NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

1-TRANS-DELTA^9-TETRAHYDROCANNABINOL

(CAS NO. 1972-08-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

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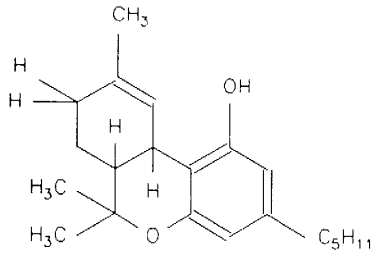
ABSTRACT



1-TRANS-DELTA^9-TETRAHYDROCANNABINOL

CAS No. 1972-08-3

Chemical Formula: $C_{21}H_{30}O_2$ Molecular Weight: 314.5

Synonyms: 3-Pentyl-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6h-dibenzo(b,d)pyran-1-ol; delta^1-tetrahydrocannabinol; (–)-delta^1-3,4-trans-tetrahydrocannabinol; delta^9-tetrahydrocannabinon; THC; delta^1-THC; delta^9-THC

Trade names: Dronabinol; Marinol

1-Trans-delta^9-tetrahydrocannabinol (THC) was nominated by the National Cancer Institute to the NTP for study because it is the major psychoactive component of marijuana and a widely used Schedule I substance. Male and female F344/N rats and B6C3F$_1$ mice received THC (97% pure) in corn oil by gavage for 13 weeks, 13 weeks with a 9-week recovery period, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse peripheral blood cells.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats received 0, 5, 15, 50, 150, or 500 mg THC/kg body weight in corn oil by gavage, 5 days per week for 13 weeks. Six male and six female rats receiving 500 mg/kg died before the end of the study. The final mean body weights and weight gains of all dosed groups of males and females, except 5 mg/kg females, were significantly lower than those of the controls. Feed consumption by dosed groups was similar to that by controls. Clinical findings observed during the study included lethargy, sensitivity to touch, convulsions, tremors, and aggressiveness. There were no clinical pathology differences considered to be directly related to the administration of THC. The absolute and relative uterus weights of 50, 150, and 500 mg/kg females were significantly lower than those of the controls. Treatment-related multifocal atrophy was observed in the testes of 150 and 500 mg/kg males; uterine and ovarian hypoplasia observed in 150 and 500 mg/kg females was also considered to be related to THC administration. Based on final mean body weights and mortality observed in the 13-week study, doses selected for the 2-year rat study were 12.5, 25, and 50 mg/kg.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice received 0, 5, 15, 50, 150, or 500 mg THC/kg body weight in corn oil by gavage, 5 days per week for 13 weeks. There were no treatment-related deaths. The final mean body weight and weight gain of 500 mg/kg males were significantly lower than those of the controls. Clinical findings included lethargy and aggressiveness, and both male and female mice in all dosed groups were easily startled. There were no absolute or relative organ weight differences, clinical pathology differences, or microscopic changes observed that were considered to be related to the administration of THC. Due to the minimal THC-related effects

observed in the 13-week study, doses selected for the 2-year mouse study were 125, 250, and 500 mg/kg.

13-Week with 9-Week Recovery Study in Rats

Groups of 10 male and 10 female rats received 0, 5, 15, 50, 150, or 500 mg THC/kg body weight in corn oil by gavage, 5 days per week for 13 weeks, and then were allowed to recover during a 9-week treatment-free period. Five male and eight female 500 mg/kg rats, five male and two female 150 mg/kg rats, and three male and two female 50 mg/kg rats died before the end of the study. During the 13-week dosing period, mean body weight gains of all dosed groups of rats were lower than those of the controls but returned to normal during the recovery period. Final mean body weights of all dosed groups were similar to those of the controls. Clinical findings observed during the recovery period included sensitivity to touch, convulsions, and aggressiveness. The absolute right testis weight of 500 mg/kg males was significantly lower than that of the controls. Treatment-related multifocal atrophy of the testis was observed in 150 and 500 mg/kg males. There were no treatment-related lesions observed in females administered THC.

