NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

1-TRANS-DELTA^9-TETRAHYDROCANNIBINOL

(CAS NO. 1972-08-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

November 1996

NTP TR 446
NIH Publication No. 97-3362

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

P.C. Chan, Ph.D., *Study Scientist*
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
R.C. Sills, D.V.M., Ph.D.
G.S. Travios, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., *Oak Ridge Associated Universities*

SRI International
Conducted 13- and 22-week studies, evaluated pathology findings

T.A. Jorgenson, M.S., Principal Investigator
E.F. Meierherny, Ph.D.
R.J. Spanggord, Ph.D.

TSI Mason Research Institute
Conducted 2-year studies, evaluated pathology findings

A.G. Braun, Sc.D., Principal Investigator
F.A. Voelker, M.S., D.V.M.
M.E.P. Goad, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

IF Hardisty, D.V.M., *Principal Investigator*
B.F. Hamilton, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats
(13 October 1992)

J.C. Seely, D.V.M., *Chairperson*
PATHCO, Inc.
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
J.R. Hailey, D.V.M.
National Toxicology Program
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report on mice
(30 July 1992)

J.C. Seely, D.V.M., *Chairperson*
PATHCO, Inc.
R. Cattley, V.M.D., Ph.D.
Chemical Industry Institute of Toxicology
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
CC. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Analytical Sciences, Inc.
Provided statistical analyses

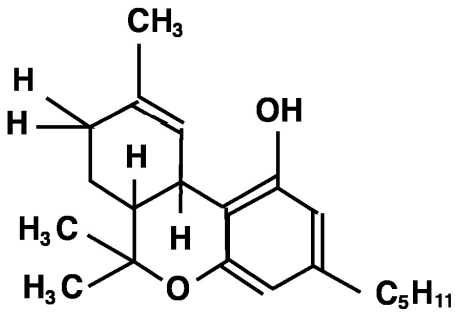
R.W. Morris, M.S, *Principal Investigator*
N.G. Mintz, B.S.
S. Rosenblum, M.S.

Biotechnical Services, Inc.
Prepared Technical Report

D.D. Lambright, Ph.D., *Principal Investigator*
S.R. Gunnels, M.A.
T.A. King-Hunter, B.S.
T.L. Rhoades, B.S.

CONTENTS

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 bodyweights 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.

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

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 1-Trans-Delta9-Tetrahydrocannabinol

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 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.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 1-Trans-delta^9-tetrahydrocannabinol on June 21, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NIT studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Arnold L. Brown, M.D., Chairperson University of Wisconsin Medical School Madison, WI

Paul T. Bailey, Ph.D. Environmental and Health Sciences Laboratory Mobil Oil Corporation Princeton, NJ

Meryl H. Karol, Ph.D. Department of Environmental Occupational Health University of Pittsburgh Pittsburgh, PA

Curtis D. Klaassen, Ph.D., Principal Reviewer Department of Pharmacology and Toxicology University of Kansas Medical Center Kansas City, KS

Claudia S. Miller, M.D. University of Texas Health Sciences Center San Antonio, TX

Janardan K. Reddy, M.D.
Department of Pathology Northwestern University Medical School Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center Philadelphia, PA

Louise Ryan, Ph.D.
Division of Biostatistics Harvard School of Public Health and Dana-Farber Cancer Institute Boston, MA

Robert E. Taylor, M.D., Ph.D., Principal Reviewer Department of Pharmacology Howard University College of Medicine Washington, DC

Matthew J. van Zwieten, D.V.M., Ph.D., Principal Reviewer Department of Safety Assessment Merck Research Laboratories West Point, PA

Mary Jo Vodicnik, Ph.D. Lilly MSG Development Center Belgium

Jerrold M. Ward, D.V.M., Ph.D. National Cancer Institute Frederick, MD

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 21, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of 1-Trans-delta^{9}-tetrahydrocannabinol (THC) received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of THC by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and bodyweight effects, and commenting on chemical-related neoplasm and nonneoplastic lesion incidences. Healso presented toxico kinetic data for male rats. The proposed conclusions were no evidence of carcinogenic activity in male and female P344/N rats and equivocal evidence of carcinogenic activity in male and female B6C3F$_1$ mice.

Dr. Klaassen, a principal reviewer, agreed with the proposed conclusions. He asked for a table in the discussion outlining decreases in neoplasm incidences and the correlation of these decreases with bodyweights. Dr. Chan said a table would be added. Dr. Klaassen asked why a 9-week recovery period was included in the present studies. Dr. Chan said that the effects of THC linger, so a recovery period was included to study the effects of the chemical, particularly on the reproductive system, and to aid in possible extrapolation of the effects in humans.

Dr. Taylor, the second principal reviewer, agreed with the proposed conclusions. Dr. Taylor asked for an expansion of the discussion of arachidonic acid metabolism modification by THC, noting it would be helpful to indicate the extent and direction and the possible therapeutic or physiologic implications. Dr. Chan agreed (page 17). Dr. Taylor said a comment explaining the selection of gavage as the route of administration should be added to the report, noting that this route differs from the typical routes of human exposure. Dr. Chan said that insufficient compound was available to perform an inhalation study, intraperitoneal injection was less akin to human routes of exposure, and only a small historical database exists for the intraperitoneal injection route.

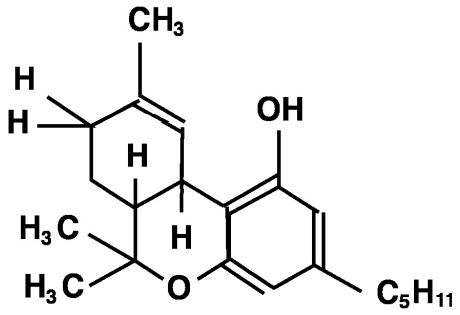
Dr. van Zwieten, the third principal reviewer, agreed with the proposed conclusions. He asked for comments on the apparent inverse dose-response relationship for the thyroid gland neoplasm incidences in mice (page 76).

Dr. Ward asked if step sectioning of mouse thyroid glands had been considered in view of the equivocal findings. Dr. M.R. Elwell, NIEHS, said that because of the small size of the gland, one cross-section is fairly representative of the entire organ. Dr. Ward asked if lower body weights of dosed groups could have been caused by exceeding maximum tolerated doses. Dr. J.R. Bucher, NIEHS, said that because THC can affect weight gain, the possibility of exceeding the maximum tolerated dose would be difficult to interpret. Dr. Chan added that because THC is taken up and stored in adipose tissue, THC buildup during chronic administration could cause the maximum tolerated dose to be exceeded. Dr. Russo asked for comments on the lower serum levels of follicle stimulating hormone and luteinizing hormone in female rats and mice when compared to male rats and mice. Dr. Bucher said although the reproductive effects of THC were well studied, there was no explanation for the difference in the hormone levels in males and females in the present studies. Dr. van Zwieten noted that many decreased neoplasm incidences observed in dosed groups were within historical control ranges from 2-year NTP gavage studies. Dr. Miller suggested including data contrasting human and animal THC plasma levels and including levels typically achieved in humans to discourage the concept of THC as a cancer inhibitor. Dr. Bucher noted that the results of these studies could be misinterpreted to demonstrate that exposure to THC could provide beneficial therapeutic effects and added that the NTP has attempted to stress that most of the observed changes were due to decreased weight gain.

Dr. Klaassen cited a report in the text that the amount of THC taken in by habitual marijuana smokers was estimated to range from 0.3 to 12.0 mg/kg, which would be comparable to doses administered to rats in the present studies. Dr. Taylor pointed out that plasma levels resulting from a dose administered via inhalation would be much higher than those resulting from the same dose administered orally.

Dr. Klaassen moved that the Technical Report on 1-Trans-delta^{9}-tetrahydrocannabinol be accepted with the revisions discussed and the conclusions as written for male and female rats, *no evidence of carcinogenic* activity, and for male and female mice, *equivocal evidence of carcinogenic activity*. Dr. Bailey seconded the motion, which was accepted unanimously with eleven votes.

INTRODUCTION



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

CHEMICAL AND PHYSICAL PROPERTIES

1-Trans-delta^9-tetrahydrocannabinol (THC) is an oil with a boiling point of 200º C. When stored, THC decomposes and becomes reddish in color. It is insoluble in water and soluble in organic solvents such as ethanol, hexane, and chloroform. It is unstable in air, light, and acidic media and at high temperatures. THC is more stable in ethanol than in carbon tetrachloride or hexane. Thin films of THC are less stable than THC in solutions. Stability is not improved by adding antioxidants. The major product of THC decomposition is cannabinol and the minor product is delta^8-THC. Due to its high lipid/aqueous partition coefficient, THC has a higher affinity for biomembranes than for aqueous media (Martin, 1986). Octanol/water and benzene/water partition coefficients of THC are about 6,000 (Pertwee, 1988).

PRODUCTION, USE, AND HUMAN EXPOSURE

More than 60 C_{21} compounds grouped together under the general designation of cannabinoids are found in the *Cannabis sativa* plant. Among the naturally occurring cannabinoids, THC is the main psychoactive component of marijuana and hashish. Marijuana is the chopped flowering tops of the female hemp plant, *Cannabis sativa*, and hashish is the resinous material derived from the flowering tops. The other pharmacologically active isomer, D^8-THC, is found only in a few strains of the plant. Maximal biological activity depends on the double bond at the D^8 or D^9 position. In most pharmacological assays, D^8-THC is approximately one-third as potent as THC. The other cannabinoids are relatively pharmacologically inactive but may interact in or influence the metabolism of THC (Bornheim, 1989).

The concentration of THC in fresh samples of cannabis is low; of the cannabinoids present, about 95% is ordinarily found in the form of D^9-tetrahydro-cannabinolic acid, a pharmacologically inactive compound. On aging and development of the plant, storage of the cut plant material, or heating, D^9-tetra-hydrocannabinolic acid is decarboxylated to form the pharmacologically active compound THC. The THC content of marijuana from American wild strains of *C. sativa* is about 0.1%. Tropical strains yield as much as 4%. Careful cultivation and genetic manipulation have raised the THC content of marijuana to much higher levels.

About one-half to three-fourths of the THC contained in a typical 500 mg marijuana cigarette (approximately 5 mg) is lost during smoking. Habitual smokers receive 0.3 to 12 mg THC/kg per day (ARF/WHO, 1981). THC is not commercially available in the U.S., but drug companies have been developing analogues of THC to exploit the medical potential of the compound without subjecting the patient to its disruptive central nervous system effects. Current production volumes of the analogues are not available.

Marijuana and hashish are among the most widely used drugs known to man. Uses of the drugs include: treatment of pain and inflammation; lowering of intraocular pressure in glaucoma; relief from nausea associated with cancer chemotherapy; appetite stimulation; decreasing intestinal motility (diarrhea); and relief from muscle spasms, epilepsy, and asthma. The drugs are also used as antirheumatics and antipyretics (Hollister, 1984). In the United States, marijuana is widely used. The social and medicinal uses of marijuana date back at least 4,000 years (Zias *et al.*, 1993).

Marijuana and cannabinoids have been classified in the U.S. as Schedule I substances under the Drug Abuse Prevention and Control Act. This classification is for compounds that currently have no accepted medical uses in the United States but have high abuse potential and/or safety risks associated with their use.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

In cats, peak plasma concentration (1.10 µg/mL) of THC was reached 1 hour after receiving a single oral dose of 2 mg/kg (McCarthy *et al.*, 1984). Peak plasma concentration of THC was reached in 4 hours in rats after an oral dose of 50 mg/kg (Abel and Subramanian, 1990). The peak plasma level of THC in rats was maintained for at least 24 hours (Bronson *et al.*, 1984). The plasma half-life of THC in dogs is 8 days, in rabbits 2 to 4 days, and in rats 5 days (Agurell *et al.*, 1986).

In plasma, approximately 97% to 99% of THC and its metabolites are bound to lipoproteins and albumin (Dewey, 1986). Two hours after rabbits were administered an intraperitoneal dose of ^{3}H-THC, the greatest amount of radiolabel was found in the kidney, urine, and bile, with lesser amounts detected in the lung and liver, and still smaller amounts in the adrenal glands, spleen, and ileum. The least amount of radiolabeled compound was detected in the brain and spinal cord. Compared to the levels in other tissues, the quantity of radiolabel had increased in adipose tissue 72 hours following administration of the dose to rabbits (Agurell *et al.*, 1970). THC is lipophilic and is accumulated in higher concentrations in the adipose tissues than in other tissues (Lemberger and Rubin, 1976; Bronson *et al.*, 1984). Preferential accumulation of THC has been observed in the gonadal fat tissues of male and female mice (Rawitch *et al.*, 1979). THC has been shown to cross the placenta and enter the fetuses of experimental animals (Kennedy and Waddell, 1972), and to transfer to suckling young via the milk of rats and monkeys (Jakubovic *et al.*, 1973; Chao *et al.*., 1976).

THC is metabolized primarily by the micsomal enzymes which are inducible (Burstein and Kupfer, 1971; Okamoto *et al.*, 1972). The isozyme involved in metabolizing THC has been identified and purified from male mice and has been designated as P_{450} MUT-2 which belongs to the P_{450} 2C subfamily (Watanabe *et al.*, 1993).

There are qualitative differences in the metabolism of THC between different organs in an animal. For example, in the rat liver the predominant metabolite is the 11-hydroxylated THC, whereas in the lung it is the 6œ-hydroxy-THC (Agurell *et al.*, 1986). There are also species differences in the biotransformation patterns of THC. In mice (strain unspecified) 6œ-hydroxylation is dominant over ß-hydroxylation, whereas in man the reverse is true (Agurell *et al.*, 1986). A general outline of the major metabolic and excretory pathway is shown in Figure 1.

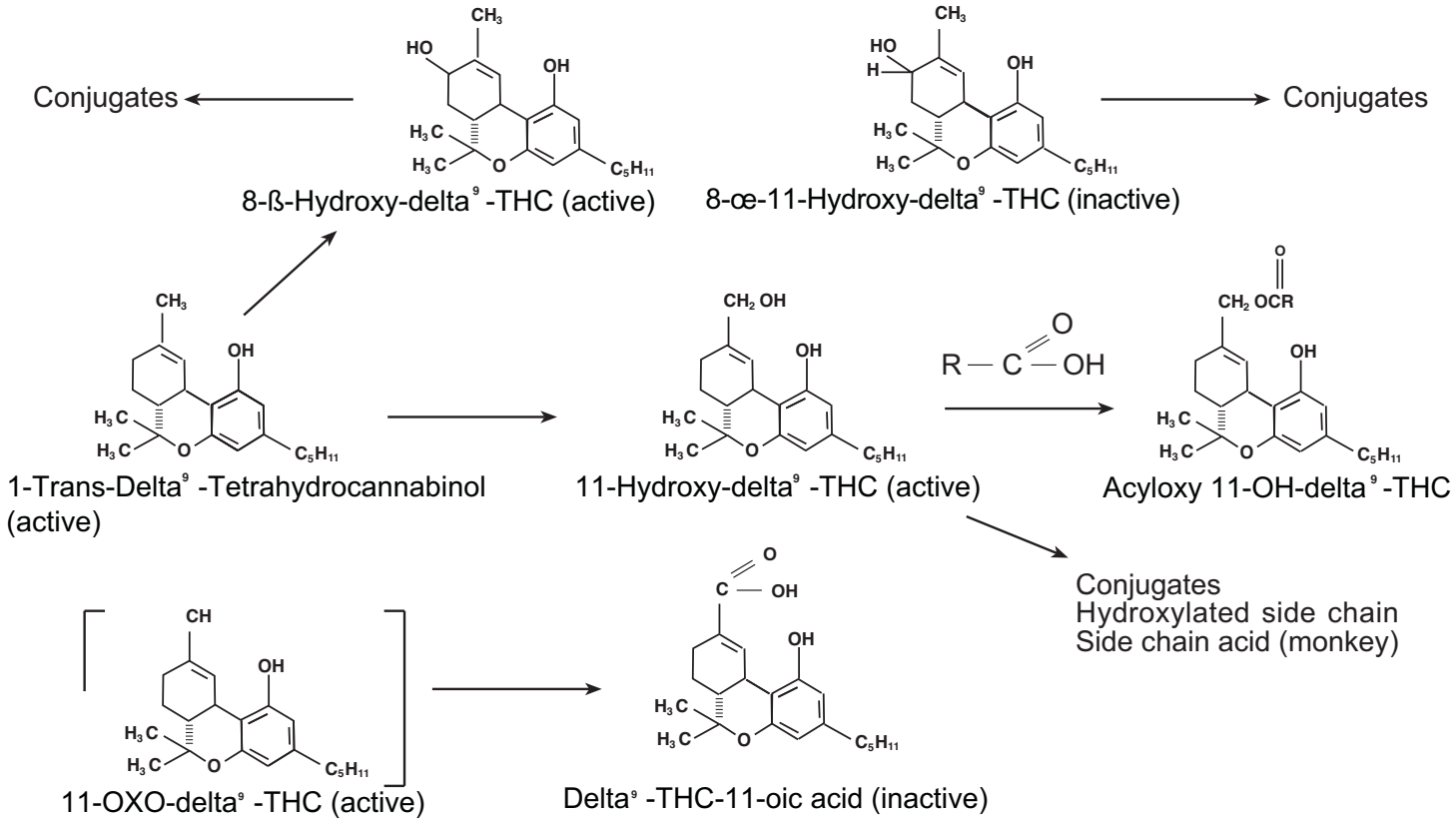


FIGURE 1
Major Metabolic and Excretory Pathway of 1-Trans-delta⁹-tetrahydrocannabinol

The pharmacokinetics of THC are not dose dependent, but are characterized by rapid disappearance of the compound from plasma and a much longer period during which THC can be detected in various body tissues. The compound remains discernable due to continuous penetration into and return from numerous body compartments. The half-life of THC in the tissues is 7 days. Complete elimination of a single dose of THC may take more than a month. THC administered daily is continuously accumulated in the body (Nahas, 1979).

In a study by Lemberger and Rubin (1976), the plasma half-life of THC in a person not previously exposed to the compound was 56 hours, whereas the half-life in a chronic marijuana smoker was only 27 hours. The shorter half-life of THC in a chronic marijuana user is probably due to higher metabolic enzyme activities. However, Hunt and Jones (1980) and Ohisson *et al.* (1982) found no significant differences in plasma profiles for chronic, moderate, and infrequent users; the half-life in all subjects studied was about 20 hours. There is no evidence that chronic THC administration alters disposition or metabolism of THC in the brain and peripheral tissues.

In humans, as in most mammalian species, the biotransformation pathway for cannabinoids is hydroxylation occurring at several positions. The most prominent pathway is hydroxylation at the allylic positions (C-8 and/or C-11).

The biological activity of THC may be largely attributable to the 11-hydroxy metabolite (Watanabe *et al.*, 1990). Hydroxylation at C-8 diminishes the biological activity. The dihydroxylated metabolites are also less potent than the parent compound (Dewey *et al.*, 1984). A trace amount of 9œ,10œ-epoxyhexahydrocannabinol has been reportedly formed in human liver (Halldin *et al.*, 1982), but the biological significance of the epoxide is not known. The secondary metabolism of THC involves glucuronidation of the carboxylic and phenolic groups. Following a single oral dose of THC in a human, the metabolites, in the form of free acid and glucuronide conjugates, are detected in the blood for up to 5 days (Law *et al.*, 1984). The glucuronides are stored in body fat for a long period of time and can be detected in urine several weeks after exposure (Mechoulam *et al.*, 1992). Generally, the poly-hydroxylated compounds, the 11-oic acids (11-nor-acids) and their conjugates, are excreted in the bile and urine. However, the biliary-excreted metabolites (the acids and the hydroxylated compounds and conjugates) were enterohepatically recirculated, which contributed to their persistence in the body (Lemberger, 1972). Biliary excretion via the feces is the major route of THC excretion. About 40% to 50% of administered THC is excreted in the feces within 5 days. Fecal metabolites are nonconjugated and contain both acidic and neutral metabolites.

Urinary excretion is a minor route of elimination of THC. In humans, about 13% to 16% of administered THC is found as metabolites in the urine within 72 hours (Well *et al.*, 1984). However, the metabolites of orally administered THC can be detected in the urine for up to 12 days (Law *et al.*, 1984). The urinary metabolites are excreted as glucuronide conjugates. The major urinary metabolite is 11-nor-THC-9-carboxylic acid (THC-COOH). Only trace quantities of neutral cannabinoids are found in the urine.

MECHANISM OF ACTION

Many of the characteristic motor and cognitive effects of THC have been shown to be mediated via a G-protein-coupled receptor (Pertwee, 1993). How- ever, since the effects of THC are diverse, many of the effects may be mediated via other mechanisms. There is evidence for the existence of THC receptors. The double bond in the 9/10 position of the A ring in the THC molecule is essential for activity, suggesting a structure-activity relationship. Interactions with binding sites and second messenger systems and the behavioral effects of cannabinoids are determined by the structural and geometric features of the cannabinoid molecules, indicating chemical selectivity and stereoselectivity.

Additionally, the potency of THC is similar to those of other types of drugs known to act through receptors, and large amount of specific, high-affinity cannabinoid binding sites in the brain have been detected through the use of antagonists (Pertwee, 1993). THC inhibits adenyl cyclase activity in a dose-dependent, reversible, stereoselective and pertussis toxin-sensitive manner, suggesting a signal transduction mechanism (interaction with second messenger systems, cAMP and calcium). Finally, a functional cannabinoid receptor (SKR6) has been cloned, and the specific binding sites in rat and human brain tissues have been identified. The identification of an endogenous cannabinoid named anandamide has strengthened the concept of the existence of cannabinoid receptors (Devane *et al.*, 1992). As a result, the idea that cannabinoids react nonspecifically with membrane lipids has been rejected (Pertwee, 1993).

Binding of THC to the receptor activates G protein to stimulate phospholipase A2 or C to hydrolyze phospholipids and release arachidonate. The release of free arachidonic acid is followed by the synthesis of eicosanoids, including prostaglandins, leukotrienes, and 5-hydroxyeicosatetraenoic acid. Many of the biological effects of THC (i.e., sedation, catalepsy, antiinflammation, analgesia, hypothermia) have been shown to be mediated via eicosanoids. For example, it has been demonstrated in rodents that sedation or catalepsy induced by THC was mediated via an increase in prostaglandin E2 in brain tissue and in plasma (Burstein, 1992).

TOLERANCE

Tolerance to THC can be divided into 2 types: pharmacokinetic and pharmacodynamic. Pharmacokinetic tolerance refers to changes in absorption, distribution, metabolism, or excretion; the changes lead to a reduction of the active form of the chemical at the site of action. Pharmacodynamic tolerance is the result of adaptational changes in the brain. Tolerance to THC is believed to be pharmacodynamic in nature (Pertwee, 1988). After repeated THC exposure, tolerance to THC in humans and animals develops (Dewey, 1986; Pertwee, 1991). The rate of onset and degree of tolerance is governed by dose and exposure frequency. Tolerance does not develop when doses are small or infrequent and the exposure duration is short. Tolerance to some chemical effects (cataleptic, ataxic, convulsive and anticonvulsive, hypothermic, hypotensive, anti- nociceptive, immunosuppressive, alterated response rates and accuracy of schedule-controlled behaviors) appears to develop more readily, or the effect may be reversed (e.g., hypothermia becomes hyperthermia [Pertwee, 1991; Abood and Martin, 1992]). Lemberger *et al.* (1971) showed that labeled THC administered intravenously had a half-life of 28 hours in the plasma of chronic marijuana users and 57 hours in the plasma of non-users. However, these results have not been confirmed by other investigators (Agurell *et al.*, 1986). Daily intramuscular administration of THC to rhesus monkeys disrupted menstrual cycles, ovulation, and cyclic serum sex hormone levels during the first few months of administration. Alter that, normal menstrual cycles and serum estradiol, progesterone, and prolactin levels resumed (Smith *et al.*, 1983). Because there are no differences in the metabolism or disposition of cannabinoids in THC-tolerant animals, tolerance could develop as a result of functional changes (loss of sensitivity to the effects of THC) (Agurell *et al.*, 1984; Dewey, 1986; Pertwee, 1991).

Neurochemicals such as acetyicholine, dopamine, 5-hydroxytryptamine, opioids, and prostaglandins may play a role in the development of tolerance (Pertwee, 1991). Smith *et al.* (1983) have suggested that tolerance is due to adaptation of neural mechanisms in the hypothalamus.

One cellular mechanism for tolerance is the down- regulation of receptors. Eldridge *et al.* (1991) demonstrated that rats repeatedly exposed to THC had decreased numbers of receptors in the brain, resulting in reduced sensitivity to THC. The amounts of THC bound in all structures measured in selected striatal levels of rat brain were reduced following daily intraperitoneal administration of THC at 10 mg/kg for 14 days; the levels in the medial septum/diagonal band were reduced 32%, and those

in the lateral caudate-putamen were reduced 52% (Oviedo *et al.*, 1993). The change in the amount of THC bound was a result of a loss of binding capacity (Bmax) rather than a change in affinity (1(D) (Oviedo *et al.*, 1993). Westlake *et al.* (1991) demonstrated that there were no irreversible changes in brain cannabinoid receptor populations in rats following their exposure to THC for 6 months and in monkeys after exposure to marijuana smoke for one year.

