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Effects of long-term oral administration of DDT on nonhuman primates

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Abstract Because of reports on tumorigenic activity in different animal species exposed to DDT a decision was made in 1969 to evaluate the long-term effects of DDT on 24 cynomolgus and rhesus monkeys. DDT (20 mg/kg) was given in the diet for 130 months, followed by an observation period that ended in 1994. The two cases of malignant tumor detected in the DDT group included a metastatic hepatocellular carcinoma in a 233-month-old male and a well-differentiated adenocarcinoma of the prostate in a 212-month-old monkey. Benign tumors detected in the DDT group included three cases of leiomyoma, two of which were uterine and one, esophageal. No tumor was detected in the control group of 17 monkeys. Fatty changes in the liver were observed in 52.9% of the DDT group and 29.4% of the control group. More specific signs of hepatotoxicity were documented microscopically in seven DDT monkeys. Severe tremors and histological evidence of CNS and spinal cord abnormalities were observed in six DDT monkeys. The present findings show clear evidence of hepatic and CNS toxicity following long-term DDT administration to cynomolgus and rhesus monkeys. However, the two cases involving malignant tumors of different types are inconclusive with respect to a carcinogenic effect of DDT in nonhuman primates.

Key words DDT · Long-term oral administration · Nonhuman primate · Monkey

This paper is dedicated to His Imperial Highness Prince Hitachi, the new, most respected Honorary Member of the Deutsche Krebsgesellschaft

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Introduction

DDT is a chlorinated hydrocarbon that was first recognized to have insecticidal properties in 1939. It has been used extensively throughout the world in vector control for malaria, typhus, and plague as well as for agricultural diseases (Fishbein 1974). Findings of mutagenic activity and tumor induction in rodents suggested that DDT might be a potential health hazard in humans. Oral administrations of DDT was reported to produce metastatic liver tumors in CF-1 mice (Tomatis et al. 1972) and to act as promoter of hepatocarcingenesis induced by N-nitrosodiethylamine (Nishizumi 1979) and 3′-methyl-4-dimethyllaminoazobenzene (Kitagawa et al. 1984). Lymphomas and lung tumors have also been documented in mice and rats treated with DDT (Levine 1991; Smith 1991). DDT alone does not produce mammary tumors in rodents (International Agency for Research on Cancer 1987; Kutz et al. 1991; Levine 1991), although it can accelerate 2-acetamidophenanthrene-induced rat-mammary tumor induction (Scribner and Mottet 1981). DDT is metabolized in the liver to DDE, which has also been shown to be carcinogenic in different rodent species (U.S. National Cancer Institute 1978; Rossi et al. 1983; Cabral 1985).

DDT and its metabolites are extremely stable and accumulate in fatty tissues. DDT has also been found in mother's milk and has been shown to cross the placenta (Fishbein 1974). In high doses, DDT causes acute toxicity in humans with symptoms of nausea, vomiting, ataxia, and paralysis (WHO 1979, 1984; Hayes 1982).

Chronic exposure also effects the nervous system, causing hyperactivity, tremors, and convulsions (U.S. National Cancer Institute 1978; Cabral 1985). Use of DDT was banned in the United States in 1972, and many other countries subsequently restricted its use.

A number of epidemiology studies have been carried out to assess the cancer risk associated with DDT exposure. Of particular interest are studies on breast cancer attributed to estrogenic effects of DDT (Krieger et al. 1994; Dess et al. 1997). The conclusion of most of these studies have been that environmental exposure to DDT does not increase the risk for breast cancer in women (Hunter et al. 1997; Lopez-Carillo et al. 1997).

Our main reason for initiating the present study in 1969 was concern over the long-term effects of DDT exposure on humans. Since nonhuman primates are phylogenetically close to humans, studies of the long-term effects of DDT could possibly provide valuable information on its carcinogenic potential in humans (Adamson and Sieber 1983). Cynomolgus and rhesus monkeys were dosed with DDT for 130 months and were then held for observation for tumor formation and other adverse effects until the age of 18–24 years.