13-Week with 9-Week Recovery Study in Mice

Groups of 10 male and 10 female mice received 0, 5, 15, 50, 150, or 500 mg THC/kg body weight in corn oil by gavage, 5 days per week for 13 weeks, and then were allowed to recover during a 9-week treatment-free period. The final mean body weights of all dosed groups were similar to those of the controls. Clinical findings observed during the study included lethargy and aggressiveness, and both male and female mice in all dosed groups were easily startled. The absolute and relative uterus weights of 150 and 500 mg/kg female mice were significantly lower than those of the controls, as was the absolute uterus weight of 50 mg/kg females.

2-Year Study in Rats

Groups of 62 vehicle control male rats, 60 low-dose male rats, 70 mid- and high-dose male rats, and 60 female rats were administered 0, 12.5, 25, or 50 mg THC/kg body weight in corn oil by gavage for 104 to 105 weeks. Nine or ten animals from each group were evaluated at 15 months.

Survival, Body Weights, and Clinical Findings

Survival of all dosed groups was generally significantly greater than that of the controls. Mean body weights of dosed groups of males and females were lower than those of the controls throughout the study. Convulsions and seizures were observed in all dosed groups of male and female rats, usually following dosing or handling.

Hematology and Clinical Chemistry

At the 15-month interim evaluation, total leukocyte and lymphocyte counts in all dosed groups of females were greater than those of the controls, and platelet counts in these groups were lower than that of the controls. Levels of follicle stimulating and luteinizing hormones in all dosed groups of males were significantly greater than those of the controls, as was the serum corticosterone level of 25 mg/kg females.

Pathology Findings

No increased incidences of neoplasms were considered related to administration of THC. The incidences of mammary gland fibroadenoma and uterine stromal polyps were decreased in dosed groups of females, as were the incidences of pituitary gland adenomas, interstitial cell adenomas of the testis, and pancreatic adenomas in dosed males.

2-Year Study in Mice

Groups of 62 vehicle control male mice, 60 low-dose male mice, 61 mid-dose male mice, and 60 high-dose male mice and 60 female mice were administered 0, 125, 250, or 500 mg THC/kg body weight in corn oil by gavage for 104 to 105 weeks (males) or 105 to 106 weeks (females).

Survival, Body Weights, and Clinical Findings

Survival of 500 mg/kg males was significantly less than that of the controls; survival of all other groups of males and of all dosed groups of females was similar to that of the controls. Mean body weights of all dosed groups were markedly lower than those of the controls throughout the study. Clinical findings in dosed groups included hyperactivity, convulsions, and seizures which occurred following dosing or handling.

Hematology

At the 15-month interim evaluation, total leukocyte and lymphocyte counts in all dosed groups of males were significantly lower than those of the controls.

Pathology Findings

Increased incidences of thyroid gland follicular cell adenoma occurred in 125 mg/kg males and females, but the increase was not dose-related. Increased incidences of thyroid gland follicular cell hyperplasia occurred in all dosed groups of males and females. Increased incidences of forestomach hyperplasia and ulcers occurred in all groups of males administered THC. Incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) occurred with a significant negative trend in male and female mice, as did incidences of eosinophilic foci and fatty change in the liver.

GENETIC TOXICOLOGY

THC was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 with or without rat and hamster liver S9 fractions. In cultured Chinese hamster ovary cells, THC induced sister chromatid exchanges at the highest dose tested in the presence of S9; at this dose level, cell cycle delay indicative of toxicity was observed. THC did not induce chromosomal aberrations in cultured Chinese hamster ovary cells with or without S9 metabolic activation enzymes. *In vivo*, no increase in the frequency of micronucleated erythrocytes was observed in the peripheral blood of male or female mice administered THC by gavage for 13 weeks.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of 1-trans-delta^9-tetrahydrocannabinol in male or female F344/N rats administered 12.5, 25, or 50 mg/kg. There was *equivocal evidence of carcinogenic activity* of THC in male and female B6C3F$_1$ mice based on the increased incidences of thyroid gland follicular cell adenomas in the 125 mg/kg groups.

Increased incidences of thyroid gland follicular cell hyperplasia occurred in male and female mice, and increased incidences of hyperplasia and ulcers of the forestomach were observed in male mice.