In a study by Dill and Howlett (1988), tolerance developed through the uncoupling of receptors from their second messenger systems. When N18TG2 neuroblastoma cells were exposed to THC, a loss of cannabinoid-mediated inhibition of adenylate cyclase activity occurred. The attenuation process is time- and dose-dependent and reversible (Dill and Howlett, 1988).

BIOLOGICAL EFFECTS

Experimental Animals Neurobehavioral effects of THC in experimental animals include central nervous system stimulation and depression, aggressiveness (rats), “popcorn effects” (mice), static ataxia (dogs), corneal areflexia (rabbits), and overt behavior (monkeys).

In squirrel monkeys injected intravenously with THC (2 to 30 mg/kg), an euphoric, quiet effect with disruption of perception was observed at low doses, stimulation and lack of coordination at medium doses, and severe psychomotor incapacitation at higher doses (Mclsaac *et al.*, 1971). Rhesus monkeys fed THC displayed increased irritability and aggressiveness (Nahas and Paton, 1979). In cats, a single intravenous, oral, or intramuscular 800 µg/kg dose of THC produced marked ataxia, vocalization, excitement, and pronounced startle behavior (McCarthy *et al.*, 1984). In rats fed up to 50 mg THC/kg body weight for 6 months, behavioral alterations varied with the time interval of treatment. Central nervous system depression, which included symptoms of ataxia, incoordination, decreased exploratory activity, and general depression, was observed initially. Prolonged treatment led to tolerance development and central nervous system stimulation symptoms which included irritability, hypersensitivity, hyperactivity, aggression, tremors, and convulsions (Luthra *et al.*, 1975). The animals also exhibited impaired specific motor and learning skills and unusual aggressive behavior toward smaller rodents (Nahas and Paton, 1979). Mice treated with a single dose of THC showed increased aggression (Leuschner et at., 1984). In monkeys exposed to marijuana via a smoking machine for 3 to 6 months, permanent brain wave changes were observed in the limbic structures that control emotion, pleasure, endocrine function, and memory storage. The brain wave changes consisted of irritative tracings with high amplitude waves or spikes. Ultrastructural examination showed that histopathologic alterations occurred in the hippocampus of the brain in monkeys exposed to 0.69 mg THC/kg body weight intravenously daily for 6 months. The histopathologic changes included presence of electron-opaque material and clumping of synaptic vesicles in the synapse, fragmentation and disorganization of the rough endoplasmic reticulum, and inclusion bodies in the nuclei. The changes persisted during an 8-month postexposure period (Heath *et al.*, 1980). Oral treatment with THC (50 mg/kg for 180 days) depressed brain acetylcholinesterase activity in females rats, but the enzyme activity was elevated in male rats (Luthra *et al.*, 1975).

THC appears to affect all major neurotransmitter systems (release and/or uptake) including the cholinergic, dopaminergic, adrenergic, serotonergic, and GABAergic systems (Martin, 1986; Pertwee, 1988). THC appears to act presynaptically to alter neurotransmitter synthesis, storage, release, and fate, and postsynaptically to alter neurotransmitter receptor- mediated events both at the level of the recognition site and at the level of second messenger systems. Evidence has been presented to demonstrate that THC affects memory via the hippocampal acetylcholine-releasing neurons, locomotor activity via dopamine-releasing neurons of the nucleus accumbens, catalepsy via the dopamine-releasing neurons of the striatum, and convulsions via y-amino- butyric acid turnover in the septum and substantia nigra (Pertwee, 1988). However, the causal relation- ships between the THC-

induced changes in neurotransmitter uptake/release and psychological, behavioral, physiological, and neuropharmacological changes in an animal remain to be established.

THC is a potent hypothermic agent. Dose-related hypothermia effects in mice were reported at doses of 5 to 100 mg/kg (Dewey, 1986). The hypothermic effect may be mediated in part by depressing thermogenesis at centers in the caudal brain stem, as well as by an action at the thermosensory neurons in the anterior hypothalamus and preoptic area (Schmeling and Hosko, 1980; Fitton and Pertwee, 1982; Howlett *et al.*, 1990). The effect may also be mediated by interference of neurotransmitter uptake and release, possibly by inhibition of the membrane-bound adenosine triphosphatase (ATPase) associated with synaptosomes or synaptic vesicles. Certain prostaglandins are known to produce hyperthermia. THC has been shown to reduce prostaglandin production in the hypothalamus (Martin, 1986). Holtzman *et al.* (1969) reported that change in brain levels of 5-hydroxy- tryptamine following THC administration correlated with the duration of hypothermia in mice. Others have reported that the hypothermic effect of THC did not correlate with the change in brain 5-hydroxy- tryptamine levels (Watanabe *et al.*, 1984) and suggested that the effect is mediated by catecholamines (Yagiela *et al.*, 1974; Singh and Das, 1976). Serotonergic mechanisms have also been suggested (Dewey, 1986). Thus, further work is required to understand the mechanism involved in hypothermia.

THC interacts with the nuclear membrane of cells and causes changes of membrane configuration leading to conformational changes of membrane bound transport and enzyme systems. This interaction causes interference with the synthesis of nucleic acids and proteins. Inhibition of the synthesis of DNA, RNA, and protein has been reported in cultured lymphocytes from THC-treated monkeys, rats, guinea pigs, and mice. Various unicellular organisms, cultured malignant cells, and human lymphocytes exposed to THC *in vitro* have also showed suppressed macromolecular synthesis (ARF/WHO 1981, Desoize *et al.*, 1991). For example, growth of transplantable Lewis lung adenocarcinoma *in vivo* was retarded by oral administration of THC (25, 50, or 100 mg/kg daily for 10 days) in a dose-dependent fashion (Munson *et al.*, 1975). The mechanism may involve dissolution of cell membrane by THC, which would prevent the transport of precursors for DNA, RNA, and protein synthesis and inhibition of acetylation and phosphorylation of chromosomal proteins. Finally, the mechanism would prevent the transcription of DNA (Nahas and Paton, 1979). The inhibitory effect of macromolecular synthesis has important implications because of the possible impact on the rapidly proliferating cells of the immune system, the intestinal mucosa, and the cells involved in spermatogenesis and fetal development. THC also inhibits ATPases, adenylate cyclase, monoamine oxidase, and a number of enzyme systems *in vivo*

(Martin, 1986). The general inhibitory effect of THC on enzymes implies perturbation of cell membranes; however, the precise biochemical process involved is not clear (Martin, 1986). The inhibitory effect on adenylate cyclase is similar to that produced by hormone-receptor interactions.

Humans Neurobehavioral effects of THC in humans include: euphoria, tranquility, difficulty in thinking or remembering, rapid flow of thoughts, dreamy states, impairment of short-term memory consolidation, altered perception of visual or auditory stimuli, and distortions in duration of time. An acute dose of marijuana induces changes in mood, perception, judgment, memory, and psychomotor coordination. These changes can include anxiety, panic, paranoia, disorientation, catatonia-like immobility, mixed anxiety and sedation, euphoria, and impaired short-term memory. The effect peaks immediately after smoking and lasts for 2 to 3 hours after a single cigarette. With oral doses, the onset of behavioral effects is delayed, and the effects last longer. Chronic effects of marijuana on behavior have also been described, but quantitative data are lacking (Comm. Inst. Med., 1982).

Discussion of Biological Effects Across Species Herkenham *et al.* (1990) first identified the stereo-specific THC receptors on brain slices sampled from rat, rhesus monkey, and man. The pattern of

binding is conserved across these species. The greatest density of receptors is observed in the globus pallidus, the substantia nigra pars reticulata, the molecular layer of the dentate gyrus of the hippocampus, and the cerebellar molecular layer. Binding at these sites appears to correlate well with behavioral alterations. There is evidence suggesting that the frontal cortex is the site where incoming information is processed and where voluntary somatosensory stimuli required for equilibrium and motor coordination are initiated. The hippocampus is the site for memory transfer and consolidation (Rawlins, 1985). Its also codes temporal and spatial relations between stimuli and responses (Eichenbaum and Cohen, 1988). The limbic area is involved in short memory recall, and the basal ganglia in cataleptic response.

THC appears to affect all major neurotransmitter systems (release and/or uptake) including the cholinergic, dopaminergic, adrenergic, serotonergic, and GABAergic systems *in vitro* (Martin, 1986; Pertwee, 1988).

In humans, THC use is associated with moderately strong cardiovascular effects. Oral doses of THC of 70 to 210 µg per day in humans initially produced tachycardia (increased heart rate, decreased standing blood pressure, and increased supine blood pressure). THC appears to act on the alpha-adrenoceptors that are located in the neighborhood of the cerebral ventricle and cause increased sympathetic outflow in the accelerans nerves (Graham, 1986a,b). But tolerance developed after repeated doses of THC and tachycardia became bradycardia (Jones and Benowitz 1976; Perez-Reyes *et al.*, 1991). In contrast, in most other mammalian species the response to acute exposure to marijuana or THC is bradycardia (Vollmer *et al.*, 1974; ARF/WHO, 1981); tolerance to this effect also developed after prolonged exposure (Adams *et al.*, 1976).

Because THC and steroids have similar chemical structures and physical properties and the brain appears to have receptors for all five classes of steroids (estrogens, progestins, androgens, glucocorticoids, and mineralocorticoids), many THC effects may be exerted at steroid hormone target sites (Martin, 1986).

THC exerts an inhibitory effect on the hypothalamo-pituitary-gonad axis. The effect disturbs the synthesis and secretion of follicle stimulating hormone, luteinizing hormone, prolactin, thyroxin, testosterone, estrogen, and progesterone, which in turn affects the maturation of germ cells and ovarian function. Conversely, THC elevates serum adrenal cortical steroids levels. Chronic exposure to THC significantly suppresses plasma levels of prolactin in male and female rats and rhesus monkeys (Chakravarty *et al.*, 1975; Kramer and Ben-David, 1978; Asch *et al.*, 1979). Smoking of marijuana cigarettes also significantly lowers plasma prolactin levels during the luteal phase of menstrual cycles in women (Mendelson *et al.*, 1985). The inhibitory effect of THC on prolactin release was observed only during the morning of estrus in female rats (Bonnin *et al.*, 1993). The effect was mediated through increases in the activity of tuberoinfundibular dopaminergic (TIDA) neurons and the sensitivity of the anterior pituitary gland to dopamine (Bonnin *et al.*, 1993). THC may also act directly on the anterior pituitary by antagonizing the effects of estradiol on anterior pituitary prolactin release in immature female rats (Murphy *et al.*, 1991, in Bonnin *et al*, 1993).

THC inhibits the midcycle luteinizing hormone surge and resultant ovulation in female rats (Ayalon *et al.*, 1977), rabbits (Asch *et al.*, 1979), and rhesus monkeys (Asch *et al.*, 1981). Smith *et al.* (1980a) reported that plasma luteinizing hormone and follicle stimulating hormone in castrated rhesus monkeys fell significantly following acute administration of THC. However, luteinizing hormone release was stimulated by luteinizing hormone-releasing factor and prolactin release was stimulated by thyrotropin-releasing hormone in the castrated monkeys. The experiment demonstrated that THC acts at the hypothalamus. THC alters the output of gonadotropin in the pituitary resulting in decreased estrogen activity. Direct interaction of THC with estrogen receptors to produce an estrogenic or

antiestrogenic effect has been suggested, but evidence shows that THC was unable to compete with estradiol in binding to cytosol from monkey and human uteri (Martin, 1986). Direct inhibitory effects on progesterone and estradiol production in rat granulosa cells (Adashi *et al.*, 1983) and isolated graafian follicles (Reich *et al.*, 1982) and on progesterone production in rat luteal cells (Lewysohn *et al.*, 1984) have been demonstrated. Treinen *et al.* (1993) showed that the inhibitory effect of THC on steroidogenesis by granulosa cells was due to inhibition of follicle stimulating hormone-activated cAMP accumulation.

A single oral dose (10 mg/kg) of THC significantly decreased serum testosterone levels in male rats (Rosenkrantz and Esber, 1980) and in men (Martin, 1986). The effect may be due primarily to the inhibition of gonadotropin secretion from the pituitary. However, chronic treatment with THC did not reduce serum testosterone in monkeys (Smith *et al.*, 1980b) or in men (Martin, 1986). THC may also have a direct action at the gonadal level. Lactate and transferrin secretions in rat Sertoli cells are stimulated when testicular tissue is exposed to THC *in vitro* (Newton *et al.*, 1993). THC also modulates

Sertoli cell response to follicle stimulating hormone. Reduction in steroidogenesis, protein and nucleic acid synthesis, glucose utilization, lactate and transferrin secretion, y-glutamyl transpeptidase activity, and cyclic AMP levels have also been demonstrated.

Acute treatment with THC (2 to 20 mg) in rats and mice produced a prompt rise in serum corticosteroid levels, presumably caused by the action of THC on the hypothalamus and pituitary gland to increase adrenocorticotropic hormone. The response was muted after repeated treatment for 7 or 14 days (Eldridge *et al.*, 1991). However, chronic treatment of rats and mice with THC led to increased adrenal weight. The adrenal cortical response to cannabinoids could not be demonstrated in other species, including humans. THC-mediated simulation of pituitary adrenocortical hormone secretion has been suggested to account for many of the behavioral, electroencephalographic, and pharmacologic actions of THC in animals (Drew and Slagel, 1973).

An oral dose (10 mg/kg) of THC for 14 days significantly decreased serum thyroxine and triiodothyronine levels in male rats (Rosenkrantz and Esber, 1980). The effect was not thought to be on the pituitary or the thyroid glands since the same dose of THC did not alter thyrotropin-releasing hormone in these animals. Prolonged administration of THC may promote thyroid hyperplasia.

THERAPEUTIC EFFECTS

THC has been shown to have antiemetic properties. The antiemetic effect may result from actions affecting the vomiting center in the brain stem or affecting connected structures such as the amygdala and neocortex that modulate the activity of the vomiting center (Howlett *et al.*, 1990). Because the mechanism of action by which THC exerts its effect is not understood, the use of THC as an antiemetic is problematic. There are questions about its efficacy against a broad range of therapeutic regimens, and there are reservations about its neurobehavioral side effects. Recently, interests on the antiemetic properties of THC are waning as use of antagonists to the 5-hydroxytryptamine receptor, 5-HT3, has become more widespread (Iversen, 1993). THC has been used to reduce intraocular pressure in the treatment of glaucoma. However, no definitive

evidence is available to explain the alteration of intraocular pressure by THC (Martin, 1986). THC may act as a vasodilator and cause a decrease in capillary pressure within the ciliary body, or the effects may be related to reduction of prostaglandins in the eye (Martin, 1986). The use of THC to treat glaucoma is impractical because it cannot be applied topically due to its insolubility in water and because of its psychoactive properties when given systemically. The use of B-adrenoceptor

blockers or pilocarpine to treat glaucoma has diminished the interest in THC as a therapeutic agent for reducing intraocular pressure (Iversen, 1993).

THC has been shown to have bronchodilating action, but very little is known about its mechanism of action. Inhibition of prostaglandin synthesis has been suggested (Martin, 1986).

THC has been shown to be antinociceptive in experimental animals in the tail flick, hot plate, Nilsen, acetic acid or phenyiquinone writhing tests, and pinch tests (Segal, 1986), but the effect is less potent than that of morphine (Dewey, 1986). The interest in the antinociceptive effect of THC is because the chemical does not induce physical dependence. Reports on the anti-inflammatory, analgesic, and antipyretic activity of THC are confusing (Dewey, 1986). THC appears to interact with a prostaglandin receptor coupled to adenylate cyclase to inhibit cAMP formation while producing the antinociceptive effect. The anti- inflammatory and antinociceptive effects of THC may be mediated via a prostaglandin pathway. However, further studies are needed to determine its action.

THC depresses feed consumption in rats in a dose- related manner (Dewey, 1986), but tolerance develops after repeated exposure. Conversely, THC has been recommended for stimulating appetite in cancer and acquired immune deficiency syndrome patients (Plasse *et al.*, 1991). The mechanism of appetite stimulation by THC is not clear.

THC induces convulsions in rabbits and mice (Martin and Consroe, 1976; Karler *et al.*, 1986; Turkanis and Karler, 1984). However, THC has also been shown to be an anticonvulsant (Fried and McIntyre, 1973; Karler *et al*, 1974, 1986; Corcoran *et al.*, 1978). Pertwee (1988) has postulated that the anticonvulsant property of THC is due to its inhibition of de- polarization-dependent Ca” uptake into brainstem synaptosomes.

TOXICITY

Experimental Animals

Signs of acute toxicity in rats include hypothermia, bradypnea, rapid weight loss, inactivity, wide stance, ataxia, muscle tremors, diarrhea, lacrimation, hyper- excitability, depression, loss of righting reflex, prostration, and dyspnea progressing to respiratory arrest. Deaths in animals after acute doses of THC are usually due to cardiac arrest or respiratory failure, and cardiac dysfunction is considered to be the major cause of death. The toxic signs disappear in 24 hours in the surviving animals. Table 1 presents LD_{50} and LC_{50} values for rat, mouse, and monkey studies.

Animals receiving chronic doses of cannabinoids exhibited behavioral changes characterized by hyper- activity, vertical jumping, fighting, and seizures (Rosenkrantz and Esber, 1980). Delayed lethality has been reported in animals receiving repeated high doses of THC, likely related to the cumulative effects of THC and/or its metabolites accumulated in the tissues (ARF/WHO, 1981).

Humans

In humans, acute toxic effects include depression of the brain reticular system and the primary sensory pathways, disorientation, dissociation of personality, euphoria, emotional excitement, uncontrolled laughter, hallucinations, illusions, distortion of the sense of time and space, increased sensitivity to sound, loss of motor control and paresthesia. Acute and subacute doses of cannabis may produce vomiting, diarrhea, and abdominal distress (ARF/WHO, 1981).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Chronic oral doses of cannabinoids induced testicular atrophy in rats (Thompson *et al.*, 1973). The weights of the ventral prostate, seminal vesicle, and epididymis were reduced in adult rats given marijuana extracts. Rats administered marijuana via inhalation through a smoking machine had significantly lower sperm counts and an increased number of abnormal sperm compared to controls; specifically, the abnormalities included dissociation of sperm heads and tails (Huang *et al.*, 1979). Treatment of mice with THC produced reversible cytolytic changes in the testes (Dixit *et al.*, 1974) and induced increased number of ring and chain translocations in primary spermatocytes (Zimmerman *et al.*, 1979).

In vitro studies of cannabinoids on rat testicular cells and testicular slices support that THC acts

TABLE 1
LD_{50} and LC_{50} Values of 1-Trans-Delta^9-Tetrahydrocannabinol in the Rat, Mouse, and Monkey

Species	Route of Administration	LD_{50}/LC_{50}	Reference
Rat	Intravenous	40 mg/kg	Rosenkrantz, 1982
	Oral	666 mg/kg	Phillips et al., 1971
	Inhalation	36-42 mg/kg	Nahas, 1979
	Intraperitoneal	372 mg/kg	Phillips et a!., 1971
Mouse	Intravenous	43 mg/kg	Rosenkrantz, 1982
	Oral	482 mg/kg	Phillips et a!., 1971
	Inhalation	40-60 mg/kg	Nahas, 1979
	Intraperitoneal	454 mg/kg	Phillips et al., 1971
Monkey	Intravenous	125 mg/kg	Rosenkrantz, 1982

Inhibition of ovulation, prolongation of estrous cycles, decrease of uterine and ovarian weights, and reduction in size of primordial ova by cannabinoids have also been observed in experimental animals (Dixit *,* 1975; Fujinioto *et al.*, 1979; Smith *et al.,* 1979). Female mice treated with THC had a higher incidence of abnormal fertilized ova (Henrich *et al.*, 1983). THC given intraperitoneally to 27-day-old female rats twice daily at 10 mg/kg body weight retarded sexual maturation; the appearance of estrus and ovulation was delayed (Field and Tyrey, 1984). The effects of cannabinoids on the female reproductive system are probably due to their inhibitory actions on the hypothalamus and the subsequent effects on follicle stimulating hormone, luteinizing hormone, and prolactm releases by the pituitary. Specifically, THC has been shown to inhibit the midcycle luteinizing hormone surge and the resultant ovulation, progesterone, and estradiol production in rat granulosa cells. THC has also been shown to compete with estrogen for estrogen receptors, but the data have not been confirmed (Purohit *et al.,* 1980).

The changes observed in sperm number and motility, endocrine profiles, and menstrual/estrous

cycle after exposure to THC are expected to influence fertility. Dalterio *et al.* (1982) reported reduced fertility, increased pre- and post-natal fetal death, and reduced litter size among offspring of male mice treated with high doses of cannabis, but Wright *et al.* (1976) reported that subchronic or chronic treatment of rodents with THC had no effect on fertility, results that may have been related to the dosage used in the studies.

Cannabinoids are embryotoxic. Treatment with cannabinoid during the first two-thirds of gestation is associated with increased frequency of fetal resorption and decreased birth weights in mice, rats, rabbits, and hamsters (ARF/WHO, 1981). Body weight at birth and fetal resorption rates were dose related. Abel *et al.* (1981) demonstrated by pair feeding that neonatal mortality and intrauterine growth retardation were not due to maternal undernutrition resulting from marijuana or THC treatment. Pregnant rabbits given marijuana extracts had small placentas. In pregnant rodents treated with THC, the greatest concentration of the chemical was observed in the mother, a smaller concentration in the placenta, and still less was observed in the fetus. THC retention in the placenta appears to serve as a barrier against THC transfer to the fetus, but the absorbed THC in the placenta disrupts placental development or function (Sassenrath *et al.,* 1979) and may contribute to abnormal fetal development and absorption. Hutchings and Dow-Edwards (1991) observed that dams treated with THC gave birth to more male offspring than female and postulated that THC may be selectively lethal to female embryos. Female rhesus monkeys treated chronically with THC and mated with untreated males had incidences of abortion and neonatal mortality four times greater than those in the control group (Sassenrath *et al.*, 1979). Rosenkrantz and Esber (1980) demonstrated that THC altered serum follicle stimulating hormone and estrogen levels in pregnant rats. It is possible that the embryotoxicity evoked by THC is due in part to hormonal imbalance in the dams. The hormonal changes may also interfere with sexual differentiation of the fetuses (Dalterio and Bartke, 1981).

Animal studies have shown that marijuana extracts and THC are teratogenic. Pregnant hamsters and rabbits given marijuana extracts had embryos with malformations in the brain, spinal cord, forelimb, and liver (Gerber and Schramm, 1969). The offspring of pregnant mice administered THC (240 mg/kg) at critical stage of development (days 12 and 13) had significantly high incidences of cleft palate (Bloch *et al.,* 1986) and exencephaly (Joneja, 1976), but abnormalities were not found in the fetuses when the pregnant mice were administered THC at 150 mg/kg during the 6th to 15th days of pregnancy (Fleischman *et al.,* 1975). Morishima (1982) observed that 48-hour-old embryos from THC-treated mice contained abnormalities resulting from segregation errors and concluded that THC acts by disrupting the mitotic apparatus of the embryonic cells.

No behavioral teratogenicity was observed in rats exposed to THC *in utero* (Abel, 1984). However, offspring of treated female rhesus monkeys reportedly present anomalies of behavior and neuroendocrine function (Nahas, 1979).

Animal studies have repeatedly shown that cannabinoids cause a reversible reduction in body weight gain. This is likely due to decreased feed consumption and altered endocrine function (ARF/WHO, 1981). The body weights return to normal levels after THC treatment is stopped. Postnatal growth of rats exposed to THC *in utero* (dams administered

50 mg/kg throughout gestation) was normal when the rats were nursed by surrogate mothers, despite lower body weights at birth (Abel *et al.,* 1981). However, Luthra (1979) reported that the offspring of rats treated during gestation and lactation with 5 or 10 mg/kg THC showed decreases in brain protein, RNA, and DNA.

Humans

Marijuana or THC appear to affect all phases of reproduction in males and females, including serum sex hormone levels, weight and functions of reproductive organs, and fetal development. These effects on reproduction may be due to the action of cannabinoids directly on the reproductive organs, indirectly by altering serum sex hormone levels, or a combination of both.

Chronic marijuana smokers had a significant decline in sperm concentration and total sperm count; sperm motility also decreased (Hembree *et al.,* 1991). The sperm nuclei of hashish users showed abnormal staining characteristics (Stefanis and Issidorides, 1976). An oligospermia associated with abnormal forms and a decrease in spermatozoa motility were observed in 16 young marijuana smokers inhaling THC in 8 to 20 cigarettes per day for 5 to 6 weeks. Because hormonal suppression of spermatogenesis normally takes longer than 4 weeks to achieve and is usually not accompanied by an increase in abnormal sperm or a decrease in sperm motility, the authors concluded that the impairment was not due to hormonal changes but due to a direct effect of the cannabinoids on the seminiferous germinal epithelium (Hembree *et al.,* 1991).