Materials and methods

The group of 24 nonhuman primates used in this study comprised 2 species: 13 *Macaca fascicularis* (cynomolgus) and 11 *M. mulatta* (rhesus) monkeys. The first group of 14 monkeys began treatment with DDT in 1969–1970 and the second group of 10 monkeys began treatment in 1975–1976. Details on the maintenance and management procedures and the methods employed to rear neonates have been described elsewhere (Adamson and Sieber 1983). The monkeys were cared for according to the standards established by the Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC). The experimental protocols used were approved by the Animal Care and Use Committee, National Cancer Institute, and were reviewed on an annual basis. The animals were given a diet consisting of high-protein Purina monkey chow (5045 Standard), apples, and a vitamin mixture spread on slices of bread. The vitamin mixture consisted of powered dry milk (5 lb.), Parvo (a folic acid supplement, 4 0Z., 20% with starch; Roche Agricultural Products), Cecon (a vitamin C supplement, 300 ml; Abbott Laboratories, Chicago, I11.), molasses (2 l), and water (500 ml). Euthanasia was performed by immobilization with ketamine hydrochloride (15 mg/kg, i.m.) followed by sodium thiamylal (40 mg/kg i.v.) The necropsies were performed immediately after euthanasia.

p,p'-DDT was purchased from Aldrich Chemical Company (Milwaukee, Wis.). Without further purification it was dissolved in Mazola corn oil. Dosing was started in newborn monkeys by the addition of DDT dissolved in corn oil to the Similac formula at a dose of 10 mg/kg, 5 days a week. When the monkeys were 2 months old, DDT dissolved in corn oil was incorporated into their vitamin spread. The appropriate dose of DDT (20 mg/kg) was mixed with 1 teaspoon of the vitamin mixture and spread on a slice of bread, which was folded in half to form a sandwich and then fed to the animal. A group of 17 control monkeys received only corn oil in the vitamin mixture. The monkeys were evaluated daily and weighed once a week. Blood was drawn every 6 months for routine hematology tests, electrolyte measurements, and assessment of liver function. Tuberculin tests were carried out on a quarterly basis.

Complete necropsies were performed on all of the monkeys. The following organs were excised and fixed in buffered formalin: brain, spinal cord, pituitary, salivary glands, thyroid, thymus, tongue, cheek pouches, trachea, esophagus, lungs, heart, aorta, liver, gallbladder, pancreas, spleen, kidneys, adrenals, stomach, duodenum, small intestine, urinary bladder, colon, uterus, ovaries, testes, seminal vesicles, prostate, lymph nodes (axillary, inguinal, hilar), skin, skeletal muscle, and bone marrow (sternum). In addition, tissue appearing abnormal was fixed for histological evaluation. The tissues were routinely processed and embedded in paraffin. Histology sections were stained with hematoxylin-eosin for histopathological examination. Liver sections were also stained with periodic acid-schiff (PAS), with and without diastase.

Serum levels of DDT and its metabolites were measured in 1975, 5–6 years after dosing had been started in the first group of 14 monkeys, and again in 1980, 4–5 years after the second group of 10 monkeys had been started on DDT. Tissue samples from the s.c. fat, omentum, liver, and brain were also measured for DDT levels in four animals in 1975. The serum samples, diluted in 4% sodium chloride solution, were extracted three times in petroleum ether and the extracts were passed through a column of anhydrous sodium sulfate. The extracts were then passed through a Florisil column and eluted with a mixture of 5% ether and petroleum ether. The eluants were concentrated to a fixed volume and assayed by gas chromatography equipped with a ^{63}Ni electron-capture detector system. The minimal sensitivity of the analysis was 0.02 ppm.