The incidences of mammary gland fibroadenomas and uterine stromal polyps were decreased in dosed groups of female rats, as were the incidences of pancreatic adenomas, pituitary gland adenomas, and interstitial cell adenomas of the testis in dosed male rats and liver neoplasms in dosed mice. These decreases were likely related to lower body weights in dosed animals.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1-Trans-Delta^9-Tetrahydrocannabinol

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 12.5, 25, or 50 mg/kg in corn oil by gavage	0, 12.5, 25, or 50 mg/kg in corn oil by gavage	0, 125, 250, or 500 mg/kg in corn oil by gavage	0, 125, 250, or 500 mg/kg in corn oil by gavage
Body weights	Dosed groups lower than controls	Dosed groups lower than controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates	22/52, 35/51, 33/52, 31/52	23/51, 40/51, 33/51, 32/50	50/62, 53/60, 45/61, 34/60	47/60, 50/60, 44/60, 41/60
Nonneoplastic effects	None	None	Forestomach: hyperplasia (7/62, 33/58, 38/58, 18/56); ulcer (5/62, 17/58, 14/58, 8/56) Thyroid gland (follicular cell): hyperplasia (16/62, 48/60, 45/61, 27/57)	Thyroid gland (follicular cell): hyperplasia (28/60, 46/60, 40/60, 33/60)
Neoplastic effects	None	None	None	None
Uncertain findings	None	None	Thyroid gland (follicular cell): adenoma (0/62, 6/60, 3/61, 1/57)	Thyroid gland (follicular cell): adenoma (4/60, 9/60, 3/60, 1/60)
Decreased incidences	Pancreas: adenoma (8/52, 0/51, 2/52, 0/52); Pituitary gland: adenoma (21/52, 19/51, 14/51, 9/52); Testis: interstitial cell adenoma (46/52, 40/51, 36/52, 43/52)	Mammary gland: fibroadenoma (15/51, 11/51, 11/51, 8/50); Uterus: stromal polyp (8/51, 5/51, 2/51, 2/50)	Liver: hepatocellular adenoma (25/62, 11/60, 6/61, 2/57); hepatocellular adenoma or carcinoma (31/62, 13/60, 9/61, 3/57); eosinophilic foci (18/62, 1/60, 0/61, 0/57); fatty change (20/62, 11/60, 1/61, 1/57)	Liver: hepatocellular adenoma (17/60, 9/60, 7/59, 3/60); hepatocellular adenoma or carcinoma (22/60, 14/60, 11/59, 4/60); eosinophilic foci (9/60, 0/60, 1/59, 1/60); fatty change (13/60, 3/60, 0/59, 2/60)
Level of evidence of carcinogenic activity	No evidence	No evidence	Equivocal evidence	Equivocal evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Positive with S9; negative without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	No increase in frequency observed

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

DISCUSSION AND CONCLUSIONS

The use of marijuana in the United States remains widespread. The major psychoactive component of marijuana and hashish is 1-trans-delta^9-tetrahydrocannabinol (THC). THC has antiemetic, analgesic, muscle relaxant, and anticonvulsant properties. The chemical has been used to reduce intraocular pressure in glaucoma patients and to treat bronchial asthma, insomnia, hypertension, and depression. Because of the widespread use of marijuana and its potential medical applications, the National Cancer Institute nominated THC for study.

In the 13-week studies, THC was administered by gavage to groups of male and female rats and mice at doses of 0, 5, 15, 50, 150, or 500 mg THC/kg body weight. In the recovery studies, male and female rats and mice were administered the same doses of THC for 13 weeks and allowed to recover for 9 weeks without further THC administration. Six male and six female 500 mg/kg rats died before the end of the 13-week study; these deaths were considered related to the administration of THC. With the exception of 5 mg/kg rats, the final mean body weights and weight gains of all dosed groups of male and female rats were significantly lower than those of the controls. Feed consumption data showed that weight gain was not due to lower feed consumption. In the recovery study, male and female rats gained weight quickly following cessation of dosing; at the end of the 9-week recovery period, their body weights were similar to those of the controls. In accord with the reported effects of THC on reproductive organs, testicular atrophy was observed in 150 and 500 mg/kg rats at the end of the 13-week study and in 500 mg/kg rats at the end of the recovery study. However, at doses of 50 mg/kg or less, testicular atrophy was not observed in either the 13-week or recovery studies. Absolute and relative uterine weights of all dosed groups of female rats were lower than those of the controls, estrous cycles were lengthened, and uterine and ovarian hypoplasia were observed in 150 and 500 mg/kg rats at the end of the 13-week study.

