aberrations, primarily exchange figures and chromatid breaks. DDT produced no significant increase in chromosome abnormalities. The Chinese hamster cell cultures exposed to DDE also had more polyploid cells.

Palmer et al (1972) reported that p,p'-DDT, p,p'-DDE, and p,p'-DDD at concentrations of 10-50 μg/ml caused chromosome abnormalities in a cultured mammalian cell line (rat-kangaroo, Potorous tridactylis). The o,p'isomers were about one half as active as the p,p' isomers. All six compounds produced single and multiple chromatid breaks and p,p'-DDT and p,p'-DDE also produced exchange figures. p,p'-DDA was inactive even at 100 μg/ml. Legator et al (1973), in a collaborative study involving four laboratories, found no significant increase in cytogenetic abnormalities of bone marrow cells of rats (strain unspecified) exposed to p,p'-DDT at doses up to 100 mg/kg.

Palmer et al (1973) reported weakly positive effects of p,p'-DDT in a dominant lethal assay in rats. A statistically significant increase was found in the proportion of females having one or more dead implants after being mated during week 3 with males given a single oral dose of 100 mg/kg. No significant effects were found in females mated with males treated with DDT intraperitoneally at the same dose.

Mahr and Miltenburger (1976) studied the effects of p,p'-DDT at 12-81 ppm, p,p'-DDD at 11-75 ppm, p,p'-DDE at 44-88 ppm, and p,p'-DDA at 20-100 ppm on Chinese hamster cells in culture (B14 E28). All four compounds caused a dose-dependent reduction in the rate of cell proliferation, with DDD and DDT being the most toxic, DDE intermediate, and DDA the least toxic. Cytogenetic effects showed the same sequence of relative toxicity: DDD and DDT caused a marked increase in chromosome gaps and breaks, DDE was intermediate in activity, and DDA was the least active, causing a significant increase only in gaps. For all four compounds, the cytogenetic effects were dose-dependent and time-dependent up to a maximum time (4-24 hours) of exposure. No induction of configuration anomalies was found in any test. Chronic exposure of cells to DDT at 8 ppm for 3 months did not alter the proliferation rate or the incidence of cytogenetic abnormalities.

Lessa et al (1976) exposed human lymphocyte cultures to technical DDT at concentrations ranging from 0.06 to 15 µg/ml. The proportion of cells with structural aberrations, mostly chromatid gaps or breaks, was increased at all DDT concentrations, the increase being statistically significant at 0.20, 4.05, and 8.72 µg/ml but less marked at the

highest concentration.

Johnson and Jalal (1973) reported chromosomal aberrations induced by DDT in BALB/c mice exposed for 3 weeks to daily intraperitoneal injections of DDT in peanut oil at concentrations of 100-400 ppm. (The precise dose was not stated.) The frequency of chromosomal stickiness was significantly increased in the mice exposed at 100 ppm. Deletions (in the form of chromosomal fragments) were significantly increased at 200 ppm and above. Ring and metacentric chromosomes were infrequent.

Larsen and Jalal (1975) reported similar effects in BALB/c mice exposed to DDT at 25-250 mg/kg in single intraperitoneal injections. The mice were killed 48 hours later. Karyotypes from bone marrow cells of femurs were analyzed for gaps, deletions, and stickiness. Gaps, stickiness, and mitotic indices were not significantly increased in exposed mice, but deletions and gaps plus deletions were significantly higher in animals exposed to DDT at 50 mg/kg or higher.

Markaryan (1966) reported a significant increase in stickiness and chromosomal damage in mice given a single dose of DDT at 10 mg/kg. He suggested that the mutagenic effect of this dose (about one-sixteenth of the LD50) in mice was equivalent to that of 24 rads of radiation.

Clark (1974) tested technical DDT for mutagenicity in mice, Drosophila melanogaster, and Neurospora crassa. Two oral doses of DDT, each at 150 mg/kg, administered to male Swiss mice, induced dominant

lethal mutations in early spermatid and spermatocyte stages, reflected in a reduction in the number of implants per female and an increase in the number of dead implants; the difference was most marked 3-6 weeks after exposure. Chronic oral doses of DDT (100 mg/kg, twice a week, for 10 weeks) caused a persistent increase in the number of dominant lethal mutations. Histologic sections showed that chronic treatment with DDT caused changes in the morphology of the seminiferous tubules and degeneration of B-type spermatogonia. Acute treatment with DDT caused an increase in spermatocyte chromosome breakage, stickiness, and precocious separation of the X and Y bivalents.

Dietary exposure of male D melangoster (Canton-S) to DDT caused an increase in dominant lethal mutations in early spermatid and spermatocyte stages. DDT also caused nondisjunction of the X and Y chromosomes at the spermatocyte state and exposed males (strain y/R (1)2, vf/$B^{s}Y_{y}+$). A shift in sex ratio in the offspring of treated males was associated with breakage of the ring-X chromosome. Exposure of a population of D melanogaster (Canton-S) to DDT at low dietary levels for 8 months caused no significant increase in the frequency of second-chromosome recessive lethal mutants (Clark 1974).

Tests for the induction of recessive lethal mutations in the ad-3 region of a N crassa heterokaryon gave inconclusive results. However, in a host-mediated assay with N crassa in mice, technical DDT did not appear to be mutagenic (Clark 1974).