Results

This long-term DDT feeding study that involved 11 rhesus and 13 cynornolgus monkeys was initiated in 1969 and ended in 1994, when a decision was made to euthanize and necropsy the 12 surviving monkeys (see Table 1). A group of eight rhesus and nine cynomolgus monkeys dosed with corn oil, the vehicle of DDT, served as controls. During the 130-month dosing period, six DDT monkeys died, including four monkeys that were euthanized after they had experienced severe tremors. The first of these four monkeys (750J, 38 months old) had undergone partial hepatecotomy for measurement of alpha-feto protein level, the second (1119R, 53 months old) had toxic hepatitis, the third (7411, 71 months old) had fatty changes in the liver, and the fourth (758J, 93 months old) had vacuolization of the white matter of the brain. Of the two remaining monkeys that died during the 130-month DDT dosing period, one (740I, 71 months old) showed coagulation necrosis of the liver and another (760J, 115 months old) surffered from hypoglycemia and showed diffuse hypatocyte vacuolization at necropsy (Table 1).

Among the 18 remaining DDT monkeys (age range 160–304 months) the most common histopathological findings were observed in the liver; these included diffuse hepatocyte vacuolization (9 cases), proliferation of bile ductular-like "oval" cells (5 cases), clear hepatocyte foci (2 cases), and liver cell necrosis (2 cases; Table 1). PAS staining with and without diastase ruled out glycogen retention in the 9 cases that showed diffuse hepatocyte vacuolization. However, excessive glycogen was observed in occasional clear hepatocyte foci, which were detected in two DDT cases and in one control. Unfortunately, the presence of fat could not be examined histochemically in the liver specimens due to inappro-

Table 1 Tumors and other pathological findings in monkeys given DDT (*Cy* cynomolgus, *Rh* rhesus, *Cum*. cumulative)

Animal number	Species	Sex	Age (days) at first dose	Dosing period (months)	Observation period (months)	Cum. dose (g)	Tumor type	Other pathological findings
7381	Cy	M	18	130	234	292.7	Hepatocellular carcinoma	Metastatic to lungs and periadrenal lymph nodes; myocardial fibrosis; hyalinization, pancreatic islet cells; transitional-cell hyperplasia, renal pelvis; ductal hyperplasia, pancreas; calcium deposits, dorsal roots of spinal cords
7391	Cy	F	30	130	171	232.1	Leiomyoma, uterus	Diabetes
7401	Rh	M	47	69	71	136	–	Coagulation necrosis, liver; congestion, liver
7411	Cy	M	37	130	276	208.2	Leiomyoma, esophagus	Fatty change, liver; hyalinization, pancreatic islet cells; atrophy, seminal vesicles
7421	Rh	M	16	44	59	52.9	–	Atrophy, testes (Sertoli-only syndrome)
7451	Cy	M	1	130	160	272.4	–	Nerve fascicular degeneration; bronchopneumonia
7491	Rh	M	13	130	304	283.3	–	Fatty change & hemosiderosis, liver; clear-cell foci, liver; atrophy, spleen and testes
750J	Rh	F	1	35	38	43.7	–	No specific abnormality
751J	Cy	F	1	130	304	183	–	Intraductal hyperplasia, breast; endometriosis; clear-cell foci, liver; hemosiderosis, liver
753J	Cy	F	1	130	206	241.6	–	Hyalinization, pancreatic islet cells; diabetes
758J	Rh	M	1	70	93	195.3	–	Vacuolization, white matter of the brain
760J	Rh	M	1	115	115	267.5	–	Fatty change, liver; hypoglycemia
763J	Rh	F	1	130	291	254.8	Leiomyoma, uterus	Fatty change, liver; oval-cell proliferation, liver; atrophy, ovaries; endometriosis
764J	Rh	F	1	130	291	242.6	–	Focal mycocardial fibrosis; hemosiderosis, liver
1107P	Cy	F	18	130	233	159.1	–	Liver cell necrosis; fatty change & hemosiderosis, liver; endometriosis; interstitial nephritis
1108P	Cy	M	5	130	233	239.3	–	Atrophy, testis; focal mycocardial necrosis
1111R	Rh	M	3	130	232	336.8	–	Liver cell necrosis; fatty change, liver; oval-cell proliferation, liver; pleural adhesion
1112R	Rh	M	1	130	232	304.1	–	Fatty change, liver; oval-cell proliferation, liver
1113R	Cy	M	1	130	232	218.9	–	Myocardial fibrosis; fatty change, liver
1114R	Cy	M	1	130	212	243.1	Adenocarcinoma, prostate	Hyalinization, pancreatic islet cells; calcification, dorsal roots of spinal cords; hypoglycemia, focal myocardial fibrosis; hemosiderosis, liver; atrophy, thyroid
1116R	Cy	F	1	130	230	171.2	–	Fatty change, liver, hyalinization, pancreatic islet cells; duct hyperplasia, pancreas; endometriosis; cyst, duodenum
1117R	Cy	F	1	128	231	180.8	–	Intraductal hyperplasia, breast; oval-cell proliferation, liver
1119R	Rh	F	1	53	53	71.2	–	Toxic hepatitis; nematodes in nostrils
1120R	Cy	F	2	130	230	150	–	Fatty change, liver, oval-cell proliferation, liver; atrophy, ovaries