Survival of male and female mice in both the 13-week and recovery studies was unaffected by the administration of THC. The final mean body weight and weight gain of 500 mg/kg male mice in the 13-week study were significantly lower than those of the controls. Final mean body weights and weight gains of all other dosed groups of male mice and of all dosed groups of female mice in the 13-week study were similar to those of the controls, as were those of all dosed groups of male and female mice in the recovery study. Feed consumption by dosed groups of male and female mice in both the 13-week and recovery studies was similar to that by controls; no histopathologic changes related to the administration of THC were observed in mice from either study.

During the course of the 13-week study, dosed groups of rats and mice initially showed clinical signs of lethargy, becoming aggressive and hyperactive later in the study. During handling of the animals, convulsions occurred in THC-dosed rats and mice in both the 13-week and recovery studies.

In the 9-week period following dosing, the rats recovered from the effects of THC on body weight depression and the ovarian effects largely resolved. However, hypersensitivity to stimulation and convulsions were observed during the recovery period in rats and mice, as were testicular atrophy and reduced leukocyte and lymphocyte counts in 500 mg/kg male rats. These effects may have persisted after cessation of treatment due to the long half-life of THC.

Dose levels selected for the 2-year studies were based on lower mean body weight gains observed in dosed rats and mice in the 13-week studies and on mortality observed in rats in the 13-week study. Fighting among dosed animals, convulsions observed in dosed groups from the present 13-week and recovery studies, reported tolerance development to THC in long-term exposure studies, and dose levels reportedly used by other investigators were also considered in the dose selection. According to calculations based on body surface area, an oral dose of 2.1 mg/kg to rats is equivalent to a human smoking one marijuana cigarette; 10 mg/kg is equivalent to the content of THC in a hashish cigarette (Luthra *et al.*, 1975; Rosenkrantz *et al.*, 1975). The amount of THC taken in by habitual smokers was estimated to range from.

0.3 to 12 mg/kg per day (ARF/WHO, 1981). THC at doses of up to 10 mg/kg administered orally to Fischer rats daily during a 21 to 22 day gestation period was considered nonteratogenic and did not cause adverse effects on the dams as determined by reproductive data, endocrine organ weights, and body weights (Luthra, 1979). THC at 50 mg/kg per day orally for 21 days during gestation did not affect litter size or pup weight at birth, although maternal weight was reduced (Abel, 1984). A 10 mg/kg dose intraperitoneally is commonly used to show clear inhibitory effects of cannabinoids on spontaneous activity in an open field test (Little *et al.*, 1988; Oviedo *et al.*, 1993). Landfield *et al.* (1988) reported that rats subcutaneously administered THC at doses of 4 and 8 mg/kg five times weekly for 8 months were irritable; their open field activity and active avoidance training were not different from those of the controls. These authors concluded that the dose was not high enough to exert behavioral effects. Thus, the dose levels of 12.5 to 50 mg/kg selected for the 2-year rat studies were considered reasonable.