In females, THC or marijuana has been shown to block ovulation and disrupt the menstrual/estrous cycles. A group of young women smoking marijuana at least three times weekly had an increased incidence of abnormal menstrual cycle (Nahas, 1979).

In humans, *in utero* exposure to marijuana has been reported to be associated with voice anomalies, short stature, low body weight, decreased head circumferences, and decreased verbal and memory scores in infants and children (Nahas, 1993). There is no information on long-term effects of marijuana or THC on growth and body weight in humans.

IMMUNOTOXICITY

Experimental Animals

THC induces immunological defects, including: elongation of allogenic skin graft survival; reduction of primary antibody production against sheep red cells and number of plaque-forming cells (Schatz *et al.,* 1993); suppression of mouse blood lymphocyte blastogenesis and splenocytes in response to plant mitogens or bacterial antigens (Friedman, 1991, Pross *et al.,* 1993); alteration of delayed hypersensitivity response; involution of the thymus; depression of bone marrow myelopoiesis; perturbations of macrophage structure, function, and mobilization (Levy *et al.,* 1974; Levy and Heppner, 1978; Johnson and Wierseman, 1974; Lefkowitz *et al.,* 1978; Desoize *et al.,* 1981); suppression of phagocytosis and spreading of mouse macrophages *in vitro* (Friedman, 1991); inhibition of natural killer cell activity, interferon production, interleukin 2 (IL-2) production, and ability to respond to Candida albicans and Legionella pneumophita infection of mouse lymphoid cells *in vitro* (Friedman, 1991); and suppression of macrophage extrinsic antiviral activity to herpes simplex virus type 2 (Cabral and Vasquez, 1992). Compared to controls, mice treated with THC were more prone to infection by Listeria monocytogenes or herpes simplex virus (Morahan *et al.,* 1979; Cabral *et al.,* 1986). The immunotoxic effects of THC are considered related to inhibition of macromolecular synthesis in response to external stimuli (Cabral and Vasquez, 1991).

However, the immunosuppressive effects of THC have been observed only at very high dose levels (Levy and Heppner, 1980). In rats, THC induced a dose-dependent increase in serum corticosterone levels (Zuardi *et al.,* 1984). It is possible that certain aspects of immune suppression observed in vivo after THC administration are mediated by increased corticosteroid release.

Humans

Reports on the effects of marijuana on human immune systems are inconsistent. Decreases in T-cell counts, responsiveness to phytohemagglutinin stimulation of lymphocytes, phagocytic activity of polymorphonuclear leukocytes, and macrophage antiviral and antitumor activities have been observed. In addition, impairment of orderly T-cell replication and cytolytic function, suppression of natural killer cell function, and suppression of interleukin-1, interleukin-2, cytokine, and interferon productions have also been reported (Donald, 1991). Conversely, other studies have reported that marijuana smoking has no effect on the human immune system and that hashish was a slight stimulant to the system. The inconsistency probably is due to the variation in doses, history of use, age, and assay systems (Comm. Inst. Med., 1982; Pross *et al.,* 1993).

Recently, a cannabinoid receptor, termed CX5, has been found in the human spleen, tonsils, thymus, and peripheral blood mononuclear and polynuclear leukocytes, suggesting a possible role in inflammatory and immune responses (Boulaboula *et al.,* 1993). The finding implies that THC may be able to modulate immunoresponses via the receptors. It also raises the possibility that the brain and peripheral cannabinoid receptors are different and that the differences can be exploited for medical use (Iversen, 1993).

NEUROTOXICITY

Rats receiving THC initially exhibit signs of central nervous system depression, but central nervous system stimulation is observed as tolerance to the depression develops. The typical signs of neurotoxicity include popcorn response (involuntary vertical jumping reaction), tremors, convulsions, and aggressive behavior (fighting). Cessation of the drug results in symptoms of withdrawal or dependence, with restlessness, irritability, and insomnia.

Alterations in the hippocampus of animals exposed to THC have been reported. Monkeys administered 5 mg/kg body weight per day for 2 months developed altered synaptic width, endoplasmic reticulum alterations, and nuclear inclusions (Heath *et al.,* 1979). Rats receiving THC orally at doses of 40 to 60 mg/kg body weight per day for 60 days had decreased density of neuronal cells, reduction in synapse number, decrease in dendritic length, and increased extra-cellular space (Scallet *et al.,* 1987). Rats administered 8 mg THC/kg body weight per day subcutaneously five times weekly for 8 months also showed decreased neuronal density and increased cytoplasmic inclusion (Landfield *et al.,* 1988).

Young Fischer rats treated subcutaneously with 10 mg/kg THC for 8 months had significantly reduced neuronal density and content of type II glucocorticoid receptors in the hippocampus. The type I glucocorticoid receptors were not affected. The degenerative changes were similar to those seen in older, untreated rats, or in rats treated with high levels of glucocorticoids (Eldridge *et al.*, 1991).

CARCINOGENICITY

Experimental Animals

THC is not structurally related to any known carcinogen. Because the metabolites of THC include allylic alcohols and an epoxide, there may exist some potential for carcinogenicity through the ability of these metabolites to function as alkylating agents.

Rodents exposed to marijuana smoke showed changes in bronchial epithelium (ARF/WHO, 1981) and bronchiolitis and alveolitis with occasional granuloma formations (Fleishman *et al.,* 1979).

Montour *et al.* (1981) reported that radiation carcinogenesis was significantly enhanced by injecting

marijuana extracts three times weekly following gamma irradiation. In this study, whether enhanced carcinogenesis was due to immunosuppression or tumor promotion by marijuana extract is not clear. The effects of THC alone could not be identified.

THC at 0.01 µg/mL transformed Fischer rat embryo cells infected with Raucher leukemia virus after 13 passages. Injection of these cells to newborn Fischer rats produced sarcomas at the injection site (Price *et al.*, 1972). Subcutaneous injection of THC into mice resulted in fibrosarcomas and mammary carcinomas at the site of injection; details of the studies are not available (Szepsenwol *et al.*, 1978; 1980).

Rats treated with THC orally at doses of 2, 10, or 50 mg/kg for 6 months showed no histopathological changes despite reduced growth rate and increased organ/body weight ratio (Rosenkrantz *et al.,* 1975).

Humans

No human epidemiological or case reports associating THC with human cancer have been found in the literature. However, regular use of marijuana reportedly has been associated with cancer of the respiratory tract (Taylor, 1988), upper digestive tract (Donald, 1991), lung (Ferguson *et al.,* 1989), and tongue

(Caplan and Brigham, 1990) in patients under the age of 40. Robison *et al.* (1989) reported increased incidences of leukemia in offspring of mothers who smoked marijuana before or during pregnancy.

Marijuana/hashish smoking has been implicated in chronic degenerative cellular changes in the lung (Abramson, 1974) and in the appearance of foci of bronchiolar ulceration, squamous metaplasia, and pigmented macrophages through much of the lung parenchyma (Morris, 1985).

Cultured human lung tissues exposed to marijuana smoke developed cellular aberrations which included abnormalities in mitosis, DNA complement and chromosomal number, and cellular proliferation (Leuchtenberger and Leuchtenberger, 1984). Dermal application of marijuana smoke condensate resulted in alterations of cell development in the sebaceous glands and in neoplasm formation.

GENETIC TOXICITY

THC has been tested for mutagenic effects in a limited number of assays, but no adverse genetic effects of THC exposure have been convincingly demonstrated. THC was not mutagenic in Salmonella (Stoeckel *et al.,* 1975; Blevins and Shelton, 1983; Zeiger *et al.,* 1988), and no induction of chromosomal aberrations was observed in cultured human lymphocytes (Stenchever and Allen, 1972).

In vivo mammalian studies with THC appear to show conflicting results, but the positive responses reported in the literature are questionable. For example, Zimmerman *et al.* (1979) reported increased frequencies of abnormal sperm in mice treated by injection with 10 mg THC/kg body weight, once daily for five consecutive days. No similar studies using intraperitoneal injection as the route of administration have been reported.

Dalterio *et al..* (1982) observed increased frequencies of ring and chain quadrivalents in diakinesis-

meta-phase I spermatocytes of male mice treated with a single oral dose of 100 mg THC/kg body weight. In addition, they observed a marked reduction in fertility of male mice exposed to 50 mg THC/kg body weight three times per week for 5 weeks. This reduction was presumably the result of induced chromosomal abnormalities in the germ cells of the treated males.

However, only eight offspring, which demonstrated gross phenotypic abnormalities, were examined cytologically and only two of the eight were confirmed translocation carriers. In contrast, Stoeckel *et al.*. (1975) reported no induction of dominant lethal mutations in an unspecified strain of male mice treated with up to 200 mg THC/kg body weight for 4 weeks. Generoso *et al.*. (1985), concerned by the implications from the Dalterio *et al.*. (1982) study of possible widespread germ cell damage among human populations exposed to marijuana, conducted a similar study. However, they increased the frequency of treatments from 3 per week to 5 per week; the dose level of 50 mg THC/kg body weight used by Dalterio *et al.* (1982) was retained. Generoso *et al.*. (1985) found no induction of either dominant lethal mutations or heritable translocations in THC-treated male (C3H x 101)F1 mice.

Results of *in vivo* micronucleus tests were mixed. Positive results were reported by Zimmerman and Raj (1980) in male B6C3F$_1$ mice administered an intraperitoneal injection of 10 mg THC/kg body weight once a day for 5 days or 5 to 20 mg/kg once. They also reported significant increases in chromosomal aberrations in bone marrow cells of mice treated five times with 10 mg THC/kg body weight. However, the lack of clarity in the protocol and data presentations in this report makes evaluation of the results difficult. No increases in the frequencies of micronucleated erythrocytes were observed in male or female Swiss mice injected intraperitoneally with 5, 10, or 20 mg THC/kg body weight twice at 24-hour intervals; bone marrow samples were taken 24 hours after the second dosing (Van Went, 1978). Additional negative micronucleus test results with THC administered by gavage were reported by Legator *et al.* (1974) and Stoeckel *et al.* (1975). THC, administered as a single subcutaneous injection (10 or 1,000 mg/kg) to Syrian hamsters, did not induce an increase in chromosomal aberrations in bone marrow cells harvested 1.5 to 96 hours after treatment (Joneja and Kaiserman, 1978).

There have been some reports of increased frequencies of chromosomal aberrations in peripheral lymphocytes of marijuana smokers (Stenchever *et al.*, 1974), but because the subjects in these human studies were not screened for conventional cigarette use, and because subjects in the Gilmour *et al.* (1971) study were users of multiple drugs in addition to marijuana, the data are unreliable. Nichols *et al.* (1974) reported no increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes from healthy male volunteers administered 20 mg THC per day orally for a period of 12 days. All of these volunteers had histories of prior marijuana use. Thus, these human studies indicate that purified THC is probably not mutagenic, but that some other components of marijuana might be capable of inducing chromosomal damage.

STUDY RATIONALE

The use of marijuana in the United States is wide-spread, and its major psychoactive component is THC. THC has been used to reduce intraocular pressure in glaucoma treatment and as an antiemetic drug during cancer chemotherapy, an analgesic, a muscle relaxant, an anticonvulsant, and to treat bronchial asthma, insomnia, hypertension, and depression. In spite of the widespread abuse of marijuana and its potential medical uses, no carcinogenicity study of THC has been reported. The National Institute of Drug Abuse requested that carcinogenicity studies of marijuana be conducted. The Food and Drug Administration needed toxicity and carcinogenicity data in view of the medical uses of THC. In conjunction with these other agencies, the Chemical Selection Working Group of the National Cancer Institute nominated THC for study by the NTP.

This document reports the results of 13-week studies, 13-week with 9-week recovery studies, and 2-year studies in which THC was administered in corn oil by gavage to male and female F344/N rats and B6C3F_1 mice. The 13-week with 9-week recovery studies were conducted to investigate the persistence of the toxic effects of THC. In addition, genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse peripheral blood cells.

The gavage route of administration was selected because the amount of THC available for the studies was limited and because the quantity of THC administered could be accurately controlled.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 1-TRANS-DELTA^9-TETRAHYDROCANNABINOL

1-Trans-delta^9-tetrahydrocannabinol (THC) was obtained from A. D. Little (Cambridge, MA) in one lot (16792-123), which was used during the 13-week and 13-week with 9-week recovery studies (recovery studies). For the 2-year studies, four lots (AJ-86.8, AJ-86.9, AJ-86.10, and AJ-86.11) were obtained from Aerojet Strategic Development Co. (Sacramento, CA) by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and assigned lot number A042487. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the THC studies are on file at the National Institute of Environmental Health Sciences. The methods and results of these studies are detailed in Appendix I.

Both lots of the chemical, a honey-colored viscous liquid, were identified as THC by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of lots 16792-123 and A042487 was determined by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, high-performance liquid chromatography, and gas chromatography. Elemental analysis for hydrogen was in good agreement with the theoretical values for THC; elemental analysis for carbon was higher than the theoretical value for THC. Karl Fischer water analysis indicated less than 1.4% water. Thin-layer chromatography showed one minor impurity spot. High-performance liquid chromatography with ultraviolet detection at 220 nm revealed a major peak and two impurities with areas of 0.5% and 1.1% of the major peak area for lot 16792-123, and a major peak and three impurities with areas of 0.2%, 0.8%, and 1.5% of the major peak area for lot A042487. Gas chromatography indicated one major peak and seven impurities with a combined peak area of 3.8% relative to the major peak for lot 16792-123 and one major peak and five impurities with a combined peak area of 2.6% relative to the major peak for lot A042487. The overall purity was determined to be approximately 96% for lot 16792-123 and approximately 97% for lot A042487.

An impurity observed in lot 16792-123 by gas chromatography was identified by capillary gas chromatography/mass spectrometry as cannabinol. Cannabinol was quantitated to be 1.0% in this sample by high-performance liquid chromatography. For lot A042487, the 0.2% and 0.8% impurity peaks were identified as cannabinol and trans-delta^8-tetrahydrocannabinol by retention time matching and by spiking with known standards.

Stability studies were performed by the analytical chemistry laboratory using high-performance liquid chromatography. These studies indicated that THC was stable as a bulk chemical for at least 2 weeks when stored in evacuated containers protected from light at temperatures up to 25° C. To ensure stability, the bulk chemical was stored at 5° C, protected from light, in evacuated glass septum vials with Teflon-lined septa. The stability of the bulk chemical was monitored by the study laboratory during the 13-week, recovery, and 2-year studies using high-performance liquid chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing THC with corn oil to give the required concentrations (Table Ii). Dose formulation stability studies per- formed by the analytical chemistry laboratory using

gas chromatography confirmed that the formulations were stable for 3 weeks at room temperature when stored under a nitrogen headspace protected from light. The dose formulations were stored for up to 3 weeks at approximately 5° C under a nitrogen or argon headspace.

Periodic analyses of the dose formulations of THC were conducted at the study laboratory and analytical chemistry laboratory using gas chromatography. During the 13-week and recovery studies, the dose formulations were analyzed 5 times; all were within 10% of the target concentrations (Table 12). During the 2-year studies, the dose formulations were analyzed approximately every 8 weeks, and were within 10% of the target concentrations 99% (68/69) of the time for both rats and mice (Table 13). Periodic analyses of the corn oil vehicle by the study laboratory demonstrated peroxide levels within the acceptable limit of 10 mEq/kg. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table 14).

13-WEEK AND 13-WEEK WITH 9-WEEK RECOVERY STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to THC and to determine the appropriate doses to be used in the 2-year studies. The 13-week with 9-week recovery studies (recovery studies) were conducted to evaluate the effect of a 9-week recovery period on the chemical-related changes observed following 13-week dosing.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats were 3 weeks old and the mice were 4 weeks old. Animals were quarantined for 13 or 14 days and were 5 or 6 weeks old on the first day of the studies. Before initiation of the studies, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on 10 male and 10 female control rats and mice using the protocols of the NT? Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice received THC in corn oil by gavage for 13 weeks at doses of 0, 5, 15, 50, 150, or 500 mg/kg. Additional groups of 10 male and 10 female rats received THC in corn oil by gavage for 13 weeks at the same dose levels, and were then allowed to recover during a 9-week treatment-free period. Feed and water were available *ad libitum*. Rats and mice were housed five per cage. Animals were observed twice daily, and clinical findings were recorded weekly. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

At the end of the 13-week and recovery studies, samples were collected from all rats and mice for sperm morphology and vaginal cytology evaluations. The parameters evaluated are listed in Table 2. Methods used were those described in the NTP General Statement of Work (April 1987). For 7 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). All male animals used in this special study were evaluated for sperm morphology, count, and motility. The right testis and right epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right

cauda epididymis was placed in buffered saline solution. Cauda were finely minced, and the tissue was incubated in saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

At the end of the 13-week and recovery studies, blood was collected for hematology from all surviving animals by cardiac puncture. Hematology analyses were performed automatically by a Coulter S560 whole blood analyzer, and leukocyte differentials were performed by microscopic identification of 200 leukocytes per animal. The hematology parameters measured are listed in Table 2. A necropsy was performed on all animals and organ weights were taken from all animals that survived to the end of the studies; organs weighed were brain, heart, right kidney, liver, lungs, right testis, thymus, and uterus. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all vehicle control and 500 mg/kg rats and mice, 150 mg/kg rats, and all rats and mice that died during the study. The organs and tissues routinely examined are listed in Table 2.

2-YEAR STUDIES

Study Design

Groups of 60 to 80 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. Groups of 60 to 80 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). Up to 18 male rats and 18 male mice were removed for special studies at 15 months; results of these special studies are not presented in this Technical Report. As many as 10 male and 10 female rats and mice from each group were evaluated at 15 months for alterations in clinical chemistry and hematology parameters.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) for use in the 2-year studies. Male rats were quarantined for 13 days and female rats were quarantined for 14 days before the beginning of the study. Male and female mice were quarantined for 15 days before the beginning of the studies. Rats and mice were approximately 7 weeks old at the beginning of the studies. Prior to study start, five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated once every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded at 4-week intervals. The animals were weighed initially, weekly for the first 13 weeks, and at 4-week intervals thereafter. A complete necropsy and microscopic examination were performed on all rats and mice. At the 15-month interim evaluation, the adrenal glands, brain, right kidney, liver, ovary, prostate gland, right

testis, seminal vesicle, spleen, thymus, and uterus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

At the 15-month interim evaluation, samples were collected from all female rats for vaginal cytology evaluations. The parameters evaluated are listed in Table 2. Methods used were those described for the 13-week and 13-week with 9-week recovery studies.

At the 15-month interim evaluation, blood was collected for clinical chemistry (rats only) and hematology from the retroorbital sinus of as many as 10 males and 10 females from each dose group. Serum hormone levels were measured using radio–immunoassay kits and reagents from various manufacturers. Hematology analyses were performed by a Sysmex TOA E-2500, computer-controlled, 18-parameter fully automated hematology analyzer. It is used for the *"in-vitro"* diagnostic testing of whole blood specimens. The clinical chemistry and hematology parameters measured are listed in Table 2.

At the end of the 2-year study, serum was collected at various intervals from three male rats from each dose group for plasma THC levels. Sampling times and methodologies are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histo-technique was evaluated. The quality assessment pathologist microscopically reviewed selected neoplasms and nonneoplastic lesions.

The quality assessment report and the reviewed slides were submitted to the NT? Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. For the 2-year studies, tissues examined in male and female rats included the forestomach (males), lung, pituitary gland, liver, pancreas (males), spleen, and testis. Tissues examined in male and female mice included the adrenal gland (females), brain, forestomach, kidney, liver, and thyroid gland. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of quality assessment pathologists, the PWG chairperson, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, CS, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, *i.e.*, the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences
The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.,* 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences
Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables
Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight

data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Clinical chemistry, hematology, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NT? personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NT? historical control database (Haseman *et al.*, 1984, 1985) are included in the NT? reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-week, 13-week with 9-week recovery, and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NT? Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of THC was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and by assessing the frequency of micronucleated erythrocytes in peripheral blood. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of THC are part of a larger effort by the NT? to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of

electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of 1-Trans-Delta^9-Tetrahydrocannabinol

13-Week Studies	Recovery Studies	2-Year Studies
Study Laboratory SRI International (Menlo Park, CA)	SRI International (Menlo Park, CA)	TSI Mason Laboratories (Worcester, MA)
Strain and Species Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source Simonsen Laboratories (Gilroy, CA)	Simonsen Laboratories (Gilroy, CA)	Taconic Farms (Germantown, NY)
Time Held Before Studies Rats: 14 days Mice: 13 days	Rats: 13 days Mice: 14 days	Rats: 13 days (males) or 14 days (females) Mice: 15 days
Age When Studies Began Rats: 5 weeks Mice: 6 weeks	Rats: 6 weeks Mice: 6 weeks	Rats: 7 weeks Mice: 7 weeks
Date of First Dose Rats: 26 August 1983 Mice: 14 September 1983	Rats: 8 September 1983 to 9 September 1983 Mice: 21 September 1983	Rats: 14 December 1988 (males) and 15 December 1988 (females) Mice: 12 May 1988 (males) and 13 May 1988 (females)
Duration of Dosing 13 weeks (5 days/week)	13 weeks (5 days/week) followed by a 60-day recovery period	Rats: 104 to 105 weeks (5 days/week) Mice: 104 to 105 weeks (5 days/week) (males) and 105 to 106 weeks (5 days/week) (females)
Date of Last Dose Rats: 27 November 1983 to 1 December 1983 Mice: 15 December 1983 to 21 December 1983	Rats: 7 December 1983 to 8 December 1983 Mice: 20 December 1983 to 21 December 1983	Rats: 6 December 1990 to 12 December 1990 (males) and 7 December 1990 to 19 December 1990 (females) Mice: 9-15 May 1990 (males) and 17-23 May 1990 (females)

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of 1-Trans-Delta^9-Tetrahydrocannabinol (continued)

13-Week Studies	Recovery Studies	2-Year Studies
Necropsy Dates		
Rats: 28 November 1983 to 2 December 1983 Mice: 21 December 1983	Rats: 6 February 1984 to 20 February 1984 Mice: 24	Rats: 15-Month interim evaluation 5-9 March 1990 (males) 12-16 March 1990 (females) Terminal - 7 December 1990 to 13 December 1990 (males) and 8 December 1990 to 20 December 1990 (females) Mice: Terminal - 10-16 May 1990 (males) and 18-24 May 1990 (females)
Average Age at Necropsy		
Rats: 18 weeks Mice: 19 weeks	Rats: 28 weeks Mice: 28 weeks	15-Month interim evaluation - Rats: 71 weeks Terminal - Rats: 110-111 weeks (males) and 110-112 weeks (females) Mice: 111-112 weeks (males) and 112-113 weeks (females)
Size of Study Groups		
10 males and 10 females	Same as 13-week studies	Special study groups - Up to 18 male rats and 18 male mice 15-Month interim evaluation - 9 or 10 male and 9 or 10 female rats Terminal - Rats: 51 or 52 males and 50 or 51 females Mice: 60 to 62 males and 60 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 13-week studies	Same as 13-week studies
Animals per Cage		
Rats: 5 Mice: 5	Rats: 5 Mice: 5	Rats: 1 Mice: 1
Method of Animal Identification		
Ear punch	Same as 13-week studies	Tail tattoo
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitnum*, changed weekly	Same as 13-week studies	Same as 13-week studies, changed twice weekly

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of 1-Trans-Delta^9-Tetrahydrocannabinol (continued)

13-Week Studies	Recovery Studies	2-Year Studies
Water Distribution Tap water (Menlo Park municipal supply) via automatic watering system (Systems Engineering, Napa, CA), available *ad libitum*	Same as 13-week studies	Tap water (City of Worcester municipal supply) via automatic watering system (Edstrom Industries Inc., Waterford, NJ), available *ad libitum*
Cages Polycarbonate (Lab Products Inc., Rochelle Park, NJ), changed twice weekly	Same as 13-week studies	Same as 13-week studies, changed weekly
Bedding Absorb-Dri (Lab Products, Maywood, NY), changed twice weekly	Same as 13-week studies	Heat-treated hardwood chips (P.J. Murphy Forest Products, Montville, NJ), changed weekly
Cage Filters Nonwoven fiber (Lab Products, Rochelle Park, NJ, or Snow Filtration, Cincinnati, OH) changed every two weeks	Same as 13-week studies	Nonwoven fiber (Snow Filtration, Cincinnati, OH) changed every two weeks
Racks Stainless steel (Lab Products Inc., Rochelle Park, NJ), changed every two weeks	Same as 13-week studies	Same as 13-week studies
Animal Room Environment Temperature: 22.8° to 25° C Relative humidity: 23% to 69% Fluorescent light: 12 hours/day Room air: 13.5 changes/hour	Temperature: 21.7° to 25.6° C Relative humidity: 20% to 75% Fluorescent light: 12 hours/day Room air: 13.5 changes/hour	Temperature: 18.9° to 26.7° C Relative humidity: 16% to 98% Fluorescent light: 12 hours/day Room air: minimum of 10 changes/hour
Doses Rats: 0, 5, 15, 50, 150, and 500 mg/kg body weight in corn oil by gavage at a volume of 5 mL/kg body weight Mice: 0, 5, 15, 50, 150, and 500 mg/kg body weight in corn oil by gavage at a volume of 10 mL/kg body weight	Same as 13-week studies	Rats: 0, 12.5, 25, and 50 mg/kg body weight in corn oil by gavage at a volume of 5 mL/kg body weight Mice: 0, 125, 250, and 500 mg/kg body weight in corn oil by gavage at a volume of 10 ml/kg body weight
Type and Frequency of Observation Animals were observed twice daily and clinical findings were recorded weekly. Body weights were recorded initially, weekly, and at the end of the studies.	Same as 13-week studies	Observed twice daily and clinical observations were recorded monthly; animals were weighed initially, weekly for the first 13 weeks, and monthly thereafter until the end of the studies.