Table 2 Major autopsy findings in control monkeys

Animal number	Species	Sex	Observation period (months)	Pathological findings
1234T	Cynomolgus	F	206	Fibrosis, papillary muscle, left ventricle
1188S	Cynomolgus	M	217	Atrophy, testes; fatty infiltration, liver; hyalinization, pancreatic islet cells; atrophy, prostate
1156R	Rhesus	F	224	No specific abnormality
947M	Rhesus	M	234	No specific abnormality
944M	Rhesus	M	237	Fatty infiltration, liver; hemorrhage & edema, lung; inguinal hernia
987M	Cynomolgus	F	240	Hypertrophy, left kidney; absence, right kidney; endometriosis
1017N	Cynomolgus	M	244	Fibrosis, papillary muscle, left ventricle
1041N	Cynomolgus	M	248	Atrophy, testes; fatty infiltration, liver; hyalinization, pancreatic islet cells; atrophy, prostate
989M	Rhesus	M	257	Nodular cortical hyperplasia, left adrenal
697I	Rhesus	M	261	Diverticulitis; emphysema & interstitial fibrosis, lung
899L	Cynomolgus	M	268	Chronic bronchitis
901L	Cynomolgus	F	277	Proliferation, follicular epithelial cell, thyroid
777J	Rhesus	M	289	Bloat; simple cyst, left kidney
778J	Rhesus	M	284	No specific abnormality
780J	Cynomolgus	M	285	Myocardial fibrosis; hyalinization, pancreatic islet cells
779J	Rhesus	F	299	Fatty infiltration, liver; clear-cell foci, liver; chronic gastritis; chronic cholecystitis; adenomyosis, uterus
687H	Cynomolgus	F	301	Fatty infiltration, liver, hyalinization, pancreatic islet cells; endometriosis; splenomegaly, hydronephrosis, right kidney

priate tissue processing for fat staining. Nevertheless, on the basis of the macrovascular appearance of the cytoplasmic hepatocyte vacuoles and the absence of glycogen, a diagnosis of fatty changes in the liver was made.

In the group of 17 control monkeys, which ranged in age from 18 to 25 years, diffuse fatty changes in the liver were observed in 5 animals and clear-cell hepatocyte foci, in 1 monkey (Table 2). The incidence of fatty changes in the liver in the DDT group was 52.9% as compared with 29.4% in the control group. A more distinct evidence of DDT-mediated hepatoxicity (toxic hepatitis, coagulation necrosis, focal liver necrosis) was found in three monkeys (740I, 1107P, and 1111R). Also, oval-cell proliferation, which reflects a response to hepatic insult, was observed in two DDT monkeys (1111R and 1112R). Two DDT monkeys (739I, 753J) were diagnosed with diabetes and two (760J, 1114R), with hypoglycemia (Table 1). At necropsy, hyalinization of the pancreatic islets was found in one diabetic monkey (753J) and one hypoglycemic (1114R) animal. Hyalinization of the pancreatic islets did not appear to be associated with DDT exposure since it was observed in 5 (20.8%) of the DDT monkeys and 4 (23.5%) of the controls (Tables 1,2). However, no case of diabetes or hypoglycemia was found in the control group.