In the 2-year studies, growth rates of dosed male and female rats were less than those of the controls. Feed consumption by rats was measured during the final 9 months of the 2-year study; there was little difference in feed consumption by dosed and control groups. The lower body weights of THC-dosed rats were probably not due to reduced feed consumption earlier in the study. Thus, it seems that growth retardation of the dosed rats was a pharmacologic effect of THC that was marked even in rats administered 12.5 mg/kg (the low dose). Increased metabolic rates may be required for the hyperactive, adaptive, and detoxification effects induced by THC treatment. Significant elevations in plasma adrenocorticotropic hormone (ACTH) and corticosterone (Zuardi *et al.*, 1984; Landfield *et al.*, 1988; Eldridge *et al.*, 1991) and increases in relative thyroid and adrenal weights (Borgen *et al.*, 1971) following THC administration have been reported. Serum corticosterone levels measured at 15 months were elevated in both male and female rats, but thyroxine levels were similar to those of the controls. The corticosterone may have played a role in the lower mean body weight gains. Data from the present studies coincided with data from the Thompson *et al.* (1973) study in which growth rates of dosed male and female Fischer rats (administered 50, 250, 400, or 500 mg THC per kg body weight by gavage for 119 days) were lower than those of the controls, but there was little difference in body weights among the dosed groups. Rosenkrantz *et al.* (1975) also reported that Fischer rats treated orally with 10 or 50 mg THC/kg body weight daily for 180 days showed weight reduction despite an elevation in feed consumption. According to Thompson *et al.* (1973), the reduced weight gain was due to depletion in body fat stores; female rats were more severely affected than males. Urinary output was also higher in the THC-dosed rats than in controls.

Survival of the dosed male and female rats was greater than that of the controls in the 2-year study; the difference was significant in each dose group except the 50 mg/kg males. The increased survival rates of the dosed male and female rats may be due to the lower mean body weights throughout the experimental period. Higher survival rates have been associated with lower body weight in diet restriction studies (Kari and Abdo, 1996).

Oviedo *et al.* (1993) administered 10 mg THC/kg body weight intraperitoneally daily for 2 weeks to male Sprague-Dawley rats. Within 10 minutes after the first dose, the rats became inactive. When placed in the center of a circular open field in the behavioral study, the rats crouched on one side. After some time, the animals started to walk in a circular fashion. They exhibited normal activity after 2 weeks. Thompson *et al.* (1973) reported that Fischer rats treated orally with up to 500 mg/kg daily for 119 days initially exhibited depression, followed by hyperactivity, aggressiveness, and convulsions. The frequency and onset of convulsions were dose-related. Luthra *et al.* (1975) reported that rats fed THC at 50 mg/kg for 6 months exhibited generalized depression and ataxia followed by irritability, hyperactivity, aggression, tremors, and convulsions. Tolerance developed after prolonged treatment. Luthra and Rosenkrantz (1974) and Luthra *et al.* (1975) demonstrated that oral treatment of male and female Fischer rats with up to 50 mg THC per/kg body weight daily for 180 days lowered the ribonucleic acid (RNA) content in the frontal cortex, parietal cortex, and subcortex of the brain. Acetylcholinesterase activity increased in the frontal cortex, parietal cortex, and subcortex of male rats, but decreased in the female rats. The degree of neurochemical alteration diminished as treatment was prolonged. Peak convulsive activity occurred near day 130; the activity fell

progressively and was not observed by 180 days. The authors believed the brain RNA and acetylcholinesterase activity and neurobehavioral changes were related.

In the present 2-year rat study, initial depression was followed by hyperactivity. Aggressive behavior was averted by housing the animals individually. The rats receiving THC had *grand mal* seizures usually induced by sensory stimulation and the time of onset and frequency appeared to correlate with dose levels. Female rats displayed seizure earlier and more frequently than male rats. The convulsive activity was still recorded during the last 6 months of the 2-year study. Apparently, tolerance did not develop. Brain lesions were not identified in the hematoxylin- and eosin-stained sections or in tissues from rats perfused with Trump's fixative. The issue of tolerance could have been more directly addressed, but evaluations of the excitatory (glutamate and aspartate) and inhibitory (γ-aminobutyric acid, glycine, and taurine) neurotransmitter amino acids and their binding sites and affinities of monoaminergic (noradrenergic/dopaminergic and serotinergic) transmitter systems and of the cholinergic system were not attempted. There was no histopathologic evidence of brain lesions in rats. However, structural and functional alterations of the hippocampal pyramidal neurons as indicated by reduced cytoplasmic and nuclear volumes and decreased synaptic density in rodents treated orally with THC (10 to 60 mg/kg) daily for 90 days have been reported (Slikker *et al.*, 1991). Landfield *et al.* (1988) also reported that rats administered THC (8 mg/kg) subcutaneously daily for 8 months had reduced numbers of neurons in striatum pyramidale of field CA1 of the hippocampus and increased cytoplasmic inclusions in hippocampal astrocytes.

