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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

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 hepatocarcinogenesis induced by N-nitrosodiethylamine (Nishizumi 1979) and 3'-methyl-4-dimethylaminoazobenzene (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 affects 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 powdered dry milk (5 lb.), Parvo (a folic acid supplement, 4 oz., 20% with starch; Roche Agricultural Products), Cenon (a vitamin C supplement, 300 ml; Abbott Laboratories, Chicago, 111.), molasses (2 l), and water (500 ml). Euthanasia was performed by immobilization with ketamine hydrochloride (15 mg/kg, i.m.) followed by sodium thiopental (40 mg/kg i.v.).

*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 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 eluates were concentrated to a fixed volume and assayed by gas chromatography equipped with a 63Ni 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 cynomolgus 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 hepatectomy 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) suffered from hypoglycemia and showed diffuse hepatocyte 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-