Microscopic examination of the brain and spinal cords of the DDT-treated monkeys revealed nerve fascicular degeneration of the spinal cord in one monkey (745I), vacuolization of the white matter of the brain in one (758J), and calcifications of the dorsal roots of the spinal cord in two animals (738I and 1114R; Table 1). No abnormal finding was observed in the brain or spinal cord of the control group.

Examination of the uterus, ovaries, and mammary glands of the 11 female monkeys in the DDT group showed 4 cases of endometriosis, 2 cases of leiomyoma of the uterus, and 2 cases of intraductal hyperplasia of the mammary glands (Table 1). Among the six females in the control group, two cases of endometriosis and one case of adenomyosis of the uterus were detected.

Malignant neoplasms developed in two of the DDT-treated monkeys. The first case was a metastatic hepatocellular carcinoma in a 234-month-old cynomolgus male (738I), and the second case was a 3-mm well-differentiated adenocarcinoma of the prostate diagnosed at necropsy of a 212-month-old cynomolgus monkey (1114R, Table 1). Benign neoplasms were found in three DDT monkeys; two were leiomyomas of the uterus (739I and 763J) and one was a leiomyoma of the esophagus (741I, Table 1). No neoplasm was found in the control group.

Measurements of serum levels of DDT and its metabolites were carried out in 1975, approximately 5 years after the first group of 14 monkeys had been started on DDT. DDT serum levels ranged from 0.393 to 4.640 ppm (Table 3). The baseline DDT levels measured in two of the corn-oil-treated controls were 0.02 and 0.04 ppm. In 1975, fatty tissue samples were obtained from two monkeys, one of which (738I) had very high DDT levels in both the omental fat (2400 ppm) and s.c. fat (960 ppm) samples (Table 4). Interestingly, this animal later developed metastatic hepatocellular carcinoma. DDT levels were also measured in the liver and brain of two other monkeys that died in 1975 (Table 4). One of these animals (742I) was diagnosed with testicular atrophy and Sertoli-only syndrome (Table 1). A second group of animals was started on DDT in 1975–1976. Serum DDT levels of this group and the original group were measured in 1980 (Table 3). This time the serum levels of DDT and its metabolites were much higher than those observed in the original group. The DDT levels ranged from 1.102 to 22.007 ppm (Table 3). The levels of four corn-oil-treated

Table 3 DDT and DDT metabolite residues detected in serum from DDT and control monkeys

Animal Number (Treatment)	Species/sex	Serum pp'DDE (ppm)		Serum pp'DDD (ppm)		Serum pp'DDT (ppm)	
		1975	1980	1975	1980	1975	1980
738I (DDT)	Cy/M	< 0.020	0.173	< 0.020	0.350	0.393	8.481
739I (DDT)	Cy/F	< 0.020	0.068	< 0.020	0.142	0.560	2.396
740I (DDT)	Rh/M	< 0.020	ND	< 0.020	ND	1.000	ND
741I (DDT)	Cy/M	< 0.020	0.130	< 0.020	0.342	0.940	6.338
742I (DDT)	Rh/M	0.156	ND	0.085	ND	4.530	ND
745I (DDT)	Cy/M	< 0.020	0.102	< 0.020	0.325	0.373	4.157
749I (DDT)	Rh/M	< 0.020	0.223	< 0.020	0.428	1.220	10.295
751J (DDT)	Cy/F	< 0.020	0.123	< 0.020	0.249	1.675	4.205
753J (DDT)	Cy/F	< 0.020	0.175	< 0.020	0.331	0.515	8.099
758J (DDT)	Rh/M	0.028		0.092		1.780	
760J (DDT)	Rh/M	< 0.020		0.038		1.710	
763J (DDT)	Rh/F	< 0.020	0.136	0.040	0.315	1.660	7.669
764J (DDT)	Rh/F	0.045	0.229	0.250	0.439	4.640	9.748
1007P (DDT)	Cy/F		0.172		0.347		6.476
1008P (DDT)	Cy/M		0.192		0.385		6.938
1111P (DDT)	Rh/M		0.089		0.184		3.015
1112P (DDT)	Rh/M		0.180		0.343		8.412
1113R (DDT)	Cy/M		0.119		0.244		3.636
1114R (DDT)	Cy/M		0.133		0.315		7.043
1116R (DDT)	Cy/F		0.133		0.323		4.166
1117R (DDT)	Cy/F		0.043		0.083		1.102
1119R (DDT)	Rh/F		0.684		0.840		22.007
1120R (DDT)	Cy/F		0.026		0.051		19.424
777J (C)	Rh/M	< 0.020		< 0.020		0.111	
778J (C)	Rh/M	< 0.020		< 0.020		0.090	
779J (C)	Rh/F	< 0.020		< 0.020		0.117	
780J (C)	Cy/M	< 0.020		< 0.020		< 0.020	