Several investigators have studied the effects of THC on the endocrine system, particularly the pituitary gland, and reported altered ACTH, corticosterone, follicle stimulating hormone (FSH), and thyroid hormone levels. Landfield *et al.* (1988) reported that rats receiving THC subcutaneously at 8 mg/kg daily had significant elevations in plasma ACTH and corticosterone levels. Borgen *et al.* (1971) reported increased relative thyroid and adrenal gland weights in pregnant female Long-Evans rats administered 100 or 200 mg/kg THC daily by gavage during the 20-day gestation period; serum thyroid hormone levels were not determined. These authors interpreted the organ weight changes to be a result of general stress response to THC administration. In the present study, there was a significant dose-related decrease in the incidence of pituitary adenoma in male rats, and serum corticosteroid levels at 15 months in male and female rats were elevated, but thyroxine levels were normal. The corticosteroid levels, body weights, and pituitary adenoma incidences in the 2-year study are probably related.

At the 15-month interim evaluation, serum FSH levels of THC-dosed males were higher than that of the controls. At the end of the 2-year study, the incidences of mammary gland neoplasms and uterine stromal polyps were lower in the 25 and 50 mg/kg females than in the controls. Kari and Abdo (1996) reported low body weights brought about by diet restriction decreased the incidence of mammary gland neoplasms and uterine stromal polyps in female rats. The lower body weights observed in THC-dosed rats from the 2-year study may have played a role in reducing the incidences of interstitial cell adenoma of the testis in males and mammary gland neoplasms and uterine stromal polyps in females. However, THC has been reported to affect the hypothalamo-pituitary-gonad axis and alter luteinizing hormone and FSH secretion (Rosenkrantz and Esber, 1980; Martin, 1986) and may also act directly at the gonadal level on steroidogenesis by the testes (Newton *et al.*, 1993) and the ovary (Treinen *et al.*, 1993). Thus, the lower incidences of interstitial cell adenoma of the testis, mammary gland neoplasms, and uterine stromal polyps observed in the 2-year study may be related to the effects of THC on the hypothalamo-pituitary-gonad axis and the gonads.

The decreased incidence of acinar cell adenomas of the pancreas in dosed male rats may have been related to decreased body weights. The incidence of acinar adenoma in the vehicle control group is greater than that in nontreated (dosed feed) control male rats and has been attributed to effects of chronic administration of corn oil (Haseman and Rao, 1992).

Survival rates of dosed mice in the 2-year study, except that of 500 mg/kg males, were similar to those of the controls; survival in the 500 mg/kg males was significantly lower than that in the controls. No specific reason for this was determined. In the 2-year mouse study, mean body weight gains of dosed male and female mice were significantly lower than those of the controls, even during the first 13 weeks. In the

13-week study, mice housed five per cage exhibited aggressive fighting behavior; therefore, mice in the 2-year study were housed individually. Mean body weight gains were not different among the dosed groups and the controls in the 13-week study. It appears that individual housing affected the growth rates of control and THC-dosed mice differently, even though feed consumption was similar. Judging from the growth rate data in the 13-week study and those during the first 13 weeks of the 2-year study, control male and female mice grew faster when housed individually. This phenomenon may account partially for the larger reduction in body weights recorded in the THC-dosed mice in the 2-year study.

Convulsions were also observed in the THC-dosed mice and the onset and frequency were dose related. Histopathologic changes in the hippocampus were not identified in mice. Abood *et al.* (1993) reported the cannabinoid receptor mRNA levels and the receptor binding capacity and affinity were not altered in whole brain homogenates of male ICR mice administered 10 mg/kg intraperitoneal injections of THC twice daily for 6.5 days. Receptor changes were not determined in the 2-year study.