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of 1-Trans-Delta^9-Tetrahydrocannabinol (continued)

13-Week Studies	Recovery Studies	2-Year Studies
Method of Sacrifice Anesthetization with sodium pentobarbital followed by exsanguination by cardiac puncture	Same as 13-week studies	Anesthetization with carbon dioxide followed by exsanguination from the retroorbital sinus.
Necropsy Necropsy performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right testis, thymus, and uterus.	Same as 13 week studies	Necropsy performed on all animals. Organs weighed were: adrenal glands, brain, right kidney, liver, ovary, prostate gland, right testis, seminal vesicle, spleen, thymus, and uterus.
Clinical Pathology Blood was collected from all animals surviving to the end of the studies by cardiac puncture for hematology. Hematology: hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and total leukocyte counts and differentials.	Same as 13 week studies	Blood was collected from 15-month interim evaluation rats and mice from the retroorbital sinus. ***Clinical Cyhemistry*** (Rats only) corticosterone, estrogen, follicle stimulating hormone, luteinizing hormone, prolactin, testosterone, THC, and thyroxine ***Hematology:*** hematocrit, hemoglobin, methemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, reticulocytes, total leukocyte counts, and differentials.
THC Plasma Analyses None	None	Samples were taken at just following administration of the final dose, 15 minutes following the final dose, and 1, 4, 8, 24, 48, 72, and 120 hours after the final dose. Blood was collected as described for clinical pathology assays, and THC levels were determined using an autoanalyzer.

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of 1-Trans-Delta^9-Tetrahydrocannabinol (continued)

13-Week Studies	Recovery Studies	2-Year Studies
Histopathology		
Complete histopathology was performed on all animals that died before the end of the study, and on 0 and 500 mg/kg rats and mice, and 150 mg/kg rats. In addition to gross lesions and tissue masses, the tissues examined were: adrenal gland, bone and marrow, brain, epididymis, esophagus, gallbladder (mouse), heart, kidney, large intestine (cecum, colon, and rectum), liver, lymph node (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord, spleen, stomach (forestomach and glandular stomach), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The following organs were examined in surviving rats administered 5, 15, and 50 mg/kg: epididymis, stomach, and testis in males and adrenal gland, ovary, stomach, liver, and uterus in females. Additional organs examined in surviving mice at 5, 15, 50, and 150 mg/kg were: adrenal gland, epididymis, liver, testis, and thyroid gland in males and liver, ovaries, spleen, stomach, and uterus in females.	Complete histopathology was performed on all animals that died before the end of the study, and on 0 and 500 mg/kg rats and mice, and 150 mg/kg rats. In addition to gross lesions and tissue masses, the tissues examined were: adrenal gland, bone and marrow, brain, epididymis, esophagus, gallbladder (mouse), heart, kidney, large intestine (cecum, colon, and rectum), liver, lymph node (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord, spleen, stomach (forestomach and glandular stomach), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The following organs were examined in surviving rats administered 5, 15, and 50 mg/kg: epididymis, stomach, and testis in males and liver and uterus in females. Additional organs examined in surviving male mice a 5, 15, 50, and 150 mg/kg were: adrenal gland, epididymis, liver, testis, and thyroid gland. The uterus of surviving 15 and 50 mg/kg females was also examined.	Complete histopathology was performed on all animals that died before the end of the study, and on 0, 12.5, 25, and 50 mg/kg rats and on 0, 125, 250, and 500 mg/kg mice. In addition to gross lesions and tissue masses, the tissues examined were: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, eyes, gallbladder (mouse), heart, kidney, large intestine (cecum, colon, and rectum), liver, lungs, lymph node (mandibular and mesenteric), mammary gland, mainstem bronchi, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord, spleen, stomach (forestomach and glandular stomach), testis, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.
Sperm Morphology and Vaginal Cytology Evaluations		
At terminal sacrifice sperm samples were collected from all male animals for sperm morphology evaluations. The parameters evaluated included: sperm density, morphology, and motility. The right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of studies from all female animals for vaginal cytology evaluations. The parameters evaluated included: relative frequency of estrous stages and estrous cycle length.	Same as 13 week studies	At the 15-month interim sacrifice, vaginal samples were collected for up to 7 consecutive days prior to the end of the study from all female rats for vaginal cytology evaluations. The parameters evaluated included: relative frequency of estrous stages and estrous cycle length.

RESULTS

RATS

13-WEEK AND 13-WEEK WITH 9-WEEK RECOVERY STUDIES

In the 13-week study, six male and six female rats receiving 500 mg/kg, two 50 mg/kg male rats, and one female administered 15 mg 1-trans-delta^{9}- tetrahydrocannabinol (THC)/kg body weight died before the end of the study (Table 3a). With the exception of 5 mg/kg rats, the final mean body weights and weight gains of all dosed groups of males and females were significantly lower than those of the controls.

In the 13-week with 9-week recovery study (recovery study), four male and seven female 500 mg/kg rats, three male 150 mg/kg rats, and one male 50 mg/kg rat died before the end of the study (Table 3b). Rats administered THC during the first 13 weeks of the recovery study gained weight quickly during the 9-week recovery period. Final mean body weights of all dosed groups were similar to those of the controls.

TABLE 3a
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Gavage Study of 1-Trans-Delta^{9}-Tetrahydrocannabinol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 1	Week 13
Male							
0	10/10	151 ± 8	331 ± 5	179 ± 7		18	14
5	10/10	153 ± 8	315 ± 7	162 ± 4*	95	16	13
15	10/10	153 ± 8	286 ± 6**	133 ± 4**	87	13	14
50	8/10[d]	154 ± 6	276 ± 6**	121 ± 7**	83	13	15
150	10/10	56 ± 6	266 ± 8**	110 ± 7**	80	11	15
500	4/10[e]	154 ± 6	242 ± 9**	75 ± 5**	73	8	15
Female							
0	10/10	116 ± 3	196 ± 4	80 ± 2		14	10
5	10/10	119 ± 3	195 ± 3	76 ± 2	99	12	9
15	9/10[f]	115 ± 4	184 ± 2*	67 ± 4**	94	10	11
50	10/10	116 ± 5	179 ± 4**	63 ± 3**	91	9	11
150	10/10	117 ± 5	173 ± 6**	56 ± 3**	88	9	10
500	4/10[g]	120 ± 4	186 ± 4*	66 ± 9**	95	7	13

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** P≤0.01
a Number of animals surviving/number initially in group
b Weights and weight changes are given as mean ± standard error.
c Feed consumption is expressed as grams per animal per day.
d Week of death: 3, 6
e Week of death: 1, 1, 1, 4, 4, 6
Week of death: 4
g Week of death:1, 5, 8, 8, 9, 12

TABLE 3b
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Gavage with 9-Week Recovery Study of 1-Trans-delta^9-tetrahydrocannabinol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]		
		Initial	Final	Change		Week 1	Week 13	Week 22
Male								
0	10/10	142 ± 7	379 ± 11	235 ± 5		30	14	18
5	10/10	147 ± 6	381 ± 9	235 ± 9	101	22	13	18
15	10/10	149 ± 8	378 ± 8	224 ± 10	100	25	15	18
50	9/10[d]	133 ± 6	376 ± 9	239 ± 10	99	23	15	18
150	7/10[e]	146 ± 6	373 ± 13	219 ± 10	99	22	14	19
500	6/10[f]	146 ± 5	373 ± 9	220 ± 11	98	29	14	20
Female								
0	10/10	119 ± 4	204 ± 5	85 ± 4		20	10	11
5	10/10	118 ± 5	206 ± 6	88 ± 3	101	16	10	11
15	10/10	117 ± 6	207 ± 3	90±3	102	20	11	11
50	10/10	115 ± 4	201 ± 7	87 ± 3	99	23	11	11
150	10/10	116 ± 4	205 ± 3	93 ± 4	101	23	9	11
500	3/10[g]	120 ± 4	212 ± 9	97 ± 7	104	11	13	13

[a] Number of animals surviving/number initially in group

[b] Weights and weight changes are given as mean ± standard error. Differences from the control group were not significant by Williams' or Dunnett's test.

[c] Feed consumption is expressed as grams per animal per day.

[d] Week of death: 13

[e] Week of death: 8, 13, 13

[f] Week of death: 1, 1, 12, 13

[g] Week of death: 1, 1, 1, 1, 2, 8, 10

Feed consumption by dosed groups of male and female rats was less than that by controls during the first part of the 13-week study, but was similar to that by controls at the end of 13 weeks (Table 3a). In the recovery study, feed consumption by 500 mg/kg females was less than that by controls during week 1, but was similar at weeks 13 and 22. Feed consump- tion by all other dosed groups of females and all dosed groups of males was similar to that by controls at weeks 1, 13, and 22 of the study (Table 3b).

Aggressive behavior became evident in both male and female rats during the 13-week and recovery studies; most of the rats had bite wounds on the tail and head. Other clinical findings observed during the studies included lethargy, sensitivity to touch, diarrhea, convulsions, and tremors. Beginning at week 5 of the 13-week study, convulsions were observed in 150 and 500 mg/kg males and females. Convulsions were observed following the dosing procedure and at feeding or cleaning of cages (when the animals were handled) and were more frequent near the end of the week. The entire sequence of events that took place during a single convulsion occurred in a time span of approximately 10 to 30 seconds, and rats were hyperexcitable to routine handling for more than 5 minutes following the initial reaction. Convulsions may have occurred at times other than the daily treatment/observation periods. Convulsions were often followed by pilo-erection or prostration, and in some cases by rapid breathing. Beginning at week 8 of the recovery study, convulsions were observed in 150 and 500 mg/kg males and females. Convulsions were often followed by hypersensitivity to touch.

At 13 weeks, the erythrocyte count and the hemato-crit and hemoglobin values of 500 mg/kg female rats were significantly greater than those of the controls (Table G1), consistent with dehydration. At the end of the recovery study, erythrocyte count and hemato-crit and hemoglobin values of 500 mg/kg females were similar to those of the controls (Table G2).

At 13 weeks, increases in the relative brain, heart, right kidney, and right testis weights of 15, 50, 150, and 500 mg/kg males were attributed to lower final mean body weights, as were the increases in relative liver weights of 150 and 500 mg/kg males (Table FI). Also at the end of the 13-week study, the right epididymal weight of 500 mg/kg males was significantly decreased, and there was an increase in the percentage of abnormal sperm in this group (Table Hi). Treatment-related multifocal atrophy was observed in the testes of 150 and 500 mg/kg males in both the 13-week studies (Table 4). Atrophic seminiferous tubules were few to moderate in number, decreased in diameter, scattered across the histological section, and contained only a few spermatogonia-type cells and/or Sertoli cells surrounding empty lumens.

At the end of the recovery study, the relative liver weights of 150 and 500 mg/kg males were significantly greater than that of the controls (Table F2). The absolute right testis weight of 500 mg/kg males was significantly lower than that of the controls.

In females at the end of the 13-week study, the absolute and relative heart, right kidney, and liver weights of 500 mg/kg females were significantly greater than those of the controls, but the absolute and relative uterus weight of 50, 150, and 500 mg/kg females were significantly lower than those of the controls (Table FI). Estrous cycle lengths of 15, 50, 150, and 500 mg/kg females were significantly longer than that of the controls (Table Hi). Uterine and ovarian hypoplasia observed in 150 and 500 mg/kg females were considered to be related to THC administration (Table 4). The small uteri had decreased cellularity and thickness of the epithelial lining and a decreased number of endometrial glands. Ovarian hypoplasia was characterized by a decrease in the size of maturing follicles.

In females at the end of the recovery study, there were no significant differences in absolute or relative organ weights (Table P2), hematology parameters (Table G2), vaginal cytology, or estrous cycle lengths (Table H2). There were no treatment-related lesions observed in females.

Histopathology was performed on the brains of rats from the 13-week and recovery studies to detect any brain lesions that might be associated with convulsions. Hematoxylin- and eosin-stained sections of brain from all male and female vehicle control and 500 mg/kg rats from both studies were examined. Three sections of brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) were contained on each slide. Sections of brain from 500 mg/kg rats that convulsed during the studies did not differ from the sections of brain from rats that did not convulse or from vehicle control rats. Review of the sections revealed no evidence of lesions associated with convulsions or other treatment-related lesions in male or female rats from both studies.

Dose Selection Rationale: Based on reduced mean body weight gains, convulsions, and mortality observed in the 13-week study, dose levels selected for the 2-year gavage study in rats were 12.5, 25, and 50 mg/kg. The anticipation of tolerance development and dose levels reported in other investigators' studies were also taken into consideration.

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Gavage Studies of 1-Trans-delta^9-tetrahydrocannabinol

Dose	Vehicle Control	5 mg/kg	15 mg/kg	50mg/kg	150 mg/kg	500mg/kg
Male						
13-Week Study						
Testis (Seminiferous Tubule)[a]	10	10	10	10	10	10
Atrophy, Multifocal[b]	0	0	0	0	5* (I.0)C	7** (1.1)
13-Week with 9-Week Recovery						
Testis (Seminiferous Tubule)	10	10	10	10	10	10
Atrophy, Multifocal	1 (1.0)	0	0	0	0	8** (1.5)
Female						
13-Week Study						
Ovary	10	10	10	10	10	10
Hypoplasia	0	0	0	0	10** (2.0)	5* (2.0)
Uterus						
Hypoplasia	10	10	10	10	10	10
	0	0	0	0	10** (2.0)	10** (2.0)

* Significantly different (P≤0.05) from the control by the Fisher exact test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (I= minimal; 2=mild; 3=moderate; 4 =marked)

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 2). Survival of dosed male and female groups was generally signifi- cantly greater than that of the controls.

Body Weights

Mean body weights of dosed groups of males and females were lower than those of the controls throughout the study, but the final mean body weights of all dosed groups were only marginally lower than those of the controls (Figure 3 and Tables 6 and 7).

Table 5
Survival of Rats in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Male				
Animals initially in study	80	60	70	70
Special study animals[a]	18	0	9	9
15-Month interim evaluation[a]	10	9	9	9
Accidental deaths[a]	1	0	2	1
Moribund	19	8	11	10
Natural deaths	10	8	6	10
Animals surviving to study termination	22	35	33	31[e]
Percent probability of survival at end of study[b]	43	69	66	61
Mean survival days[c]	650	684	652	663
Survival analysis[d]	P=0.237N	P=0.016N	P=0.041N	P=0.120N
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	9	9	9	10
Accidental deaths[a]	2	0	3	3
Moribund	18	9	9	10
Natural deaths	8	2	6	5
Animals surviving to study termination	23	40[e]	33	32[f]
Percent probability of survival at end of study	48	78	69	68
Mean survival days	644	695	681	656
Survival analysis	P=0.130N	P=0.002N	P=0.021N	P=0.047N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or a lower mortality in a dose group is indicated by N.
[e] Includes one animal that died during the last week of the study
[f] Includes two animals that died during the last week of the study

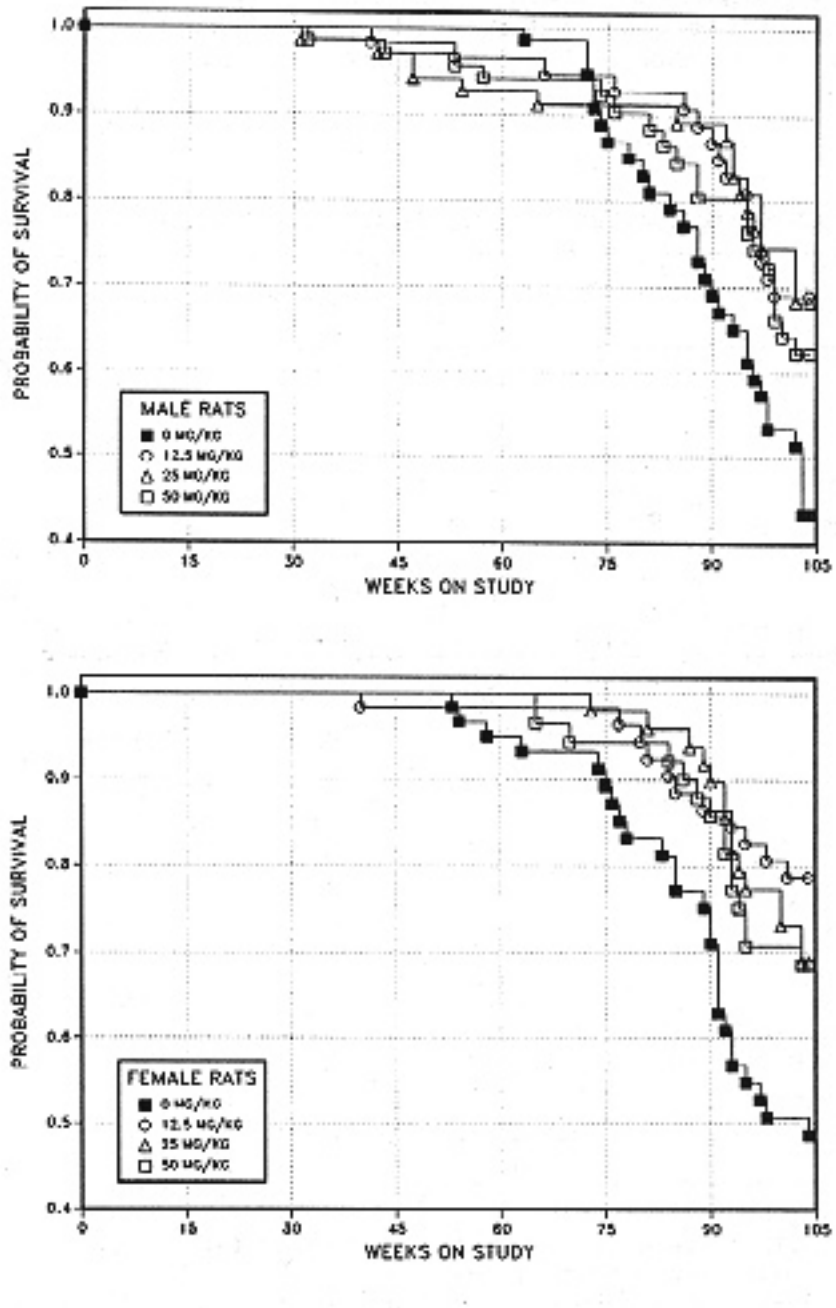


Figure 2
Kaplan-Meier Survival Curves For Male And Female Rats Administered THC in Corn Oil by Gavage for 2 Years

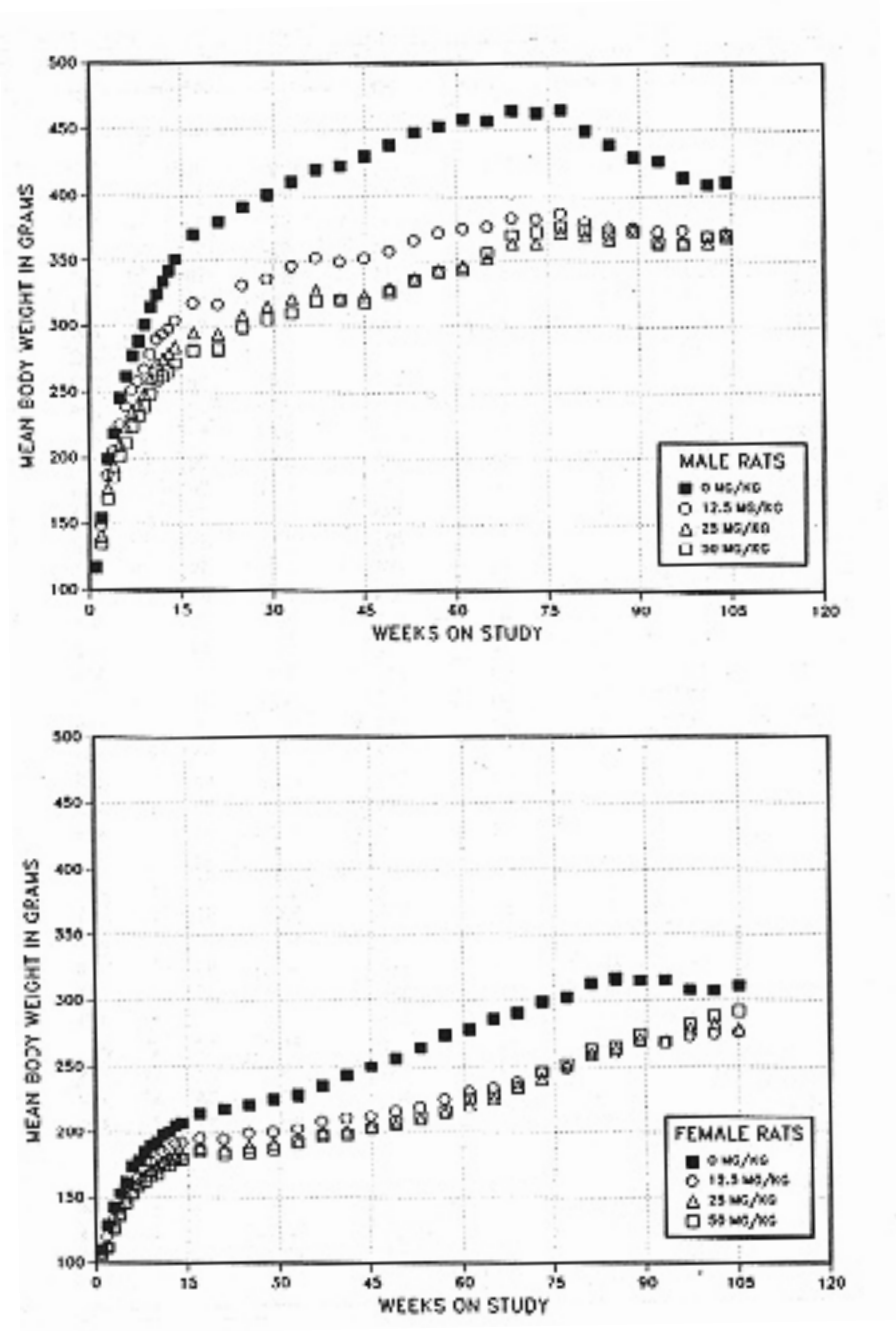


Figure 3
Growth and Survival Curves For Male And Female Rats Administered THC in Corn Oil by Gavage for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Weeks on Study	Vehicle Control		12.5 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	118	80[a]	117	99	60	117	100	70[b]	118	100	70[c]
2	154	80	149	96	60	141	92	70	135	88	70
3	199	80	187	94	60	177	89	70	169	85	70
4	219	80	205	94	60	194	89	70	186	85	70
5	245	80	226	92	60	211	86	70	201	82	70
6	262	80	239	91	60	224	85	70	211	81	70
7	277	80	252	91	60	236	85	70	224	81	70
8	289	80	259	90	60	242	84	70	232	80	70
9	301	80	267	89	60	249	83	70	239	80	70
10	315	80	279	89	60	261	83	70	248	79	70
11	324	80	290	89	60	272	84	70	259	80	70
12	334	80	293	88	60	275	82	70	262	79	70
13	342	80	298	87	60	279	82	70	266	78	70
14	351	80	304	87	60	284	81	69	272	78	70
17	370	80	317	86	60	295	80	69	282	76	70
21	380	79	317	83	60	294	77	69	283	74	70
25	392	79	331	85	60	308	79	69	299	76	70
29	401	79	336	84	60	315	79	69	305	76	70
33	411	79	346	84	60	321	78	68	310	76	69
37	420	79	352	84	60	327	78	68	319	76	69
41	423	79	349	83	60	322	76	68	320	76	69
45	430	79	352	82	59	323	75	67	318	74	68
49	438	79	358	82	59	329	75	65	326	74	68
53	448	79	366	82	59	336	75	65	335	75	68
57	452	79	372	82	58	343	76	64	341	76	67
61	458	79	375	82	58	346	76	64	343	75	66
65[d]	457	76	377	83	56	352	77	60	356	78	62
69	464	50	383	83	48	364	78	45	370	80	48
73	463	47	383	83	48	364	79	45	373	81	48
77	465	44	387	83	47	372	80	45	377	81	46
81	449	42	381	85	47	370	82	45	374	83	46
85	439	40	376	86	47	367	84	45	372	85	43
89	429	37	376	88	45	374	87	43	373	87	38[e]
93	427	34	373	87	42	364	85	42	365	85	40
97	415	30	374	90	41	366	88	37	363	88	37
101	409	27	369	90	35	364	89	36	369	90	32
104	411	22	372	91	35	369	90	33	370	90	31
Mean for weeks											
1-13	260		235	90		221	85		212	82	
14-52	402		336	84		312	78		303	75	
53-104	442		376	85		361	82		363	82	