In contrast to the positive results obtained by Clark (1974) and Palmer et al (1973) in dominant lethal assays in Swiss mice and in

rats, Epstein and Shafner (1968) and Epstein et al (1972) found no significant effects of DDT in dominant lethal assays in ICR/Ha mice. Buselmaier et al (1972, 1973) reported that DDT and DDE gave "ambiguous" results in dominant lethal assays in NMRl mice. However, they reported that DDD was strongly mutagenic in a host-mediated assay with Serratia marcesscens in mice, whereas DDT, DDE, and DDA were inactive. None of the compounds was mutagenic in Salmonella typhimurium in a host-mediated assay in mice.

In other experiments with Drosophila, Lüers (1953) reported that DDT had no mutagenic effect. However, Vogel (1972) reported that p,p'-DDA was significantly mutagenic and p,p'-DDT was weakly active in Drosophila, inducing sex-liked recessive lethals; p,p'-DDE, p,p'-DDD, and p,p'-DDOM were not significantly active.

McCann et al (1975) and McCann and Ames (1976) reported that p,p'-DDE gave negative results for mutagenicity in the S typhimurium reversion bioassay (Ames Test), with and without activation by rat liver microsomal preparations (S-9). They used four strains of S typhimurium: TA 1535, a base-pair substitution mutant; TA 1537, a frameshift mutant; and the more sensitive strains TA 98 and TA 100. Van Dijck and Van de Voorde (1976) similarly reported that p,p'-DDT and p,p'-DDE were negative in this bioassay with activation by mouse liver microsomes. Marshall et al (1976) reported that DDT at 2,500 µg/plate and DDE at 1,000 µg/plate were not mutagenic in the S typhimurium bioassay, with or without rat liver homogenates. They used four strains of S typhimurium: TA 1535, TA 1536, TA 1537, and

TA 1538. The more sensitive strains TA 98 and TA 100 were not used. Shirasu et al (1976) also reported that p,p'-DDT was not mutagenic in these four strains of S typhimurium or in two tryptophaneless strains of E coli (B/r try WP2 and WP2 try hcr), but they did not attempt activation with microsomal enzymes. Shirasu et al (1976) classified p,p'-DDT as negative in recombination assays with B subtilis strains H17 REC^+ and M45 Rec^-, but they did not provide specific data. Swenberg et al (1976) reported negative results for DDT at concentrations of 0.1-3.0 mM in an in vitro alkaline elution assay for DNA dammage in Chinese hamster (V79) cell culture with rat liver microsomal activation. Both McCann and Ames (1976) and Swenberg et al (1976) interpreted the results with DDE and DDT as "false negatives" because the systems utilized in their experiments otherwise usually give positive results with carcinogens.

Langenbach and Gingell (1975) tested p,p'-DDT, p,p'-DDD, p,p'-DDE, and p,p'-DDA in an in vitro mouse embryo cell culture susceptible to malignant transformation. All four compounds at concentrations of 2.8-43 μM caused transformations, with DDD being considerably more active than the other three compounds. However, none of the transformed foci had the typical malignantly transformed morphology produced by 7,12-dimethylbenz(a)anthracene (DMBA). None of 11 transformed cell foci from cultures exposed to DDT, DDE, DDD, or DDA caused tumors when innoculated into syngeneic mice, whereas 3/7 DMBA-transformed tumors did so.

Walker et al (1970a,b) reported that p,p'-DDT markedly inhibited the growth of Ehrlich ascites tumor cells in vivo. Technical DDT had similar but less marked effects. DDT also inhibited the incorporation of amino acid precursors of DNA, RNA, and protein into Ehrlich ascites tumor cells.

Chung et al (1967) reported that DDT at concentrations above 0.5 ug/ml reduced leucine incorporation by 20% in HeLa S cells in vitro. Uridine and thymidine incorporation were both decreased at low concentrations of DDT (0.5 µg/ml) but unaffected at higher concentrations (50 µg/ml). The small magnitude of the effect and its inconsistent relation to dose make its significance questionable.

Wyrobeck and Bruce (1975) reported that p,p'-DDT did not cause sperm abnormalities in mice after five daily intraperitoneal injections at 10 or 15 mg/kg. In the same experiment, many, but not all, mutagens and carcinogens caused sperm abnormalities.

Wallace et al (1976) conducted a multigeneration screening test for recessive mutations in the descendants of treated mice. Mice of an inbred strain (CF1, genotype aabbcc) were exposed to technical DDT at a dietary concentration of 250 ppm through five generations (Tomatis et al 1972) and were maintained under various degrees of inbreeding through the 12th generation, with appropriate controls. The stock did not suffer unduly from litter competition, and the breeding program used is stated to have provided a sensitive test. There was no evidence for a greater incidence of recessive invisible mutations in the exposed stock than in the controls. Two recessive visible mutations (black-and-tan and

exencephaly) were identified in the exposed stock, which had been derived from 60 females in the eighth generation. The authors concluded that, if DDT is mutagenic in this strain of mice, the effect is unlikely to have been a major one.

Grosch and Valcovic (1967) exposed the parthenogenetically reproducing wasp Habrobracon juglandis to DDT at nearly lethal levels. They reported no evidence of an increase in dominant or recessive lethal mutants, but the sensitivity of the system is doubtful.