Table 4 DDT and DDT metabolite residues detected in the liver, brain and fatty tissues of DDT monkeys

Animal number/organ Site	Species/sex	pp'DDE (ppm) 1975	pp'DDD (ppm) (1975)	pp'DDT (ppm) (1975)
738I s.c fat	Cy/M	50.00	280.00	960.00
738I omental fat	Cy/M	60.00	8.80	2400.00
740I liver	Rh/M	3.70	18.80	21.70
740I brain	Rh/M	2.45	1.00	16.00
740I serum	Rh/M	0.83	0.33	5.33
742I liver	Rh/M	0.54	3.88	15.28
742I brain	Rh/M	1.60	1.40	5.80
742I serum	Rh/M	0.20	0.07	2.80
750I liver	Rh/F	9.50	140.00	52.00
750I brain	Rh/F	2.17	2.66	51.00
750I serum	Rh/F	0.47	1.14	10.40

controls ranged from 0.184 to 0.341 ppm. The monkey with the highest DDT serum level (1119R) died at 53 months of toxic hepatitis. Serum DDT measurements performed on selected animals for the second time in 1975 also showed levels that were more consistent with those observed in 1980 (see Table 4).

Discussion

The present report documents the results of a 25-year study on 24 cynomolgus and rhesus monkeys that were dosed with DDT for a period of 130 months. There were evidence of liver and CNS toxicity in the DDT group. With regard to neoplastic development in the DDT group, one metastatic hepatocellular carcinoma was diagnosed in a 19.5-year-old cynomolgus male and a small, well-differentiated adenocarcinoma of the prostate was discovered at necropsy of a 17.5-year-old cynomolgus monkey. In addition, benign neoplasms were found in three DDT monkeys, which included two cases of leiomyoma of the uterus and one leiomyoma of the esophagus. Although no tumor was detected in the control group of 17 monkeys, the spontaneous malignant tumor rate among 373 control animals during the 34-year history of this monkey colony was 1.5% in cynomolgus monkeys and 2.8% in rhesus monkeys (Thorgeirsson et al. 1994). The occurrence of two different types of malignant tumors in animals at 7 and 14 years after the cessation of DDT dosing, respectively does not settle the question of its carcinogenic potential in higher primates.

The acute toxicity of DDT manifess in its effect on the nervous system. Species differences were reported in earlier experiments, where tremors were common in rats given 10.5 mg/kg but hamsters dosed with up to 40 mg/kg DDT for life showed no sign of neurotoxicity (U.S. National Cancer Institute 1978; Cabral 1985). During the first 7 years of the present study, six monkeys had to be euthanized after they had developed irreversible tremors. Interestingly, five of the six were rhesus monkeys, suggesting that they were more susceptible to DDT-mediated neurotoxicity. A possible explanation

for this discrepancy may be differences in DDT metabolism between these monkey species. Alternatively, release of DDT and its metabolites from body fat during periods of anorexia may have accounted for the CNS signs noted. The results of serum analyses for levels of DDT and its metabolites (Table 3) showed generally higher levels of DDT and DDD in the rhesus versus cynomolgus monkeys, although the levels did not correlate with the documented neurotoxicity.