[a] Special studies were performed on 18 vehicle control males.
[b] Special studies were performed on nine 25 mg/kg males.
[c] Special studies were performed on nine 50 mg/kg males.
[d] Interim or special study evaluation occurred during weeks 63 and 64.
[e] The number of animals weighed for this week is fewer than the number of animals surviving.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Weeks on Study	Vehicle Control		12.5 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	110	60	108	98	60	107	98	60	107	97	60
2	129	60	121	94	60	113	88	60	111	86	60
3	143	60	133	93	60	128	90	60	127	89	60
4	154	60	142	92	60	138	90	60	137	89	59
5	163	60	151	93	60	147	90	60	146	90	59
6	174	60	160	92	60	155	89	60	153	88	58
7	179	60	165	92	60	161	90	60	159	89	58
8	185	60	172	93	60	167	91	60	163	89	58
9	190	60	177	94	60	172	91	60	167	88	58
10	192	60	179	93	60	173	90	60	168	88	58
11	198	60	185	93	60	179	90	60	175	88	58
12	201	60	187	93	60	180	90	60	175	87	58
13	205	60	191	93	60	182	89	60	178	87	58
14	207	60	192	93	60	183	89	60	180	87	58
17	214	60	195	91	60	189	88	59	185	87	58
21	218	60	195	89	60	187	86	59	183	84	58
25	221	60	199	90	60	189	86	59	185	84	58
29	225	60	200	89	60	191	85	59	187	83	58
33	229	60	203	89	60	193	84	59	192	84	58
37	235	60	208	89	60	200	85	59	197	84	58
41	243	60	211	87	59	201	83	59	198	81	58
45	251	60	212	85	59	204	81	59	203	81	58
49	256	60	216	85	59	206	81	59	208	81	58
53	264	59	219	83	59	210	80	59	212	80	58
57	274	58	226	82	59	215	79	59	216	79	58
61	278	57	231	83	59	221	80	59	226	81	58
65[a]	286	55	234	82	59	226	79	58	229	80	56
69	290	46	238	82	50	234	81	49	235	81	45
73	299	46	245	82	50	241	81	49	246	82	44
77	302	43	248	82	50	251	83	48	253	84	44
81	313	41	259	83	48	259	83	48	263	84	44
85	317	40	261	82	46	264	83	47	265	84	43
89	316	38	270	85	45	271	86	46	275	87	41
93	316	30	269	85	44	270	86	41	269	85	38
97	308	27	273	89	42	279	90	37	282	91	33
101	308	24	275	90	41	282	92	35	288	94	33
Mean for weeks											
1-13	171		159	93		154	90		151	88	
14-52	230		203	88		194	84		192	83	
53-101	298		250	84		248	83		251	84	

[a] Interim evaluation occurred during weeks 65 and 66

Feed Consumption, Clinical Findings, and Organ Weights

Feed consumption was measured at 4-week intervals from week 65 to the end of the study. Feed consumption by dosed groups was similar to that by controls (Table 8). A slight but consistent trend of lower feed consumption was observed in vehicle control females. Convulsions and seizures were observed in all dosed groups of male and female rats. Convulsions were observed beginning at week 35 in 50 mg/kg males and week 22 in 50 mg/kg females, at week 41 for 25 mg/kg males, at week 31 for 25 mg/kg females, at week 66 for 12.5 mg/kg males, and at week 49 for 12.5 mg/kg females. The number of animals convulsing peaked at approximately weeks 62 through 65, when 47 males and 43 females administered 50 mg/kg were observed with convulsions. Convulsions/seizures were more frequent in females than in males, and frequency appeared to be dose related (Figure 4); however, the intensity and duration of convulsions were similar in males and females. At the 15-month interim evaluation, relative brain and liver weights were generally significantly increased in dosed groups of males (Table F3). Relative brain, liver, and adrenal gland weights of dosed groups of females were also increased. Relative thymus weights of dosed groups of females were decreased. In females at the 15-month interim evaluation, there were no significant differences in vaginal cytology or estrous cycle lengths (Table H3). There were no treatment-related lesions observed in females.

TABLE 8
Feed Consumption by Rats in the 2-Year Gavage Study of 1-Trans-delta^9-tetrahydrocannabinol

	Vehicle Control		12.5 mg/kg		25 mg/kg		50 mg/kg	
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)
Males								
65	15.1	457	14.5	377	14.4	352	15.0	356
73	13.9	463	13.3	383	13.9	364	14.8	373
77	14.6	465	13.5	387	15.1	372	15.7	377
81	13.6	449	13.9	381	14.6	370	16.4	374
85	13.8	439	13.3	376				
89	13.4	429	13.1	376	14.9	374	14.8	373
93	15.6	427	14.6	373	15.0	364	15.1	365
97	15.8	415	14.7	374	15.3	366	15.7	363
101	16.1	409	14.8	369	15.3	364	16.8	369
Mean	14.7	439	14.0	377	14.8	366	15.5	369
Females								
65	10.6	286	11.0	234	11.3	226	11.6	229
69	10.6	290	11.4	238	11.4	234	12.3	235
73	11.7	299	11.4	245	11.5	241	12.5	246
77	9.6	302	9.9	248	11.1	251	11.4	253
81	11.4	313	11.1	259	11.7	259	12.5	263
89	9.6	316	11.3	270	11.7	271	12.1	275
93	11.0	316	11.5	269	11.5	270	12.1	269
97	10.1	308	11.1	273	11.6	279	12.1	282
101	9.8	308	10.7	275	11.4	282	12.0	288
Mean	10.5	304	11.1	257	11.5	257	12.1	260

a Grams of feed consumed per animal per day

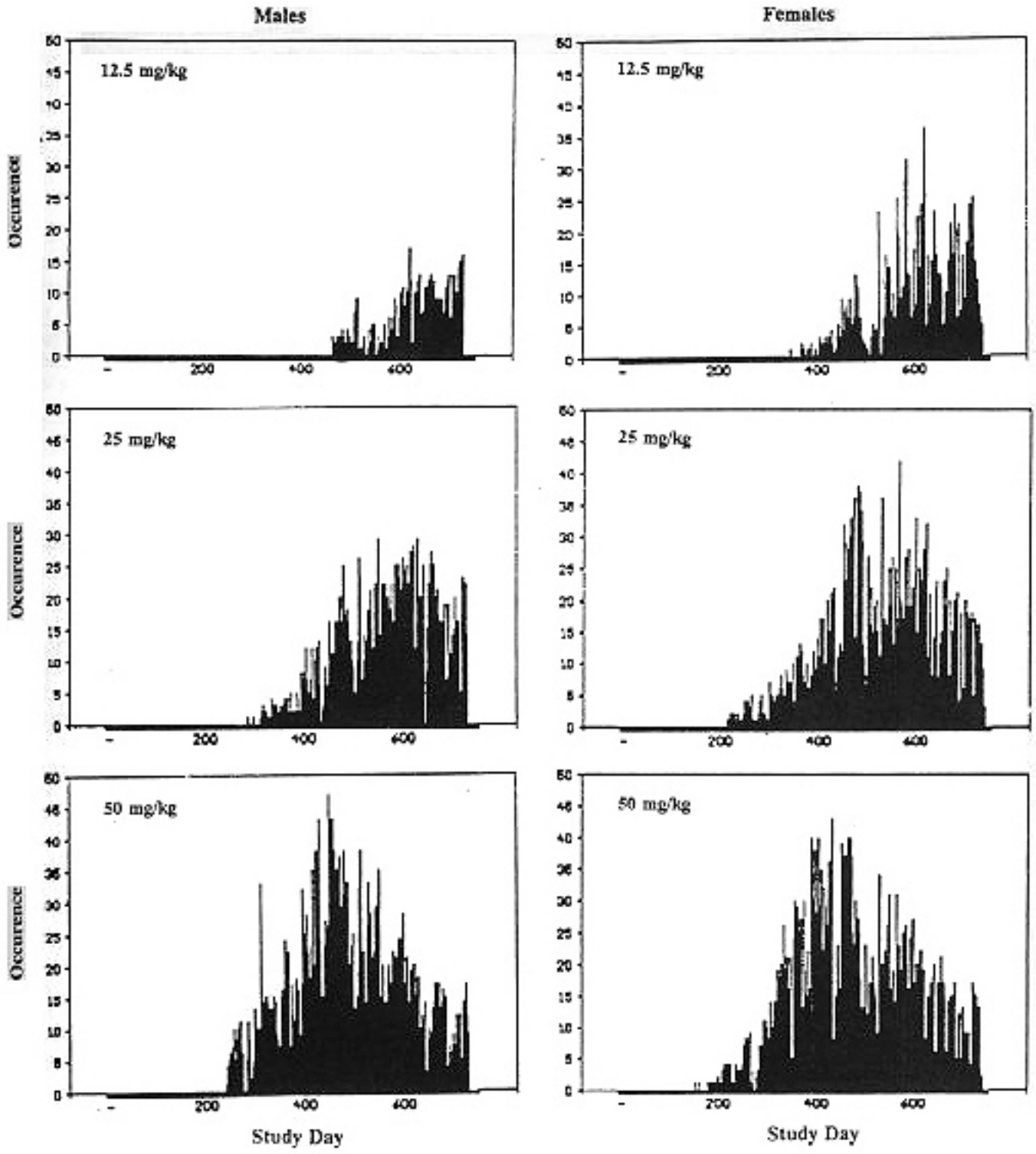


FIGURE 4
Daily Convulsion Incidence for Rats Administered THC in Corn Oil by Gavage for 2 Years

Hematology and Clinical Chemistry

Total leukocyte and lymphocyte counts in 25 and 50 mg/kg females were significantly greater than those of the controls at the 15-month interim evaluation (Table G3). The lymphocyte count of 12.5 mg/kg females was also significantly greater than that of the control. Concentrations of follicle stimulating and luteinizing hormones were increased in all male dosed groups.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the pancreas, pituitary gland, testis, mammary gland, uterus, lung, and brain and in the incidences of mononuclear cell leukemia in females. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats. The incidences of benign and malignant neoplasms in male (Table A3) and female (Table B3) rats were decreased in a dose-related manner.

Mononuclear Cell Leukemia: At the end of the 2-year study, the incidence of mononuclear cell leukemia was marginally increased in 25 mg/kg females (Tables 9 and B3); however, the increase was not significant by life table analysis (the most appropriate test for these generally fatal neoplasms), and there was no significant trend. The increased incidence was due in part to the longer survival of dosed groups of animals, and the increase was not considered to be related to the administration of THC.

TABLE 9
Incidences of Mononuclear Cell Leukemia in Female Rats in the 2-Year Gavage Study of 1-Trans-delta^9-tetrahydrocannabinol

Dose	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Mononuclear Cell Leukemia[a]				
Overall rate[b]	9/51 (18%)	17/51 (33%)	20/51 (39%)	13/50 (26%)
Adjusted rate[c]	32.3%	38.1%	47.6%	32.3%
Terminal rate[d]	6/23 (26%)	13/40 (33%)	12/33 (36%)	6/32 (19%)
First incidence (days)	524	534	509	454
Life table test[e]	P=0.460	P=0.407	P=0.130	P=0.481
Logistic regression test[e]	P=0.292	P=0.102	P=0.027	P=0.246

a Historical incidence of lymphocytic, monocytic, mononuclear cell, or undifferentiated cell type leukemia for 2-year NTP gavage studies with corn oil vehicle control groups (mean ± standard deviation): 277/1,070 (25.9% ± 7.2%); range, 12%-38%
b Number of neoplasm-bearing animals/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 is the P value 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 logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.

Lung: Increased incidences of foreign bodies in the lung occurred in all dosed groups of males (vehicle control, 8/52; 12.5 mg/kg, 26/51; 25 mg/kg, 26/52; 50 mg/kg, 15/52; Table A5). All dosed groups of females had increased incidences of chronic inflammation at the 15-month interim evaluation (3/9, 3/9, 5/9, 4/10) and at the end of the 2-year study (25/51, 48/51, 43/51, 42/50; Table B5). Foreign body in the lung was characterized by droplets of yellow oil in alveolar spaces. In general, the presence of the droplets was not associated with the inflammatory process. Chronic inflammation was minimal to mild in severity. The incidence of chronic inflammation was approximately equal across the dose groups. Although the incidence was increased in dosed female rats, approximately one-half of the vehicle control females had a similar lesion. In addition, the incidence of chronic inflammation in males was approximately equal across all groups (37/52, 40/51, 40/52, 36/52). Therefore, this lesion was probably not due to a systemic effect of the chemical.

Brain: As in the 13-week and recovery studies, brain tissues from animals evaluated at 15 months and at the end of the 2-year study were subjected to a special review. Tissues from rats with a history of convulsions or seizures were examined; additional or special procedures were performed to facilitate detection of neuropathologic changes. Brain tissues from two vehicle control females and six 50 mg/kg females were step-sectioned in their entirety and examined. In addition, step sections were performed on the brain tissues of three 50 mg/kg males and one 50 mg/kg female killed moribund during the study and fixed by perfusion with Trump's fixative, vehicle control and 50 mg/kg males and females from the 15-month interim evaluation, and 50 mg/kg males and females that survived to the end of the 2-year study. No microscopic lesions were observed in any tissues evaluated by step section; no treatment- or convulsion-related lesions were observed. Neuronal necrosis was present in the cerebral cortex (25 mg/kg, 1/52), hippocampus (vehicle control, 4/52; 12.5 mg/kg 1/50), or cerebellar cortex (25 mg/kg, 1/52) in male rats and in the hippocampus (12.5 mg/kg, 1/51) and cerebellar cortex (12.5 mg/kg, 1/51) in female rats. Some of these animals also developed mononuclear cell leukemia, which may have resulted in localized ischemia (neuronal necrosis) due to neoplastic cells within vessels.

Decreased Neoplasm Incidences: Incidences of neoplasms were decreased in various organs in male and female rats (Tables 10, A3, and B3). These included pancreatic acinar cell adenomas in males (significantly decreased in all dose groups), pituitary gland adenomas in males (significantly decreased in 50 mg/kg males), uterine stromal polyps (significantly decreased in 25 and 50 mg/kg females), and mammary gland fibroadenomas (decreased in all dosed groups of females). Many of the decreased incidences may have been associated with decreased mean body weights in dosed groups of rats.

Incidences of interstitial cell adenomas of the testis were also significantly decreased in 12.5 and 25 mg/kg male rats. The decreased incidence was more prominent for bilateral interstitial cell adenomas (Tables 10 and A1). A similar response was observed at 15 months where nine vehicle control males and one 12.5 mg/kg male had interstitial cell adenomas. As in the 2-year study, this response was more striking for bilateral interstitial cell adenomas, where adenomas were observed in six vehicle controls, but none were observed in dosed groups. Although there was a decrease in the incidence of interstitial cell adenomas, the incidence of hyperplasia at 15 months and at 2 years was slightly increased. Proliferative lesions involving the interstitial cells of the testis in F344/N rats are common age-related changes. The decreased incidence of interstitial cell adenomas was considered to be related to THC administration.

THC Plasma Concentration Analyses

The concentration of THC in plasma from dosed male rats was measured at various time points following the end of the 2-year study (Figures 5 and 6). THC was detectable in samples 120 hours after the final dose was administered, and the levels were proportional to the amount of THC administered. Throughout the 2-year study, serum THC levels likely fluctuated near the ranges reflected in Figure 6 at 24 hours following the final dose.

TABLE 10
Decreased Incidences of Selected Neoplasms in Rats in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Dose	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Male				
15-Month Interim Evaluation				
Pituitary Gland (Pars Distalis)[a]				
Adenoma[b]	1/10 (10%)	2/9 (22%)	2/9 (22%)	0/9 (0%)
Testis				
Bilateral Interstitial Cell Adenoma	6/10 (60%)	0/9 (0%)	0/9 (0%)	0/9 (0%)
Interstitial Cell Adenoma	3/10 (30%)	1/9 (11%)	0/9 (0%)	0/9 (0%)
2-Year Study				
Pancreas				
Adenoma[c]				
Overall rate[d]	8/52 (15%)	0/51 (0%)	2/52 (4%)	0/52 (0%)
Adjusted rate[e]	33.8%	0.0%	5.7%	0.0%
Terminal rate[f]	7/22 (32%)	0/35 (0%)	1/33 (3%)	0/31 (0%)
First incidence (days)	647	–[h]	709	–
Logistic regression test[g]	P=0.002N	P=0.001N	P=0.019N	P=0.002N
Pituitary Gland (Pars Distalis)				
Adenoma[i]				
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
Testis				
Interstitial Cell Adenoma[j]				
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

(continued)

TABLE 10
Decreased Incidences of Selected Neoplasms in Rats in the 2-Year Gavage Study of l-Trans-Delta^9-Tetrahydrocannabinol (continued)

Dose	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Female				
15-Month Interim Evaluation				
Mammary Gland				
Fibroadenoma	1/9 (11%)	0/9 (0%)	0/8 (0%)	0/10 (0%)
Uterus				
Stromal Polyp	1/9 (11%)	0/9 (0%)	1/9 (11%)	1/10 (10%)
2-Year Study				
Mammary Gland				
Fibroadenoma[k]				
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[l]				
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=0.046N

(T) Terminal sacrifice
[a] Number of animals with organ examined microscopically
[b] Number of animals with neoplasm
[c] Historical incidences for 2-year NTP gavage studies with corn oil vehicle control groups (mean ± standard deviation): 68/1,060 (6.4% ± 8.3%); range, 0%-32%
[d] Number of neoplasm-bearing animals/number of animals examined microscopically.
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value 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 logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dosed group is indicated by N.
[h] Not applicable; no neoplasms in these dose groups
[i] Historical incidence: 344/1,046 (32.9% ± 9.1%); range, 18%-49%
[j] Historical incidence (incidences reflect all adenomas of the testis): 933/1,062 (87.9% ± 5.8%); range, 76%-94%
[k] Historical incidence: 387/1,070 (36.2% ± 10.2%); range, 16%-56%
[l] Historical incidence: 207/1,070 (19.4% ± 6.4%); range, 4%-32%

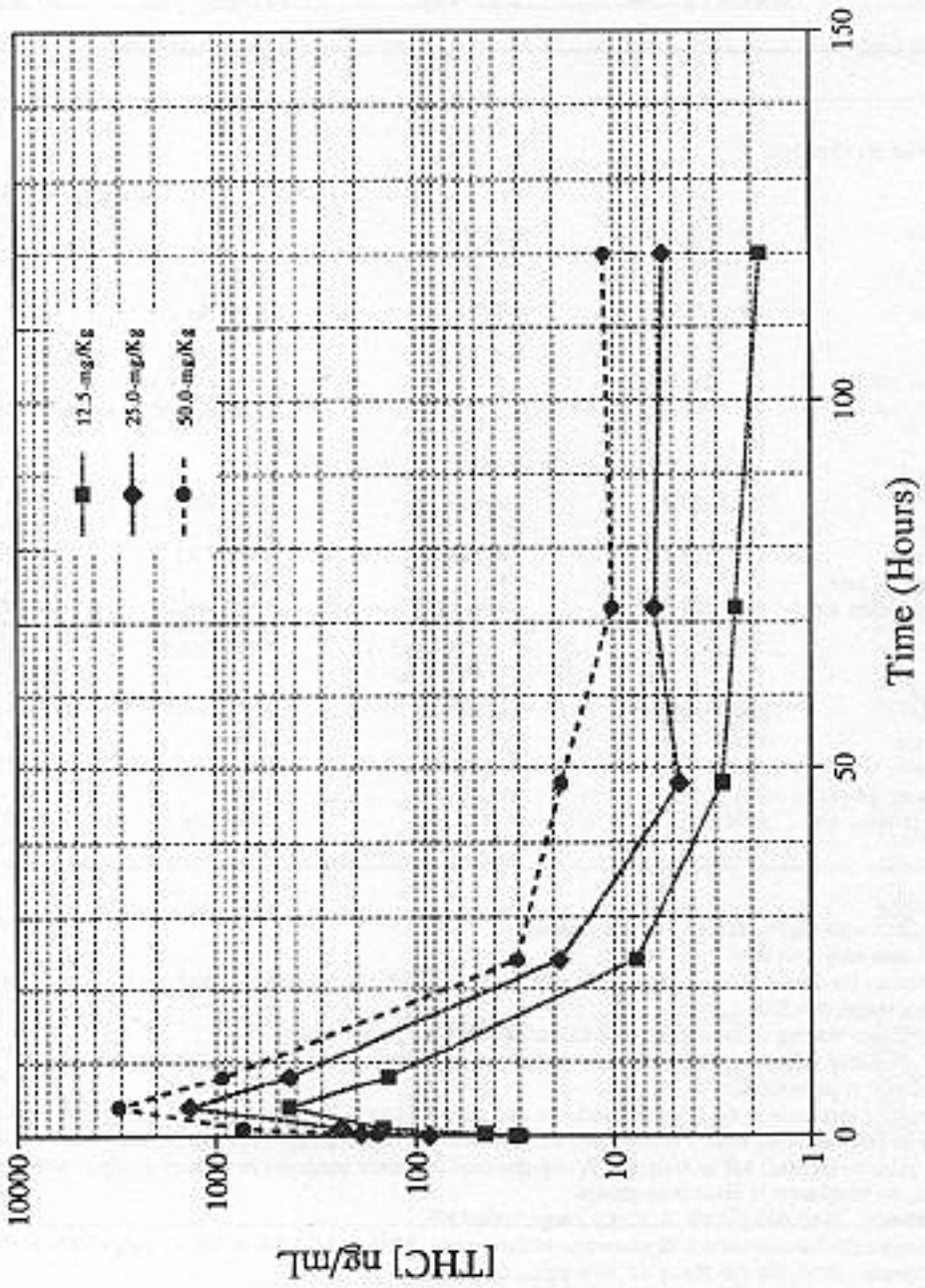


FIGURE 5
Mean THC Plasma Concentrations of Male Rats Administered THC in Corn Oil by Gavage for 2 Years

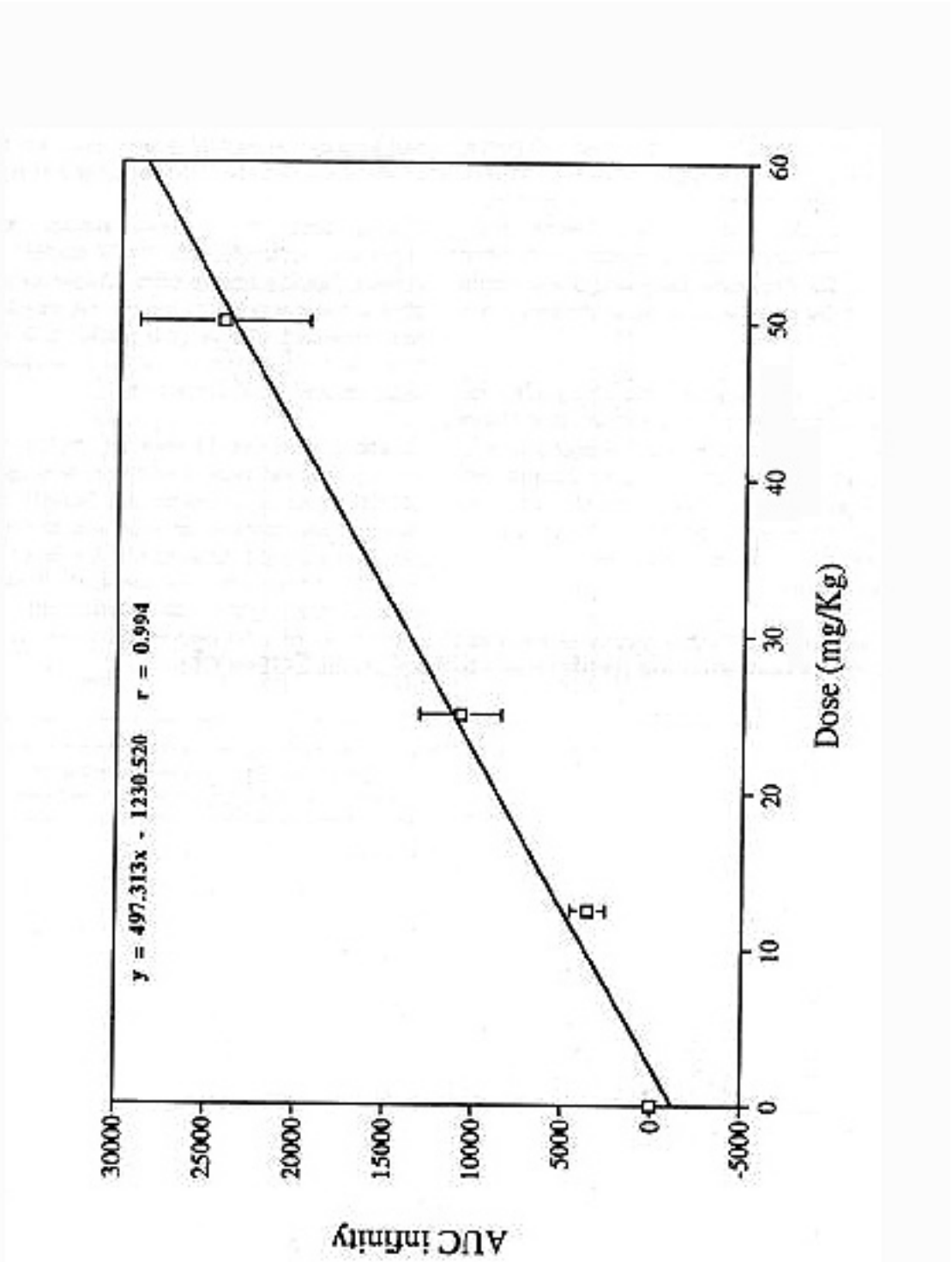


FIGURE 6
Area Under the Curve versus Dose for Male Rats Administered THC in Corn Oil by Gavage for 2 Years

MICE
13-WEEK AND 13-WEEK WITH 9-WEEK RECOVERY STUDIES

One 500 mg/kg male, three 150 mg/kg females, one male and one female administered 50 mg/kg, one 15 mg/kg female, one male and two females administered 5 mg/kg, and one vehicle control male died prior to the end of the 13-week study (Table 11a). The deaths were considered related to gavage error. The final mean body weight and weight gain of 500 mg/kg males were significantly lower than those of the controls.