Incidences of eosinophilic foci, fatty change, and hepatocellular adenoma and carcinoma (combined) of dosed male and female mice were significantly lower than those of the controls in the 2-year study. The decrease was dose related. Incidences of hepatocellular neoplasms correlate well with body weights in male and female B6C3F$_1$ mice (Rao *et al.*, 1990; Turturro *et al.*, 1993). However, the lower body weights of the THC-dosed mice were not due to lower feed consumption. The dose-related decrease in the incidence of hepatocellular neoplasms in the present study was probably related to decreases in body weights resulting from physiological and hormonal changes brought about by THC administration as discussed above.

Incidences of thyroid gland follicular cell hyperplasia were significantly increased in all dosed male groups and in 125 and 250 mg/kg female mice in the 2-year study. The severity of hyperplasia did not increase with increasing dose. Hyperplasia of the thyroid gland follicular epithelium was not observed in the 13-week study; marginally increased incidences of thyroid gland follicular cell adenoma occurred in the 125 mg/kg males and females, but the incidences did not increase with increasing dose. Additionally, single carcinomas were observed in a vehicle control male and a 125 mg/kg female. There was no clear developmental progression from hyperplasia to adenoma to carcinoma by the end of the study. Serum thyroid hormone levels in dosed mice were not determined. Thyroid gland follicular cell neoplasms are relatively uncommon in historical control corn oil gavage mice. The NTP historical incidence for mouse thyroid gland follicular cell neoplasms from 2-year gavage studies is 1.6% for males and 2.0% for females. Thus, the incidences of 10% and 17% observed in the 125 mg/kg males and females were higher than the historical control ranges. The incidences of thyroid gland follicular cell neoplasms in the 250 and 500 mg/kg groups were lower than that observed in the 125 mg/kg groups. There were no marked differences in survival or body weights among dosed groups that could account for this lack of dose response. Thus, the evidence of carcinogenic activity of THC in male and female mice is considered to be "equivocal."

The primary effect of the 2-year administration of THC in the present studies was to lower body weight gains in male and female Fischer rats and B6C3F$_1$ mice. THC also induced lethargy, followed by aggressive behavior, convulsions, and hyperactivity. The total number of benign and malignant neoplasms in male and female rats and mice decreased in a dose-related manner (Tables 18, A3, B3, C3, and D3), as did mortality rates of dosed male and female rats; both effects may be related to reduced body weights.

TABLE 18
Summary of Final Mean Body Weights and Selected Decreased Neoplasm Incidences in Male and Female Rats and Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Rats				
Male				
Final Mean Body Weights[a]	411	372	369	370
Pancreas: Adenoma				
Overall rate[b]	8/52 (15%)	0/51 (0%)	2/52 (4%)	0/52 (0%)
Adjusted rate[c]	33.8%	0.0%	5.7%	0.0%
Terminal rate[d]	7/22 (32%)	0/35 (0%)	1/33 (3%)	0/31 (0%)
First incidence (days)	647	–[f]	709	–
Logistic regression test[e]	P=0.002N	P=0.001N	P=0.019N	P=0.002N
Pituitary Gland (Pars Distalis or Unspecified Site): Adenoma				
Overall rate	21/52 (40%)	19/51 (37%)	14/51 (27%)	9/52 (17%)
Adjusted rate	70.5%	46.8%	35.0%	23.8%
Terminal rate	14/22 (64%)	14/35 (40%)	8/33 (24%)	4/31 (13%)
First incidence (days)	556	610	595	578
Logistic regression test	P=0.003N	P=0.225N	P=0.063N	P=0.004N
Testes: Adenoma				
Overall rate	46/52 (88%)	40/51 (78%)	36/52 (69%)	43/52 (83%)
Adjusted rate	97.8%	92.9%	92.2%	95.5%
Terminal rate	21/22 (95%)	32/35 (91%)	30/33 (91%)	29/31 (94%)
First incidence (days)	438	527	592	563
Logistic regression test	P=0.270N	P=0.037N	P=0.006N	P=0.214N
Female				
Final Mean Body Weights	308	275	282	288
Mammary Gland: Fibroadenoma				
Overall rate	15/51 (29%)	11/51 (22%)	11/51 (22%)	8/50 (16%)
Adjusted rate	40.9%	24.8%	30.3%	23.5%
Terminal rate	4/23 (17%)	7/40 (18%)	9/33 (27%)	6/32 (19%)
First incidence (days)	528	584	562	659
Logistic regression test	P=0.074N	P=0.415N	P=0.216N	P=0.071N
Uterus: Stromal Polyp				
Overall rate	8/51 (16%)	5/51 (10%)	2/51 (4%)	2/50 (4%)
Adjusted rate	25.6%	12.1%	6.1%	6.3%
Terminal rate	3/23 (13%)	4/40 (10%)	2/33 (6%)	2/32 (6%)
First incidence (days)	546	659	725 (T)	725 (T)
Logistic regression test	P=0.020N	P=0.227N	P=0.038N	P=0.044N