There was a wide range in the serum levels of DDT and metabolites detected among the experimental animals, all of which were dosed at 20 mg/kg. The basis for this variation is not clear, but it could be attributable to a variety of factors, such as differences in the amount of body fat or the extent of absorption, metabolism, and secretion of DDT. Fluctuations in serum levels over time may also have contributed to this finding. Studies of DDT metabolism in humans have shown marked fluctuations in serum levels recorded for the same individual over time (Morgan and Roan 1971). This observation in humans may also help explain the marked discrepancy in the serum levels recorded for the same monkey in 1975 and 1980, although changes in methodology cannot be ruled out. Considering the inconsistent results of the DDT measurements, it cannot be determined whether there was an association between high DDT levels and disease development. However, it is intriguing that the monkey with the highest DDT serum levels died of toxic hepatitis.

Administration of DDT can induce different isoforms of cytochrome P-450 in humans and rats (Campbell et al. 1983; Li et al. 1995), thus potentially affecting steroid metabolism (International Agency for Research on Cancer 1987; Kutz et al. 1991; Levine 1991; Smith 1991). The mouse, on the other hand, appears to be relatively resistant in this regard, again demonstrating species variation in response to DDT exposure (Chabra and Fouts 1973). Studies on the early hepatic changes caused by DDT in rats have demonstrated a focal necrosis and regenerative liver response (Kostka et al. 1996). In the present study, chronic dosing with DDT appeared to affect the liver in some of the monkeys as evidenced by liver cell necrosis, oval-cell proliferation, and clear hepatocyte foci. However, there was no indication of hyperplastic nodules or adenomas.

Among the 24 monkeys in the DDT group, 2 developed diabetes and 2 developed hypoglycemia. This may suggest that long-term DDT exposure affected glucose metabolism or insulin production in these monkey species. This finding is in keeping with earlier reports on rodents, showing that exposure to DDT lowered blood glucose levels in rats (Berdanier and de Dennis 1977) and inhibited insulin secretion in mice (Yau and Mennear 1977). The question then arises as to whether there is a link between diabetes or hypoglycemia and the development of malignant tumors in the DDT monkeys. It has been shown that diabetic patients may develop hepatomegaly due to glycogenosis (Chatila and West 1996) and are at increased risk of developing primary liver cancer or malignancies at other sites (Adami et al. 1996). Increased incidence of hepatocellular carcinomas has also been observed in patients with glycogen storage disease type I (Bianchi 1993). In the monkey study, excessive glycogen was not present in the liver samples (except in the clear hepatocyte foci) from the DDT group. Hypoglycemia, however, was observed in one of the two DDT monkeys that developed carcinomas. Considering the low number of malignant tumors involved, it is purely a matter of speculation as to whether hypoglycemia or diabetes played a role in the induction of the two different types of malignant tumors that developed in an 18- and a 20-year-old monkey, respectively.

Estrogenic effects of DDT were recently reported to cause uterine enlargement in CBA mice (Morozova et al. 1977). In the DDT-treated group, 6 of the 11 female monkeys had uterine enlargement, caused by either endometriosis or leiomyomas. Two cases of endometriosis were seen in the control group. In view of the observation that endometriosis was not uncommon in breeders and females dosed with other test compounds in our monkey colony, this finding is probably not related to DDT exposure. However, the uterine leiomyomas (two cases) and intraductal hyperplasia of the breast (two cases) observed in the DDT females, but not in the control group, may reflect estrogenic effects of DDT.

In summary, the present long-term DDT study in cynomolgus and rhesus monkeys emphasizes the neurotoxic effects of DDT. The findings also suggest that DDT has hepatotoxic and, possibly, estrogenic effects. The two malignant tumors occurring in old monkeys may not have been induced by DDT exposure.

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