In the 13-week with 9-week recovery study, one 50 mg/kg male and five 15 mg/kg males died before the end of the study, as did one 500 mg/kg female, two 150 mg/kg females, four 50 mg/kg females, two 15 mg/kg females, one 5 mg/kg female, and one vehicle control female (Table 11b). The final mean body weights of all dosed groups were similar to those of the controls.

Feed consumption by all dosed groups of males and females in the 13-week study was similar to that by controls (Table 11a). During the recovery study, average feed consumption by dosed groups of males and females was slightly greater than that by controls (Table 11b), but the difference was not significant.

During both the 13-week studies, mice were aggressive, lethargic, and easily startled. In both studies, fighting among mice became more frequent after a few weeks of treatment. A number of mice were observed with wounds and/or hair loss on the head and/or abdomen, labored breathing, pilo-erection, and brief convulsions.

At the end of the 13-week study, the hematocrit, hemoglobin, and mean erythrocyte hemoglobin levels of 500 mg/kg males were significantly lower than those of the controls, as were the hemoglobin and mean erythrocyte hemoglobin levels of 500 mg/kg females (Table G4). At the end of the recovery study, there were no statistically significant differences in erythrocyte variables of male and female mice (Table G5).

TABLE 11a
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	9/10[d]	23.8 ± 0.5	31.0 ± 0.8	7.0 ± 0.4		2.8	4.2
5	9/10[e]	23.9 ± 0.4	30.4 ± 0.5	6.5 ± 0.4	98	3.0	4.6
15	10/10	24.6 ± 0.5	30.1 ± 0.5	5.5 ± 0.4	97	3.1	4.1
50	9/10[d]	23.7 ± 0.3	30.6 ± 0.6	6.8 ± 0.5	99	3.1	4.1
150	10/10	22.8 ± 0.6	30.0 ± 0.8	7.1 ± 0.4	97	2.7	3.6
500	9/10[f]	23.0 ± 0.5	28.2 ± 0.6**	4.9 ± 0.5**	91	2.8	4.1
Female							
0	10/10	18.9 ± 0.3	24.2 ± 0.5	5.3 ± 0.4		3.3	4.2
5	8/10[g]	18.4 ± 0.5	24.8 ± 0.4	5.8 ± 0.4	102	3.5	4.3
15	9/10[d]	18.3 ± 0.4	24.5 ± 0.6	6.2 ± 0.4	101	3.5	4.2
50	9/10[d]	18.9 ± 0.3	25.1 ± 0.5	6.2 ± 0.4	104	3.4	4.4
150	7/10[h]	18.9 ± 0.6	25.2 ± 0.5	5.8 ± 0.5	104	3.1	4.6
500	10/10	18.8 ± 0.4	23.1 ± 0.5	4.3 ± 0.3	95	3.2	3.8

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.
[d] Week of death: 3
[e] Week of death: 4
[f] Week of death: 6
[g] Week of death: 1, 1
[h] Week of death: 1, 3, 3

TABLE 11b
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Gavage with 9-Week Recovery Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]		
		Initial	Final	Change		Week 1	Week 13	Week 22
Male								
0	10/10	23.0 ± 0.3	35.2 ± 1.1	12.2 ± 1.1		3.0	2.8	4.8
5	10/10	23.1 ± 0.4	35.7 ± 0.7	12.6 ± 0.6	102	3.3	3.2	5.0
15	5/10[d]	23.6 ± 0.4	35.1 ± 0.7	11.7 ± 0.8	100	3.4	3.4	6.0
50	9/10[e]	23.3 ± 0.7	34.7 ± 0.6	11.2 ± 0.6	99	3.5	3.3	5.3
150	10/10	23.3 ± 0.5	34.8 ± 1.1	11.5 ± 0.8	99	3.5	3.0	5.3
500	10/10	23.2 ± 0.3	35.2 ± 0.3	12.1 ± 0.4	100	3.1	3.6	5.1
Female								
0	9/10[f]	18.1 ± 0.3	27.6 ± 1.0	9.6 ± 0.8		3.0	2.9	5.2
5	9/10[g]	18.7 ± 0.4	25.8 ± 1.0	7.3 ± 0.7	93	2.9	3.2	4.9
15	8/10[h]	18.5 ± 0.3	28.5 ± 1.0	10.1 ± 0.8	103	2.9	3.2	5.6
50	6/10[i]	18.2 ± 0.3	26.8 ± 0.5	8.7 ± 0.5	97	2.3	3.5	4.8
150	8/10[j]	17.9 ± 0.3	24.9 ± 0.6	6.7 ± 0.6*	90	3.5	3.3	5.0
500	9/10[k]	18.3 ± 0.3	27.4 ± 0.7	9.1 ± 0.7	99	3.1	3.2	4.6

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
a Number of animals surviving/number initially in group
b Weights and weight changes are given as mean ± standard error.
c Feed consumption is expressed as grams per animal per day.
d Week of death: 1, 13, 14, 14, 14
e Week of death: 17
f Week of death: 2
g Week of death: 12
h Week of death: 2, 2
i Week of death: 2, 2, 7, 13
j Week of death: 7, 17
k Week of death: 18

At the end of the 13-week study, the relative liver weights of 500 mg/kg males and females were significantly greater than those of the controls (Table F4). Estrous cycle lengths of 5, 15, and 500 mg/kg females were significantly longer than for the controls (Table H4).

At the end of the recovery study, the absolute and relative uterus weights of 150 and 500 mg/kg females and the absolute uterus weight of 50 mg/kg females were significantly lower than those of the controls (Table F5). Sperm concentration in 500 mg/kg males was significantly lower than that in controls (Table H5); there were no other significant differences in sperm morphology, vaginal cytology, or estrous cycle length parameters.

Dose Selection Rationale: Due to the absence of significant histopathologic lesions and marked effects on mean body weight gains in the 13-week study, doses selected for the 2-year mouse study were 125, 250, and 500 mg/kg.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 7). Survival of 500 mg/kg males was significantly less than that of the controls; survival of all other dosed groups of males and of all dosed groups of females was similar to those of the controls.

Body Weights

Mean body weights of all dosed groups were markedly lower than those of the controls throughout the 2-year study (Figure 8 and Tables 13 and 14).

TABLE 12
Survival of Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
Male				
Animals initially in study	80	60	70	70
Special study animals[a]	18	0	9	10
Accidental death[a]	0	0	2	1
Moribund	3	2	3	4
Natural deaths	9	5	11	21
Animals surviving to study termination	50	53	45	34[e]
Percent probability of survival at end of study[b]	81	88	77	58
Mean survival (days)[c]	706	718	656	584
Survival analysis[d]	P<0.001	P=0.348N	P=0.608	P=0.003
Female				
Animals initially in study	60	60	60	60
Accidental deaths[a]	0	0	0	2
Moribund	3	5	3	3
Natural deaths	10	5	13	14
Animals surviving to study termination	47	50	44	41
Percent probability of survival at end of study	78	83	73	71
Mean survival days	702	716	678	634
Survival analysis	P=0.162	P=0.610N	P=0.583	P=0.392

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A lower mortality in a dose group is indicated by N.
[e] Includes one animal that died during the last week of the study

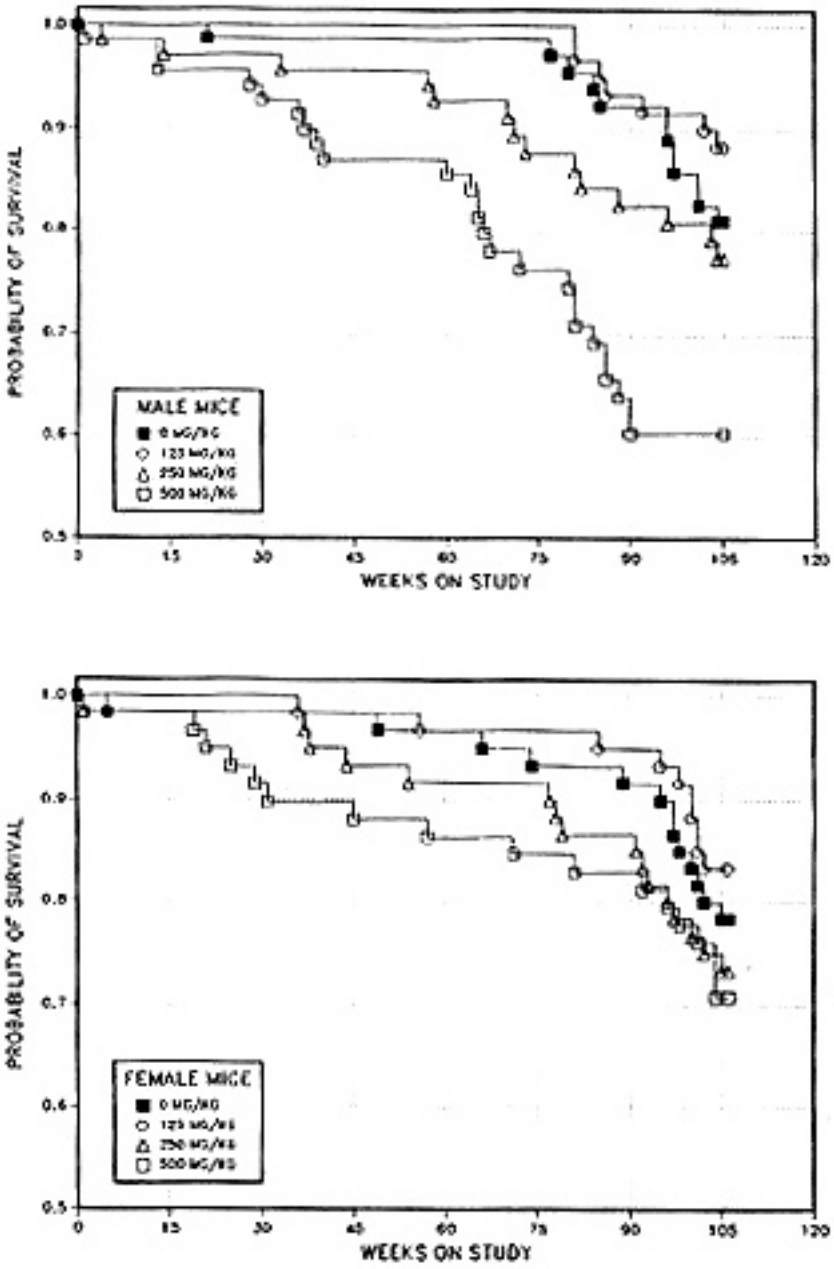
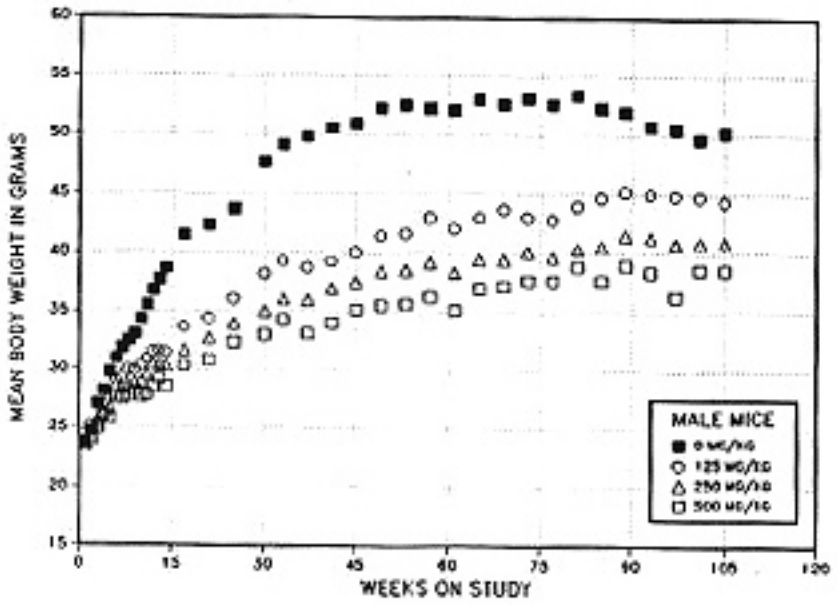


FIGURE 7
Kaplan-Meier Survival Curves for Male and Female Mice Administered THC in Corn Oil by Gavage for 2 Years



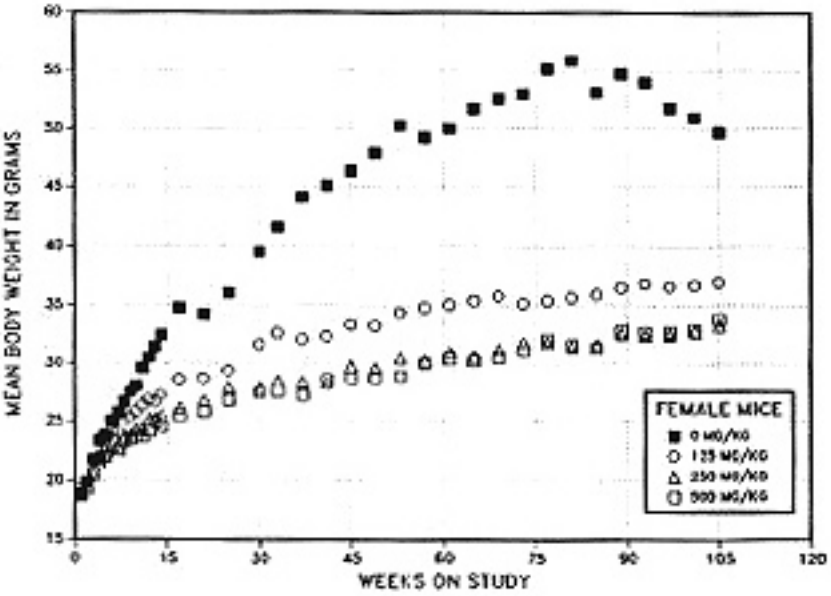


FIGURE 8
Growth and Survival Curves for Male and Female Mice Administered THC in Corn Oil by Gavage for 2 Years

TABLE 13
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Weeks on Study	Vehicle Control Av. Wt. (g)	Vehicle Control No. of Survivors	125 mg/kg Av. Wt. (g)	125 mg/kg Wt. (% of controls)	125 mg/kg No. of Survivors	250 mg/kg Av. Wt. (g)	250 mg/kg Wt. (% of controls)	250 mg/kg No. of Survivors	500 mg/kg Av. Wt. (g)	500 mg/kg Wt. (% of controls)	500 mg/kg No. of Survivors
1	23.7	80[a]	23.6	100	60	23.5	99	70[b]	24.4	103	70[c]
2	24.8	80	25.2	102	60	24.3	98	70	23.9	96	69
3	27.0	80	25.1	93	60	25.6	95	70	24.9	92	69
4	28.1	80	25.9	92	60	26.3	94	70	26.1	93	69
5	29.7	80	27.4	92	60	26.6	90	69	25.8	87	69
6	30.9	80	29.0	94	60	28.6	93	69	27.5	89	69
7	31.8	80	29.2	92	60	28.3	89	69	27.5	87	69
8	32.4	80	29.9	92	60	28.5	88	69	27.7	86	69
9	33.0	80	29.9	91	60	28.8	87	69	28.0	85	69
10	34.3	80	30.2	88	60	28.9	84	69	27.6	81	69
11	35.5	80	30.8	87	60	29.5	83	69	27.8	78	69
12	36.7	80	31.5	86	60	30.4	83	69	28.5	78	69
13	37.7	80	31.6	84	60	30.3	80	69	29.1	77	69
14	38.6	80	31.4	81	60	30.3	79	69	28.4	74	66
17	41.4	80	33.6	81	60	31.7	77	68	30.3	73	66
21	42.2	80	34.4	82	60	32.7	78	68	30.8	73	66
25	43.6	79	36.1	83	60	33.9	78	68	32.4	74	66
30	47.6	79	38.2	80	60	35.0	74	68	33.0	69	65
33	49.0	79	39.3	80	60	36.1	74	68	34.3	70	64
37	49.7	79	38.7	78	60	36.0	72	66	33.1	67	63
41	50.5	79	39.3	78	60	37.0	73	66	34.0	67	60
45	50.9	79	40.0	79	60	37.5	74	66	35.2	69	60
49	52.2	79	41.4	79	60	38.4	74	66	35.6	68	60
53	52.5	79	41.6	79	60	38.5	73	66	35.6	68	60
57	52.2	79	43.0	82	60	39.2	75	66	36.3	70	60
61	52.1	79	42.0	81	60	38.4	74	64	35.1	67	59
65	53.0	79	43.0	81	60	39.4	74	64	37.0	70	58
69[d]	52.6	61	43.7	83	60	39.4	75	54	37.2	71	44
73	53.1	61	43.0	81	60	40.1	76	52	37.6	71	43
77	52.6	61	42.8	81	60	39.7	76	51	37.6	72	43
81	53.4	59	44.0	82	60	40.4	76	51	38.9	73	41
85	52.3	58	44.7	86	58	40.6	78	49	37.6	72	39
89	52.0	57	45.2	87	56	41.5	80	48	39.0	75	36
93	50.7	57	44.9	89	55	41.4	82	48	38.4	76	34
97	50.5	54	44.8	89	55	40.8	81	47	36.3	72	34
101	49.7	53	44.8	90	55	40.9	82	47	38.6	78	34
105	50.2	50	44.3	88	53	40.9	82	45	38.6	77	34
Mean for weeks											
1-13	31.2		28.4	91		27.7	89		26.8	86	
14-52	46.6		37.2	80		34.9	75		32.7	70	
53-105	51.9		43.7	84		40.1	77		37.4	72	

[a] Special studies were performed on 18 vehicle control males.
[b] Special studies were performed on nine 250 mg/kg males.
[c] Special studies were performed on ten 500 mg/kg males.
[d] Mice were removed for special study evaluation during week 66.

TABLE 14
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Weeks on Study	Vehicle Control Av. Wt (g)	Vehicle Control No. of Survivors	125 mg/kg Av. Wt (g)	125 mg/kg Wt (% of controls)	125 mg/kg No. of Survivors	250 mg/kg Av. Wt (g)	250 mg/kg Wt (% of controls)	250 mg/kg No. of Survivors	500 mg/kg Av. Wt (g)	500 mg/kg Wt (% of controls)	500 mg/kg No. of Survivors
1	18.8	60	19.0	101	60	18.7	100	60	19.3	103	60
2	19.8	60	19.8	100	60	19.6	99	59	19.3	98	59
3	21.7	60	21.5	99	60	20.6	95	59	20.5	95	59
4	23.3	60	22.0	94	60	21.5	92	59	21.7	93	59
5	23.8	60	22.4	94	60	21.9	92	59	22.1	93	59
6	25.0	59	24.0	96	60	22.9	92	59	23.4	94	59
7	25.7	59	24.0	93	60	22.7	88	59	22.4	87	59
8	26.6	59	25.1	94	60	23.7	89	59	23.2	87	59
9	27.5	59	25.3	92	60	23.8	87	59	23.4	85	59
10	28.0	59	25.7	92	60	24.2	86	59	23.7	85	59
11	29.6	59	26.3	89	60	24.3	82	59	23.7	80	59
12	30.5	59	26.8	88	60	25.2	83	59	24.2	79	59
13	31.4	59	26.6	85	60	25.2	80	59	24.8	79	58
14	32.4	59	27.2	84	60	25.4	78	59	24.5	76	58
17	34.7	59	28.6	82	60	26.1	75	59	25.5	74	58
21	34.2	59	28.7	84	60	26.7	78	59	25.8	75	57
25	36.1	59	29.4	81	60	27.9	77	59	26.7	74	56
30	39.5	59	31.6	80	60	27.8	70	59	27.4	69	54
33	41.6	59	32.6	78	60	28.5	69	59	27.5	66	52
37	44.1	59	32.1	73	59	28.3	64	59	27.2	62	52
41	45.1	59	32.3	72	59	28.2	63	57	28.5	63	52
45	46.3	59	33.3	72	59	29.7	64	56	28.7	62	52
49	47.9	58	33.2	69	59	29.5	62	56	28.7	60	51
53	50.2	58	34.4	69	59	30.5	61	56	28.9	58	51
57	49.2	58	34.8	71	58	30.2	61	55	30.0	61	51
61	50.0	58	35.0	70	58	30.9	62	55	30.4	61	50
65	51.7	58	35.4	69	58	30.6	59	55	30.2	58	50
69	52.5	57	35.8	68	58	31.2	59	55	30.5	58	50
73	53.0	57	35.2	66	58	31.8	60	55	31.1	59	49
77	55.2	56	35.4	64	58	31.8	58	55	32.1	58	49
81	55.9	56	35.6	64	58	31.4	56	52	31.6	57	49
85	53.1	56	35.9	68	58	31.6	60	52	31.3	59	48
89	54.7	56	36.5	67	57	32.5	59	52	32.8	60	48
93	54.0	55	36.8	68	57	32.4	60	50	32.6	60	47
97	51.8	54	36.5	71	56	32.5	63	48	32.7	63	46
101	50.9	50	36.7	72	52	32.8	64	46	32.8	64	44
105	49.7	48	37.0	74	50	33.3	67	45	33.7	68	41
Mean for weeks											
1-13	25.5		23.7	93		22.6	89		22.4	88	
14-52	40.2		30.9	77		27.8	69		27.1	67	
53-105	52.3		35.8	68		31.7	61		31.5	60	

Feed Consumption and Clinical Findings

Feed consumption by male and female mice, measured at 4-week intervals beginning at week 94 of the study, was similar to that by controls (Table 15). Clinical findings in dosed groups included hyperactivity, convulsions, and seizures. Convulsions occurred following handling of the mice and were observed initially in 250 and 500 mg/kg males and females during the fourth month of the study, in male mice during the eleventh month of the study, and in 125 mg/kg female mice during the ninth month of the study. No convulsions were observed in vehicle control groups, and if any convulsions occurred at times other than daily treatment or handling intervals, they were not recorded. Convulsions were not induced by auditory stimuli (i.e., hand clapping). Animals displayed periods of hyperactivity following convulsions. The frequency of convulsions is presented in Figure 9.

Hematology

Total leukocyte and lymphocyte counts in all dosed groups of males were significantly lower than those in the controls (Table G6). No other biologically significant differences in hematology parameters were observed in the remaining dosed groups of male mice or in dosed groups of females.