(continued)

TABLE 18
Summary of Final Mean Body Weights and Selected Decreased Neoplasm Incidences in Male and Female Rats and Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol
(continued)

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Mice				
Male				
Final Mean Body Weights	50.2	44.3	40.9	38.6
Liver: Hepatocellular Adenoma				
Overall rate	25/62 (40%)	11/60 (18%)	6/61 (10%)	2/57 (4%)
Adjusted rate	45.3%	19.8%	12.8%	5.6%
Terminal rate	20/50 (40%)	9/53 (17%)	4/45 (9%)	1/34 (3%)
First incidence (days)	672	566	716	611
Logistic regression test	P<0.001N	P=0.010N	P<0.001N	P<0.001N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	31/62 (50%)	13/60 (22%)	9/61 (15%)	3/57 (5%)
Adjusted rate	54.3%	23.0%	18.7%	8.4%
Terminal rate	24/50 (48%)	10/53 (19%)	6/45 (13%)	2/34 (6%)
First incidence (days)	554	563	574	611
Logistic regression test	P<0.001N	P=0.001N	P<0.001N	P<0.001N
Female				
Final Mean Body Weights	49.7	37.0	33.3	33.7
Liver: Hepatocellular Adenoma				
Overall rate	17/60 (28%)	9/60 (15%)	7/59 (12%)	3/60 (5%)
Adjusted rate	34.4%	18.0%	15.5%	7.3%
Terminal rate	15/47 (32%)	8/49 (16%)	6/44 (14%)	3/41 (7%)
First incidence (days)	659	714	694	737 (T)
Logistic regression test	P=0.001N	P=0.053N	P=0.032N	P=0.002N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	22/60 (37%)	14/60 (23%)	11/59 (19%)	4/60 (7%)
Adjusted rate	43.0%	27.3%	23.8%	9.4%
Terminal rate	18/47 (38%)	12/49 (24%)	9/44 (20%)	3/41 (7%)
First incidence (days)	659	661	674	701
Logistic regression test	P<0.001N	P=0.071N	P=0.035N	P<0.001N

(T)Terminal sacrifice
[a] Weights are presented in grams.
[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, pancreas, pituitary gland, testes, and uterus; for other tissues, denominator is number of animals necropsied.
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[f] Not applicable; no neoplasms in animal group

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of 1-trans-delta^9-tetrahydrocannabinol in male or female F344/N rats administered 12.5, 25, or 50 mg/kg. There was *equivocal evidence of carcinogenic activity* of THC in male and female B6C3F$_1$ mice based on the increased incidences of thyroid gland follicular cell adenomas in the 125 mg/kg groups.

Increased incidences of thyroid gland follicular cell hyperplasia occurred in male and female mice, and increased incidences of hyperplasia and ulcers of the forestomach were observed in male mice.

The incidences of mammary gland fibroadenomas and uterine stromal polyps were decreased in dosed groups of female rats, as were the incidences of pancreatic adenomas, pituitary gland adenomas, and interstitial cell adenomas of the testis in dosed male rats, and liver neoplasms in male and female mice. These decreases were likely related to lower body weights in dosed animals.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.