TABLE 15
Feed Consumption by Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

	Vehicle Control		125 mg/kg		250 mg/kg		500 mg/kg	
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)
Male								
93	5.1	50.7	5.4	44.9	6.1	41.4	6.7	38.4
97	5.0	50.5	4.8	44.8	5.1	40.8	5.1	36.3
101	6.7	49.7	7.1	44.8	7.5	40.9	8.0	38.6
105	5.8	50.2	5.3	44.3	5.8	40.9	6.5	38.6
Mean	5.7	50.3	5.6	44.7	6.1	41.0	6.6	38.0
Female								
93	4.9	54.0	5.5	36.8	5.8	32.4	5.6	32.6
97	5.1	51.8	5.0	36.5	5.3	32.5	4.9	32.7
101	6.7	50.9	6.6	36.7	5.9	32.8	7.0	32.8
105	6.0	49.7	5.7	37.0	5.2	33.3	5.2	33.7
Mean	5.7	51.6	5.7	36.8	5.5	32.7	5.7	32.9

[a] Grams of feed consumed per animal per day

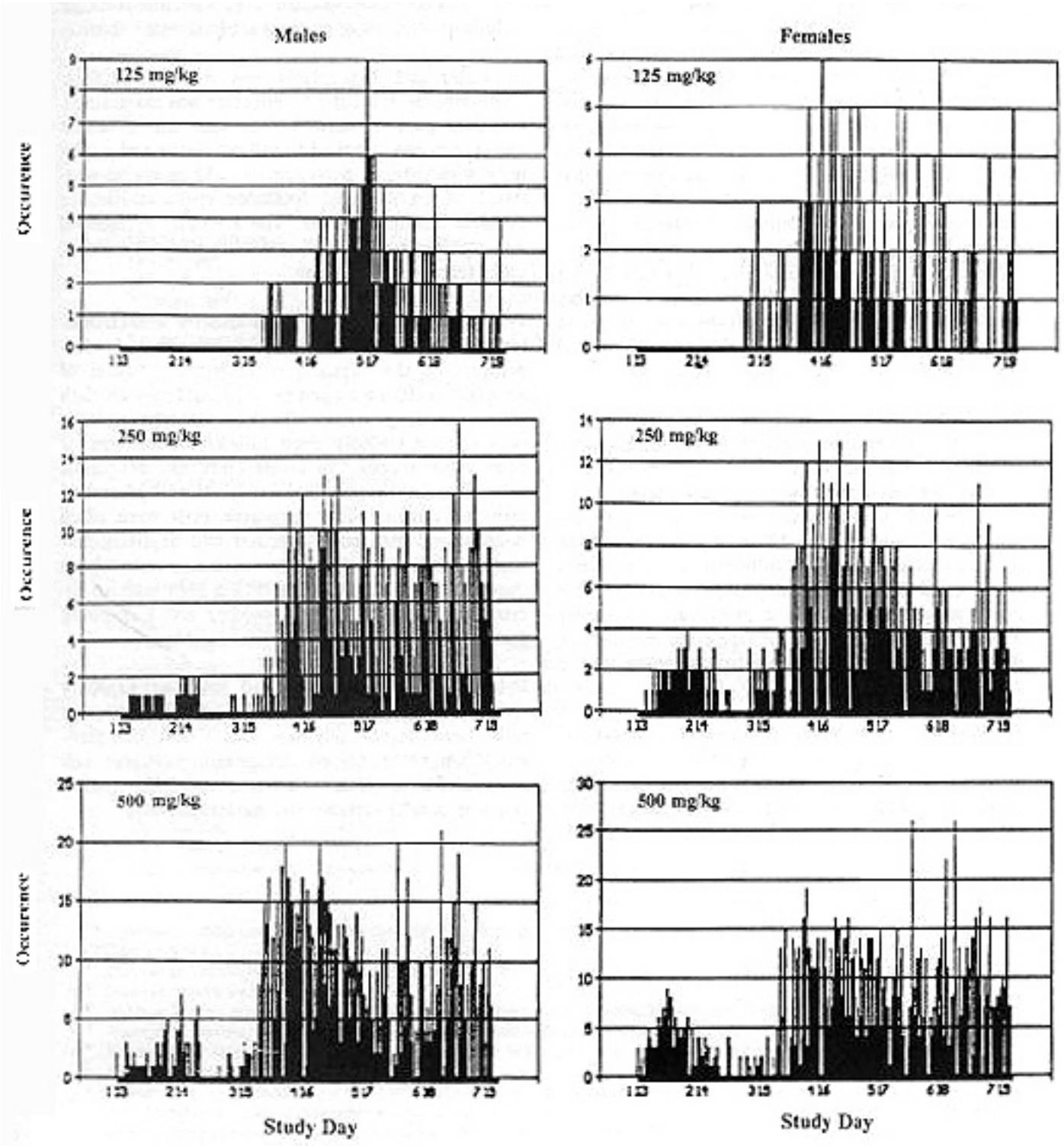


FIGURE 9
Daily Convulsion Incidence for Mice Administered THC in Corn Oil by Gavage for 2 Years. Note scale differences in the figures.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the thyroid gland, liver, forestomach, and urinary tract. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice. The total number of neoplasm-bearing mice and the incidences of benign and malignant neoplasms in male (Table C3) and female (Table D3) mice were decreased in a dose-related manner.

Thyroid Gland: Marginally increased incidences of follicular cell adenoma occurred in 125 mg/kg males and females (Tables 16, C3, and D3), but the incidences did not increase with increasing dose. Additionally, one carcinoma was observed in a vehicle control male and one in a 125 mg/kg female, and the incidence of thyroid gland follicular cell hyperplasia was increased in all dosed groups of mice. This would suggest an increase in proliferative follicular cell lesions, but no clear developmental progression from hyperplasia to adenoma to carcinoma by the end of the study.

Proliferation of follicular cells is generally considered to follow a developmental progression from hyperplasia to adenomas and carcinomas. As with other endocrine glands, clear distinction between these categories is sometimes difficult because morphologic criteria are not always predictive of biologic behavior.

Follicular cell hyperplasia was focal or diffuse. Generally, the follicular architecture was maintained. Follicles were of variable size and the follicular epithelium was cuboidal to tall columnar and nuclei were sometimes hyperchromatic. Hyperplasia consisted of an enlarged follicular space containing multiple smaller follicles. The follicular epithelium was multilayered in small areas, but was not a prominent feature of the lesion.

Follicular cell adenomas were usually well-circumscribed, expansile lesions that often caused compression of the adjacent parenchyma. Nuclei of neoplastic cells were often more hyperchromatic than the surrounding thyroid follicular cells and neoplastic cells formed variably sized follicular structures or large cystic spaces. In larger cysts, the neoplastic cells often formed papillary structures that protruded into the lumen. The neoplastic cells were often multilayered with both follicular and papillary patterns. Follicular cells in the adenoma varied from cuboidal to columnar, often with a high nucleus-to-cytoplasm ratio. Nuclear crowding was a common feature and the mitotic rate was variable.

Follicular cell carcinomas had solid to papillary follicular cell patterns. Occasionally, the follicular cells were highly pleomorphic. Cellular pleomorphism often helped distinguish follicular cell carcinoma from follicular cell adenoma. The mitotic rate was usually variable and moderately high.

TABLE 16
Incidences of Thyroid Gland Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Dose	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
Male				
Thyroid Gland[a]	62	60	61	57
Follicular Cell Hyperplasia[b]	16 (1.1)[c]	48** (1.6)	45** (2.0)	27**(1.7)
Follicular Cell Adenoma				
Overall rate[d]	0/62 (0%)	6/60 (10%)	3/61 (5%)	1/57 (2%)
Adjusted rate[e]	0.0%	11.1%	6.7%	3.0%
Terminal rate[f]	0/50 (0%)	5/53 (9%)	3/45 (7%)	1/33 (3%)
First incidence (days)	–[h]	725	730 (T)	730 (T)
Logistic regression test[g]	P=0.504	P=0.020	P=0.104	P=0.417
Follicular Cell Adenoma or Carcinoma (Combined)[i]				
Overall rate	1/62 (2%)	6/60 (10%)	3/61 (5%)	1/57 (2%)
Adjusted rate	1.8%	11.1%	6.7%	3.0%
Terminal rate	0/50 (0%)	5/53 (9%)	3/45 (7%)	1/33 (3%)
First incidence (days)	678	725	730 (T)	730 (T)
Logistic regression test	P=0.537N	P=0.059	P=0.262	P=0.690
Female				
Thyroid Gland	60	60	60	60
Follicular Cell Hyperplasia	28 (1.5)	46** (1.7)	40** (1.5)	33 (1.4)
Follicular Cell Adenoma				
Overall rate	4/60 (7%)	9/60 (15%)	3/60 (5%)	1/60 (2%)
Adjusted rate	8.5%	18.0%	6.8%	2.4%
Terminal rate	4/47 (9%)	9/50 (18%)	3/44 (7%)	1/41 (2%)
First incidence (days)	737 (T)	737 (T)	737 (T)	737 (T)
Logistic regression test	P=0.075N	P=0.143	P=0.536N	P=0.223N
Follicular Cell Adenoma or Carcinoma (Combined)[j]				
Overall rate	4/60 (7%)	10/60 (17%)	3/60 (5%)	1/60 (2%)
Adjusted rate	8.5%	20.0%	6.8%	2.4%
Terminal rate	4/47 (9%)	10/50 (20%)	3/44 (7%)	1/41 (2%)
First incidence (days)	737 (T)	737 (T)	737 (T)	737 (T)
Logistic regression test	P=0.065N	P=0.095	P=0.536N	P=0.223N

** Significantly different (P≤0.01) from the control by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)
[d] Number of animals with neoplasm per number of animals with examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value 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 logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dosed group is indicated by N.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year NTP gavage studies with corn oil vehicle control groups (mean ± standard deviation): 15/929 (1.6% ± 1.4%); range, 0%-4%
[j] Historical incidence: 19/934 (2.0% ± 2.6%); range, 0%-8%

Liver: Significantly decreased incidences of hepatocellular adenomas and carcinomas occurred in dosed groups of males and females, as did decreased incidences of eosinophilic foci and fatty change (Tables 17, Cl, C5, D1, and D5). The incidence of hepatocellular adenoma or carcinoma (combined) in 500 mg/kg males was below that observed in recent NT? 2-year gavage studies (range, 14%-72%). The decrease was probably related to decreased body weight (Haseman *et al.*, 1994; Seilkop, 1995).

Forestomach: Increased incidences of forestomach hyperplasia (vehicle control, 7/62; 125 mg/kg, 33/58; 250 mg/kg, 38/58; 500 mg/kg, 18/56) and ulcers (5/62, 17/58, 14/58, 8/56) occurred in all groups of males administered THC (Table CS). No increased incidences of forestomach hyperplasia or ulcers were observed in females. The increased incidence of ulcerations and hyperplasia in males may have been secondary to the gavage process.

TABLE 17
Incidences of Hepatocellular Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol

Dose	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
Male				
Clear Cell Foci				
Overall rate[a]	7/62 (11%)	1/60 (2%)	0/61 (0%)	0/57 (0%)
Logistic regression test[b]	P=0.004N	P=0.028N	P=0.014N	P=0.031N
Eosinophilic Foci				
Overall rate	18/62 (29%)	1/60 (2%)	0/61 (0%)	0/57 (0%)
Logistic regression test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Fatty Change				
Overall rate	20/62 (32%)	11/60 (18%)	1/61 (2%)	1/57 (2%)
Logistic regression test	P<0.001N	P=0.040N	P<0.001N	P<0.001N
Hepatocellular Adenoma				
Overall rate	25/62 (40%)	11/60 (18%)	6/61 (10%)	2/57 (4%)
Adjusted rate[c]	45.3%	19.8%	12.8%	5.6%
Terminal rate[d]	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
Hepatocellular Carcinoma				
Overall rate	10/62 (16%)	3/60 (5%)	5/61 (8%)	1/57 (2%)
Adjusted rate	18.2%	5.4%	10.5%	2.9%
Terminal rate	6/50 (12%)	2/53 (4%)	3/45 (7%)	1/34 (3%)
First incidence (days)	554	563	574	730 (T)
Logistic regression test	P=0.014N	P=0.052N	P=0.165N	P=0.020N
Hepatocellular Adenoma or Carcinoma (Combined)[e]				
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

(continued)

TABLE 17
Incidences of Hepatocellular Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of 1-Trans-Delta^9-Tetrahydrocannabinol (continued)

Dose	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
Female				
Clear Cell Foci				
Overall rate	1/60 (2%)	3/60 (5%)	0/59 (0%)	0/60 (0%)
Logistic regression test	P=0.172N	P=0.310	P=0.513N	P=0.527N
Eosinophilic Foci				
Overall rate	9/60 (15%)	0/60 (0%)	1/59 (2%)	1/60 (2%)
Logistic regression test	P=0.008N	P=0.002N	P=0.013N	P=0.017N
Fatty Change				
Overall rate	13/60 (22%)	3/60 (5%)	0/59 (0%)	2/60 (3%)
Logistic regression test	P=0.001N	P=0.007N	P<0.001N	P=0.006N
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
Hepatocellular Carcinoma				
Overall rate	6/60 (10%)	5/60 (8%)	4/59 (7%)	1/60 (2%)
Adjusted rate	12.2%	9.8%	8.8%	2.2%
Terminal rate	4/47 (9%)	4/49 (8%)	3/44 (7%)	0/41 (0%)
First incidence (days)	706	661	674	701
Logistic regression test	P=0.058N	P=0.494N	P=0.420N	P=0.082N
Hepatocellular Adenoma or Carcinoma (Combined)[f]				
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] Number of neoplasm-bearing animals/number of animals examined microscopically.
[b] Beneath the control incidence is the P value 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 logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Historical incidence for 2-year NTP gavage studies with corn oil vehicle control groups (mean ± standard deviation): 388/951 (40.8% ± 15.1%); range, 14%-72%
[f] Historical incidence: 133/948 (14.0% ± 8.0%); range, 2%-34%

Urinary Tract: Slightly increased incidences of focal to multifocal chronic inflammation in the renal pelvis occurred in 500 mg/kg males (1/62, 2/60, 5/61, 12/60; Table C5). Similarly, focal to multifocal chronic inflammation of the urinary bladder occurred in 500 mg/kg males (0/62, 0/60, 4/61, 9/58). Urinary bladder transitional epithelium hyperplasia also occurred in 500 mg/kg males (0/62, 0/60, 2/61, 8/58). The epithelial hyperplasia in the urinary bladder was not considered to be directly related to the administration of THC, but was considered to be secondary to the inflammatory lesions.

GENETIC TOXICOLOGY

There is little evidence for mutagenic activity attributable to THC *in vitro* or *in vivo*. THC (100 to 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Zeiger *et al.*, 1988; Table EI). In cytogenetic tests with cultured Chinese hamster ovary cells, THC induced dose-related increases in sister chromatid exchanges in the presence of S9; however, only at the highest scorable dose (12.5 µg/mL) was the response significantly different from the control level (Table E2). Significant slowing of the cell cycle was observed at doses of 10 µg/mL and above, necessitating a delayed harvest to allow sufficient cells to accumulate for evaluation. No induction of chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with THC, with or without S9 (Table E3). Severe toxicity was noted at the highest dose scored in the absence of S9 (15 µg/mL) and only 28 cells were evaluated for chromosomal aberrations at this dose level.

The single *in vivo* assay that was performed with THC provided no evidence of induced chromosomal damage. No increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples obtained from male and female mice at the end of the 13-week study (Table E4).

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-tetrahydro–cannabinol (THC). TUC 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, TI-IC 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 TUC. 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 TUC 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 TUC 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 (y-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 TUC-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 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 TI-IC 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 NT? 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 TI-IC 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. TUC 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-Delta⁹-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	–[e]	709	–
Logistic regression test[f]	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–delta9–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.

* 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.

Abel, E.L. (1984). Effects of delta^9-THC on pregnancy and offspring in rats. *Neurobehav. Toxicol. Teratol.* **6**, 29-32.

Abel, E.L., and Subramanian, M.G. (1990). Effects of low doses of alcohol on delta-9-tetrahydro-cannabinol's effects in pregnant rats. *Life Sci.* **47** 1677-1682.

Abel, E.L., Bush, R., Dintcheff, B.A., and Ernst, C.A.S. (1981). Critical periods for marihuana-induced intrauterine growth retardation in the rat. *Neurobehav. Toxicol. Teratol.* **3**, 351-354.

Abood, M.E., and Martin, B.R. (1992). Neurobiology of marijuana abuse. *Trends Pharmacol. Sci.* **13**, 201-206.

Abood, M.E., Sauss, C., Fan, F., Tilton, C.L., and Martin, B.R. (1993). Development of behavioral tolerance to delta^9-THC without alteration of cannabinoid receptor binding or mRNA levels in whole brain. *Pharmacol. Biochem. Behav.* **46**, 575-579.

Abramson, H.A. (1974). Respiratory disorders and marijuana use. *J. Asthma Res.* **11**, 97.

Adams, M.D., Chait, L.D., and Earnhardt, J.T. (1976). Tolerance to the cardiovascular effects of delta^9-tetrahydrocannabinol in the rat. *Br. J. Pharmacol.* **56**, 43-48.

Adashi, E.Y., Jones, P.B., and Hsueh, A.J. (1983). Direct antigonadal activity of cannabinoids: Suppression of rat granulosa cell functions. *Am. J. Physiol.* **244**, 177-185.

Addiction Research Foundation/World Health Organization (ARF/WHO) (1981). Report of an Addiction Research Foundation/World Health Organization Scientific Meeting on Adverse Health and Behavioral Consequences of Cannabis Use. Addiction Research Foundation, Toronto.

Agurell, S., Nilsson, I.M., Ohlsson, A., and Sandberg, F. (1970). On the metabolism of tritium-labeled delta-1-tetrahydrocannabinol in the rabbit. *Biochem. Pharmacol.* **19**, 1333-1339.

Agurell, S., Dewey, R.E., and Willette, R.E. (1984). In *The Cannabis: Chemical, Pharmacologic, and Therapeutical Aspects* (S. Agurell, W.L. Dewey, and R.E. Willette, Eds.). Academic Press, New York.

Agurell, S., Halldin, M., Lindren, J.E., Ohlsson, A., Widman, M., Gillespie, H., and Hollister, L. (1986). Pharmacokinetics and metabolism of delta1- tetrahydrocannabinol and other cannabinoids with emphasis on man. *Pharmacol. Rev.* **38**, 21-43.

Armitage, P. (1971). *Statistical Methods in Medical Research,* pp. 362-365. John Wiley and Sons, New York.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., and Puaerstein, C.J. (1979). Acute decreases in serum prolactin concentrations caused by delta-9-tetrahydro-cannabinol in nonhuman primates. *Fertil. Steril.* **32**, 571-575.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., Paurstein, C.J. (1981). Effects of delta 9-tetrahydrocannabinol during the follicular phase of the rhesus monkey (*Maccaca mulata*). *J. Clin. Endocrinol. Metab.* **52**, 50-55.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., and Lindner, H.R. (1977). Acute effect of delta1–tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in the rat. *Neuroendocrinology* **23**, 31-42.

Blevins, R.D., and Shelton, M.S. (1983). Response of Salmonella typhimurium mutants to D^9-THC and in conjunction with known mutagens. *J. Environ. Sch. Health* **A18**, 413-443.

Bloch, E., Fishman, R.H.B., Morrill, G.A., and Fujimoto, G.I. (1986). The effect of intragastric administration of D^9-tetrahydrocannabinol on the growth and development of fetal mice of the A/J strain. *Toxicol. Appl. Pharmacol.* **82**, 378-382.

Bonnin, A., Ramos, J.A., Rodriguez de Fonseca, F., Cebeira, M., and Fernández-Ruiz, J.J. (1993). Acute effects of delta^9-tetrahydrocannabinol on tuberoinfundibular dopamine activity, anterior pituitary sensitivity to dopamine and prolactin release vary as a function of estrous cycle. *Neuroendocrinology* **58**, 280-286.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Borgen, L.A., Davis, W.M., and Pace, H.B. (1971). Effects of synthetic D^9-tetrahydrocannabinol on pregnancy and offspring in the rat. *Toxicol. Appl. Pharmacol.* **20**, 480-486.

Bornheim, L.M. (1989). Effect of cannabidiol on drug metabolism. In *Biochemistry and Physiology of Substance Abuse* (R.R. Watson, Ed.), Vol. 1, pp. 22-35. CRC Press, Boca Raton, FL.

Boulaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., Le Fur, G., and Casellas, P. (1993). Cannabinoid-receptor expression in human leukocytes. *Eur. J. Biochem.* **214**, 173-180.

Bronson, M., Latour, C., and Nahas, G.G. (1984). Distribution and disposition of delta–9–tetrahydrocannabinol (THC) in different tissues of the rat. In *The Cannabis: Chemical, Pharmacologic, and Therapeutic Aspects* (S. Agurell, W.L. Dewey, and R.E. Willette, Eds.), pp. 309-317. Academic Press, New York.

Burstein, S. (1992). Eicosanoids as mediators of cannabinoid action. In *Marihuana/Cannabinoids Neurobiology and Neurophysiology* (L. Murphy and A. Bartke, Eds.), pp. 73-91. CRC Press, Boca Raton, FL.

Burstein, S., and Kupfer, D. (1971). Hydroxylation of trans–delta–1–tetrahydrocannabinol by hepatic microsomal oxygenases. *Ann.. N.Y. Acad. Sci.* **191**, 61-67.

Cabral, G.A., and Vasquez, R. (1991). Marijuana decreases macrophage antiviral and antitumor activities. *In Physiopathology of Illicit Drugs: Cannabis, Cocaine, Opiates. Advances in the Biosciences* (G.G. Nahas and C. Latour, Eds.), pp. 93-105. Pergamon Press, Oxford.

Cabral, G.A., and Vasquez, R. (1992). Delta-9-tetrahydrocannabinol suppresses macrophage extrinsic anti-herpes virus activity. *Proc. Soc. Exp. Biol. Med.* **192**, 205-263.

Cabral, G.A., Lockmuller, J.C., and Mishkin, E.M.
(1986). Delta^9-tetrahydrocannabinol decreases alpha! beta interferon response to herpes simplex virus type 2 in the B6C3F$_1$ mouse. *Proc. Soc. Exp. Biol. Med.* **181**, 305-311.

Caplan, G.A., and Brigham, B.A. (1990). Marijuana smoking and carcinoma of the tongue. *Cancer* **66**, 1005-1006.

References

Chakravarty, I., Sheth, A.R., and Ghosh, J.J. (1975). Effect of acute delta-9-tetrahydrocannabinol treatment on serum luteinizing hormone and prolactin levels in adult female rats. *Fertil. Steril.* **26**, 947-948

Chao, F., Green, D.E., Forrest, I.S., Kaplan, J.N., and Winship-Ball, A. (1976). The passage of 14-C-delta-9-tetrahydrocannabinol into the milk of lactating squirrel monkeys. *Res. Commun. Chem. Pathol. Pharmacol.* 15, 303-317.

Code of Federal Regulations (CFR) 21, Part 58.

Committee of the Institute of Medicine (Comm. Inst. Med.) (1982). Marijuana and Health (A.S. Relman, Chairman). National Academy Press, Washington, DC.

Corcoran, M.E., McCaughrin, J.A. Jr., Wada, J.A. (1978). Antiepileptic and prophylactic effects of tetrahydrocannabinols in amygdaloid-kindled rats. *Epilepsia* **19**, 47-55.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology: Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehiman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dalterio, S., and Bartke, A. (1981). Fetal testosterone in mice: Effect of gestational age and cannabinoid exposure. *J. Endocrinol.* **91**, 509-514.

Dalterio, S., Badr, F., Bartke, A., and Mayfield, D. (1982). Cannabinoids in male mice: Effects on fertility and spermatogenesis. *Science* **216**, 315-316.

Desoize, B., Nahas, G.G., Leger, C., and Banchereau, J. (1981). Cannabinoids and the immunity system. *In Immunologic Considerations in Toxicology* (R.P. Sharma, Ed.), Vol. 2, pp. 61-82. CRC Press, Boca Raton, FL.

Desoize, B., Nahas, G.G., and Latour, C. (1991). Inhibition of macromolecular synthesis by cannabinoids in replicating cells. In *Physiopathology of Illicit Drugs: Cannabis, Cocaine, Opiates. Advances in the Biosciences* (G.G. Nahas and C. Latour, Eds.), pp. 107-118. Pergamon Press, Oxford.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946-1949.

Dewey, W.L. (1986). Cannabinoid pharmacology. *Pharmacol. Rev.* **38**, 151-173.

Dewey, W.L., Martin, B.R., and May, E.L. (1984). Canabioid stereoisomers. Pharmacological effects. In *CRC Handbook of Stereoisomers: Drugs in Psychopharmacology* (D.F. Smith, Ed.), pp. 317-326. CRC Press, Boca Raton, FL.

Dill, J.A., and Howlett, A.C. (1988). Regulation of adenylate cyclase by chronic exposure to cannabimimetic drugs. *J. Pharmacol. Exp. Ther.* **244**, 1157-1163.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl.. Statist.* **32**, 236-248.

Dixit, V.P., Sharma, V.N., and Lohiya, N.K. (1974). The effect of chronically administered cannabis extract on testicular function of mice. *Europ. J. Pharmacol.* **26**, 111-114.

Dixit, V.P., Arya, M., and Lohiya, N.K. (1975). The effect of chronically administered cannabis extract on the female genital tract of mice and rats. *Endokrinologie* **66**, 365-368.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis,* 1st ed., pp. 145-147. McGraw- Hill Book Company, Inc., New York.

Donald, P.J. (1991). Marijuana and upper aerodigestive tract malignancy in young patients. In *Physiopatho!ogy of Illicit Drugs: Cannabis, Cocaine, Opiates. Advances in the Biosciences* (G.G. Nahas and C. Latour, Eds.), Vol 80, pp. 39-54. Pergamon Press, Oxford.

Drew, W.G., and Slagel, D.E. (1973). Delta^9-TUC: Selective impairment of corticosterone uptake by limbic structures of the rat. *Neuropharmacology* **12**, 909-914.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eichenbaum, H., and Cohen, N.J. (1988). Representation in the hippocampus: What do hippocampal neurons code? *Trends Neurosci.* **11**, 244-248.

Eldridge, J.C., Murphy, L.L., and Landfield, P.W. (1991). Cannabinoids and the hippocampal glucocorticoid receptor: Recent findings and possible significance. *Steroids* **56**, 226-231.

Ferguson, R.P., Hasson, J., and Walker, S. (1989). Metastasic lung cancer in a young marijuana smoker. *JAMA* **261**, 41-42.

Field, E., and Tyrey, L. (1984). Delayed sexual maturation in the female rat during chronic exposure to delta-9-tetrahydrocannabinol. *Life Sci.* **35**, 1725-1730.

Fitton, A.G., and Pertwee, R.G. (1982). Changes in body temperature and oxygen consumption rate of conscious mice produced by intrahypothalamic and intracerebroventricular injections of delta^9- tetrahydrocannabinol. *Br. J. Pharmacol.* **75**, 409-414.

Fleischman, R.W., Hayden, D.W., Rosenkrantz, H., and Braude, M.C. (1975). Teratologic evaluation of delta-9-tetrahydrocannabinol in mice, including a review of the literature. *Teratology* **12**, 47-50.

Fleischman, R.W., Baker, J.R., and Rosenkrantz, H. (1979). Pulmonary pathologic changes in rats exposed to marihuana smoke for one year. *Toxicol. Appl. Pharmacol.* **47**,557-566.

Fried, P.A., and McIntyre, D.C. (1973). Electrical and behavioral attenuation of the anti-convulsant properties of delta^9-TNC following chronic administrations. *Psychopharmacologia* **31**, 215-227.

Friedman, H. (1991). Cannabis and immunity. In *Physiopathology of Illicit Drugs: Cannabis, Cocaine, Opiates. Advances in the Biosciences* (G.G. Nahas and C. Latour, Eds.), Vol. 80, pp. 79-92. Pergamon Press, Oxford.

Fujimoto, G.I., Rosenbaum, R.M., and Zieglar, D. (1978). Effects of marihuana extract given orally on male rat reproduction and gonads. 60th Annual Meeting of the Endocrine Society, p. 373.

Fujimoto, G.I., Kostellow, A.B., Rosenbaum, R., Morrill, G.A., and Bloch, E. (1979). Effects

of cannabinoids on reproductive organs in the female Fischer rat. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction, and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 441-447. Pergamon Press, New York.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Garrett, E.R. (1979). Pharmacokinetics and disposition of delta^9-tetrahydrocannabinol and its metabolites. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain.* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 105-121. Pergamon Press, New York.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.

Generoso, W.M., Cain, K.T., Cornett, C.V., and Shelby, M.D. (1985). Tests for induction of dominant-lethal mutations and heritable translocations with tetrahydrocannabinol in male mice. *Mutat. Res.* **143**, 51-53.

Gerber, W.F., and Schramm, L.C. (1969). Effect of marihuana extract on fetal hamsters and rabbits. *Toxicol. Appl. Pharmacol.* **14**, 276-282.

Graham, J.D.P (1986a). The bronchodilator action of cannabinoids. In *Cannabinoids as Therapeutic Agents* (R. Mechoulam, Ed.), pp. 147-158. CRC Press, Inc., Boca Raton, FL

Graham, J.D.P. (1986b). The cardiovascular action of cannabinoids. In: *Cannabinoids as Therapeutic Agents* (R. Mechoulam, Ed.), pp. 159-166. CRC Press, Inc., Boca Raton, FL.

Halldin, M.M., Widman, M., Bahr, C.V., Lindgren, J.E., and Martin, B.R. (1982). Identification of *in vitro* metabolites of D1-tetrahydrocannabinol formed by human livers. *Drug Metab. Dispos.* **10**, 297-301.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., and Rao, G.N. (1992). Effects of corn oil, time-related changes, and inter-laboratory variability on tumor occurrence in control Fischer 344 (F344/N) rats. *Toxicol. Pathol.* **20**, 52-60.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F1 (B6C3F$_1$) mice. *JNCI* **75**, 975-984.

Haseman, J.K., Bourbina, J., and Eustis, S.L. (1994). The effect of individual housing and other factors on tumor incidence in B6C3F$_1$ mice. *Fundam. Appl. Toxicol.* **23**, 44-52.

Heath, R.G., Fitzjarrell, A.T., Garey, R.E., and Myers, W.A. (1979). Chronic marihuana smoking: Its effect on function and structure of the primate brain. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 713-730. Pergamon Press, New York.

Heath, R.G., Fitzjarrell, A.T., Fontana, C.J., and Garey, R.E. (1980). Cannabis sativa: Effects on brain function and ultrastructure in rhesus monkeys. *Biol. Psychiatry* **15**, 657-690.

Hembree, W.C., III, Nihas, G.G., Zeidenberg, P., and Huang, H.F.S. (1991). Changes in human spermatozoa associated with high dose marihuana smoking. In *Physiopathology of Illicit Drugs: Cannabis, Cocaine, Opiates. Advances in the Biosciences* (G.G. Nahas and C. Latour, Eds.), p. 80. Pergamon Press, Oxford.

Henrich, R.T., Shinohara, 0., Nogawa, T., and Moroshima, A. (1983). Effects of chronic administration of THC on early embryonic development of mice. In *The Cannabinoids: Chemical, Pharmacologic and Therapeutic Aspects* (S. Agurell, W.L. Dewey, and R.E. Willette, Eds.). Academic Press, New York.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., DeCosta, B.R., and Rice, K.C. (1990). Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci.* **87**, 1932-1936.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods,* pp. 120-123. John Wiley and Sons, New York.

Hollister, L.E. (1984). Health aspects of cannabis. *Pharmacol Rev.* 38, 1-20.

Holtzman, D., Lovell, R.A., Jaffe, J.H., and Freedman, D.X. (1969). 1-Delta-9-tetrahydrocannabinol: Neurochemical and behavioral effects in the mouse. *Science* **163**, 1464-1467.

Howlett, A.C., Bidaut-Russell, M., Devane, W.A., Melvin, L.S., Johnson, M.R., and Herkenham, M. (1990). The cannabinoid receptor: Biochemical, anatomical and behavioral characterization. *Trends Neurosci.* **13**, 420-423.

Huang, H.F.S., Nahas, G.G., and Hembree, W.C., III. (1979). Effects of marihuana inhalation on spermatogenesis of the rat. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 419-427. Pergamon Press, New York.

Hunt, C.A., and Jones, R.T. (1980). Tolerance and disposition of tetrahydrocannabinol in man. *.J. Pharmacol. Exp. Ther.* **215**, 35-44.

Hutchings, D.E., and Dow-Edwards, D. (1991). Animal models of opiate, cocaine, and cannabis use. *Clin. Perinatol.* **18**, 1-22.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Assay Data Management & Statistical Software Package (version 1.4). P.O. Box 13501, Research Triangle Park, NC 27709.

Iversen, L.L. (1993). Medical uses of marijuana? *Nature* **365**, 12-13.

Jakubovic, A., and McGeer, P.L. (1977). Biochemical changes in rat testicular cells *in vitro* produced by cannabinoids and alcohol: Metabolism and incorporation of labeled glucose, amino acids, and nucleic acid precursors. *Toxicol. Appl. Pharmacol.* **41**, 473-486.

Jakubovic, A., Hattori, T., and McGeer, P.L. (1973). Radioactivity in suckled rats after giving 14C-tetrahydrocannabinol to the mother. *Eur. J. Pharmacol.* **22**, 221-223.

Jakubovic, A., McGeer, E.G., and McGeer, P.L. (1979). Effects of cannabinoids on testosterone and protein synthesis in rat testis Leydig cells *in vitro*. *Mol. Cell. Endocrinol.* **15**, 41-50.

Johnson, R.J., and Wierseman, V. (1974). Repression of bone marrow leukopoiesis by delta-9- tetrahydrocannabinol. *Res. Commun. Chem. Pathol. Pharmacol.* **7**, 613-616.

References

Jonckheere, A.R. (1954). A distribution-free k-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Joneja, M.G. (1976). A study of teratological effects of intravenous, subcutaneous, and intragastric administration of D^9-tetrahydrocannabinol in mice. *Toxicol. Appl. Pharmacol*. **36**, 151-162.

Joneja, M.G., and Kaiserman, M.Z. (1978). Cytogenetic effects of delta-9-tetrahydrocannabinol (Delta^9-THC) on hamster bone marrow. *Experientia* **34**, 1205-1206.

Jones, R.T., and Benowitz, N. (1976). The 30-day trip - Clinical studies of cannabis tolerance and dependence. In *Pharmacology of Marihuana* (M.C. Braude and S. Szara, Eds.), Vol. 2, pp. 627-642. Raven Press, New York.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc*. **53**, 457-481.

Kari, F.W., and Abdo, K.M. (1996). Feed restriction desensitizes the NTP bioassay for carcinogen hazard evaluation. (in press).

Karler, R., Cely, W., and Turkanis, S.A. (1974). Anticonvulsant properties of delta^9-tetrahydrocannabinol and other cannabinoids. *Life Sci*. **15**, 931-947.

Karler R., Calder, L.D., and Turkanis, S.A. (1986). Prolonged CNS hyperexcitability in mice after a single exposure to delta-9-tetrahydrocannabinol. *Neuropharmacology* **25**, 441-446.

Kennedy, J.S., and Waddell, W.J. (1972). Whole-body autoradiography of the pregnant mouse after administration of 14C-delta^9-THC. *Toxicol. Appl. Pharmacol*. **22**, 252-258.

Kramer, J., and Ben-David, M. (1978). Prolactin suppression by (-)delta-9-tetrahydrocannabinol (THC): Involvement of serotonergic and dopaminergic pathways. *Endocrinology* **103**, 452-457.

Landfield, P.W., Cadwallader, L.B., and Vinsant, S. (1988). Quantitative changes in hippocampal structure following long–term exposure to D^9-tetrahydrocannabinol: Possible mediation by glucocorticoid systems. *Brain Res*. **443**, 47-62.

Law, B., Mason, P.A., Moffat, A.C., Gleadle, R.I., and King, L.J. (1984). Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin. *J. Pharm. Pharmacol*. **36**, 289-294.

Lefkowitz, S.S., Kiager, K., Nemeth, D., and Pruess, M. (1978). Immunosuppression of mice by delta–9-tetrahydrocannabinol. *Res. Commun. Chem. Pathol. Pharmacol*. **19**, 101-107.

Legator, M.S., Weber, E., Connor, T., and Stoeckel, M. (1974). Failure to detect mutagenic effects of delta^9-tetrahydrocannabinol in the dominant lethal test, host-mediated assay, blood–urine studies, and cytogenic evaluation with mice. In *The Pharmacology of Marijuana* (M.C. Braude and S. Szara, Eds.), Vol. 2, pp. 699-709. Raven Press, New York.

Lemberger, L. (1972). The metabolism of the tetrahydrocannabinols. *Adv. Pharmacol. Chemother.*
10, 221-256.

Lemberger, L., and Rubin, A. (1976). *Physiologic Disposition of Drugs of Abuse,* pp. 269-310. Spectrum Publications, Inc., New York.

Lemberger, L., Axeirod, J., and Kopin, I.J. (1971). Metabolism and disposition of tetrahydrocannabinol in naive subjects and chronic marijuana users. *Ann. NY Acad. Sci*. **191**, 142-154.

Leuchtenberger, C., and Leuchtenberger, R. (1984). The effects of naturally occurring metabolites (L-cysteine, Vitamin C) on cultured human cells exposed to smoke of tobacco or marijuana cigarettes. *Cytometry* **5**, 396-402.

Leuschner, J.T.A., Wing, D.R., Harvey, D.J., Brent, G.A., Dempsey, C.E., Watts, A., and Paton, W.D.M. (1984). The partitioning of delta-1- tetrahydrocannabinol into erythrocyte membranes in vivo and its effect on membrane fluidity. *Experientia* **40**, 866-868.

Lewysohn, 0., Corfova, T., Nimrod, A., and Ayalon, D. (1984). The suppressive effect of delta-1- tetrahydrocannabinol on the steriodogenic activity of rat granulosa cells in culture. *Horm. Res.* **19**, 43-51.

Levy, J.A., and Heppner, G. (1978). Alterations in murine delayed type hypersensitivity responses by delta-8-THC and cannabinol. *J. ImmunopharmacoL* **1**, 105-114.

Levy, J.A., and Heppner, G.H. (1980). Immunosuppression by marihuana and its cannabinoid constituents. *J. Immunopharinacol.* **2**, 159-177.

Levy, J.A., Munson, A.E., Harris, L.S., and Dewey, L.W. (1974). Effects of delta-8 and delta-9- tetrahydrocannabinol on the immune response in mice. *Pharmacologist* **16**, 259.

Little, P.J., Compton, D.R., Johnson, M.R., and Martin, B.R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.* **247**,1046-1051.

Luthra, Y. (1979). Brain biochemical alterations in neonates of dams treated orally with delta^9-tetrahydrocannabinol during gestation and lactation. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 531-537. Pergamon Press, New York.

Luthra, Y.K., and Rosenkrantz, H. (1974). Cannabinoids: Neurochemical aspects after oral administration to rats. *ToxicoL AppL PharmacoL* **27**, 158-168.

Luthra, Y.K., Rosenkrantz, H., Heyman, l.A., and Braude, M.C. (1975). Differential neurochemistry and temporal pattern in rats treated orally with delta^9-tetrahydrocannabinol for periods up to six months. *ToxicoL Appl. Pharmacol.* **32**, 418-431.

McCarthy, L.E., Flora, KP., and Vishnuvajjala, B.R. (1984). Disparities in the antiemetic and behavioral actions of delta-9-tetrahydrocannabinol and its 11-OH metabolite in the cat. *Res. Commun. SubsL Abuse* **5**, 103-114.

McConnell, E.E., Solleveld, HA, Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., and Langlois, R.G. (1983). A simple fluorescent staining procedure for micronuclei and RNA in erythrocytes using Hoechst 33258 and pyronin Y. *Mutat. Res.*, **120**, 269-275.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Mclsaac, W.M., Fritchie, G.E., Idanpaan-Heikkila, J.E., Ho, B.T., and Englert, L.F. (1971). Distribution of marihuana in monkey brain and concomitant behavior effects. *Nature* **230**, 583-584.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

References

Margolin, B.H., Resnick, M.A., Rimpo, J.Y., Archer, P., Galloway, S.M., Bloom, A.D., and Zeiger, E. (1986). Statistical analyses for *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen.* **8**, 183-204.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *ToxicoL Pathol.* **10**, 71-80.

Martin, B.R. (1986). Cellular effects of cannabinoids. *Pharmacol. Rev.* **38**, 45-74.

Martin, P., and Consroe, P. (1976). Cannabinoid induced behavioral convulsions in rabbits. *Science* **194**, 965-967.

Mechoulam, R., Devane, W.A., and Glaser, R. (1992). Cannabinoid geometry and biological activity. In *Marijuana/ Cannabinoids. Neurobiology and Neurophysiology* (L. Murphy and A. Bartke, Eds.), pp. 1-33. CRC Press, Boca Raton, FL.

Mendelson, J.H., Mello, N.K., and Ellingboe, J. (1985). Acute effects of marihuana smoking on prolactin levels in human females. *J. PharmacoL Exp. Therap.* **232**, 220-222.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Montour, J.L., Dutz, W., and Harris, L.S. (1981). Modification of radiation carcinogenesis by marihuana. *Cancer* **47**, 1279-1285.

Morahan, P.S., Klykken, P.C., Smith, S.H., Harris, L.S., and Munson, A.E. (1979). Effects of cannabinoids on host resistance to Listeria monocytogenes and herpes simplex virus. *Infect. 1mmun.* **23**, 670-674.

Morishima, A. (1982). Drugs of abuse: Their effects on embryos and fetuses. *Acta Paediatr. Jpn.* **24**, 36-45.

Morris, R.R. (1985). Human pulmonary histopathological changes from marijuana smoking. *J. Forensic Sci* **30**, 345-349.

Morrison, D.F. (1976). *Multivariate Statistical Methods,* 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

Munson, A.E., Harris, L.S., Friedman, M.A., Dewey, W.L., and Carchman, R.A. (1975). Antineoplastic activity of cannabinoids. *J. Natl. Cancer Inst.* **55**, 597-602.

Murphy, L.L., Newton, S.C., Dhali, J., and Chavez, D. (1991). Evidence for a direct anterior pituitary site of delta-9-tetrahydrocannabinol action. *Pharmacol. Biochem. Behav.* **40**, 603-607.

Nahas, G.G. (1979). Current status of marijuana research. *JAMA* **242**, 2775-2778.

Nahas, G. (1993). General toxicity of cannabis. In *Cannabis Physiopathology, Epidemiology, and Detection* (G.G. Nahas and C. Latour, Eds.), pp. 5-17. CRC Press, Boca Raton, FL.

Nahas, G.G., and Paton, W.D.M. (1979). *Marihuana: Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Advances in the Biosciences,* Pergamon Press, Oxford and New York.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1987). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated December 1987). Research Triangle Park, NC.

Newton, S.C., Murphy, L.L., and Bartke, A. (1993). *In vitro* effects of psychoactive and non-psychoactive cannabinoids on immature rat Sertoli cell function. *Life Sc*i **53**, 1429-1437.

Nichols, W.W., Miller, R.C., Heneen, W., Bradt, C., Hollister, L., and Kanter, S. (1974). Cytogenetic studies on human subjects receiving marihuana and delta-9-tetrahydrocannabinol. *Mutat. Res.* **26**, 413-417.

Ohlsson, A., Lindgren, J.E., Wahlen, A., Agurell, S., Hollister, L.E., and Gillespie, H.K. (1980). Plasma delta-9-tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. *Clin. Pharmacol. Ther.* **28**, 409-416.

Ohlsson, A., Lindgren, J.E., Wahlen, A., Agurell, S., Hollister, L.E., and Gillespie, H.K. (1982). Single-dose kinetics of deuterium-labelled delta-1-tetrahydrocannabinol in heavy and light users. *Biomed. Mass Spectrom.* **9**, 6-10.

Okamoto, T., Chan, P.C., and So, B.T. (1972). Effect of tobacco, marijuana and benzo[a]pyrene on aryl hydrocarbon hydroxylase in hamster lung. *Life Sci.* **11**, 733-741.

Oviedo, A., Glowa, J., and Herkenham, M. (1993). Chronic cannabinoid administration alters cannabinoid receptor binding in rat brain: A quantitative autoradiographic study. *Brain Res.* **616**, 293-302.

Perez-Reyes, M., White, W.R., McDonald, S.A., Hicks, R.E., Jeffcoat, A.R., and Cook, C.E. (1991). The pharmacologic effects of daily marijuana smoking in humans. *PharmacoL Biochem. Behav.* **40**, 691-694.

Pertwee, R.G. (1988). The central neuropharmacology of psychotropic cannabinoids. *Pharmac. Ther.* **36**, 189-261.

Pertwee, R.G. (1991). Tolerance to and dependence on psychotropic cannabinoids. In *The Biological Bases of Drug Tolerance and Dependence* (J.A. Pratt, Ed.). Academic Press, NY.

Pertwee, R. (1993). The evidence for the existence of cannabinoid receptors. *Gen. Pharmacol.* **24**, 811-824.

Phillips, R.N., Turk, R.F., and Forney, R.B. (1971). Acute toxicity of delta^9-tetra-hydrocannabinol in rats and mice. Proc. Soc. Exp. Biol. Med. 136, 260-263.

Plasse, T.F., Gorter, R.W., Krasnow, S.H., Lane, M., Shepard, K.V., and Wadleigh, R.G. (1991). Recent clinical experience with dronabinol. *PharmacoL Biochem. Behav.* **40**, 695-700.

Price, P.3., Suk, W.A., Spahn, G.J., and Freeman, A.E. (1972). Transformation of Fischer rat embryo cells by the combined action of murine leukemia virus and (-)-trans-delta^9-tetrahydro-cannabinol. *Proc. Soc. Exp. Biol. Med.* **140**, 454-456.

Pross, S., Nakano, Y., Widen, R., McHugh, S., and Friedman, H. (1993). Age related immunomodulation in murine splenocytes induced by delta-9-tetra-hydrocannabinol (THC). *Mech. Ageing Dev.* **68**, 11-26.

References

Purohit, V., Ahluwahlia, B.S., and Vigersky, R.A. (1980). Marihuana inhibits dihydrotestosterone binding to the androgen receptor. *Endocrinology* **107**, 848-850.

Rawitch, A.B., Rohmer, R., and Vandaris, R.M. (1979). Delta-9-tetrahydrocannabinol uptake by adipose tissues. Preferential accumulation in gonadal fat organs. *Gen. Pharmacol.* **10**, 525-529.

Rao, G.N., Haseman, J.K., Grumbein, S., Crawford, D.D., and Eustis, S.L. (1990). Growth, body weight, survival, and tumor trends in (C57B1/6 x C3H/HeN) F1 ($B6C3F_1$) mice during a nine-year period. *ToxicoL PathoL* **18**, 71-77.

Rawlins, J.N.P. (1985). Associations across time: The hippocampus as a temporary memory store. Behav. Brain Sci. 8, 479-496.

Reich, R., Laufer, N., Lewysohn, 0., Cordova, T., Ayalon, D., and Tsafriri, A. (1982). *In vitro* effects of cannabinoids on follicular function in the rat. *Biol. Reprod.* **27**, 223-231.

Robison, L.L., Buckley, J.D., and Daigle, A.E. (1989). Maternal drug use and risk of childhood nonlymphoblastic leukemia among offspring. *Cancer* **63**, 1904-1910.

Rosenkrantz, H. (1982). Cannabis, marihuana and cannabinoid toxicological manifestations in man and animals. In *Adverse Health and Behavioral Consequences of Cannabis Use* (K.O. Fehr and H. Kalant, Eds.).

Rosenkrantz, H., and Esber, H.J. (1980). Cannabinoid-induced hormone changes in monkeys and rats. *J. Toxicol. Environ. Health* **6**, 297-313.

Rosenkrantz, H., Sprague, R.A., Fleischman, R.W., and Braude, M.C. (1975). Oral delta^9–THC toxicity in rats treated for period up to six months. *Toxicol. Appl. Pharmacol.* **32**, 399-417.

Sassenrath, E.N., Chapman, L.F., and Goo, G.P. (1979). Reproduction in rhesus monkeys chronically exposed to delta-9-tetrahydrocannabinol. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 501-512. Pergamon Press, New York.

Scallet, A.C., Uemura, E., Andrews, A., Ali, S.F., McMillan, D.E., Paule, M.G., Brown, R.M., and Slikker, W., Jr. (1987). Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydro- cannabinol (THC). *Brain Res.* **436**, 193-198.

Schatz, A.R., Koh, W.S., and Kaminski, N.E. (1993). Delta^9-tetrahydrocannabinol selectively inhibits T-cell dependent humoral immune responses through direct inhibition of accessory T-cell function. *Immunopharmacology* **26**, 129-137.

Schmeling, W.T., and Hosko, M.J. (1980). Effect of delta^9-tetrahydrocannabinol on hypothalamic thermosensitive units. *Brain Res.* **187**, 431-442.

Schmid, W. (1976). The micronucleus test for cytogenetic analysis. In *Chemical Mutagens, Principles and Methods for their Detection* (A. Hollaender, Ed.), Vol. 4, pp. 31-53. Plenum Press, New York.

Segal, M. (1986). Cannabinoids and analgesia. In *Cannabinoids as Therapeutic Agents* (R. Mechoulam, Ed.), pp. 105-120. CRC Press, Boca Raton, FL.

Seilkop, S.K. (1995). The effect of body weight on tumor incidence and carcinogenicity testing in $B6C3F_1$ mice and F344 rats. *Fundam. Appl. Toxicol.* **24**, 247-259.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. **Biometrics 33**, 386-389.

Singh, P.P., and Das, P.K. (1976). Role of catecholamines in the hypothermic activity of cannabis in albino rats. *Pychopharmacology* **50**, 199-204.

Slikker, W., Jr., Paule, M.G., Au, S.F., Scallet, A.C., and Bailey, J.R. (1991). Behavioral, neurochemical, and neurohistological effects of chronic marijuana smoke exposure in the nonhuman primate. In *Marijuana/Canabinoids. Neurobiology and Neurophysiology* (L. Murphy and A. Bartke, Eds.), pp. 219-273. CRC Press, Roca Raton, FL.

Smith, C.G., Smith, M.T., Besch, N.F., Smith, R.G., and Asch, R.H. (1979). Effects of delta^9–tetrahydrocannabinol (11-IC) on female reproductive function. In *Marihuana Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction and Brain* (G.G. Nahas and W.D.M. Paton, Eds.), pp. 449-467. Pergamon Press, New York.

Smith, C.G., Almirez, R.G., Berenberg, J., and Asch, R.H. (1980a). Tolerance develops to the disruptive effects of delta^9–tetrahydrocannabinol on primate menstrual cycle. *Science* **219**, 1453-1455.

Smith, C.G., Besch, N.F., and Asch, R.H. (1980b). Effects of marihuana on the reproductive system. In *Advances in Sex Hormone Research* (J.A. Thomas and R.L. Singhal, Eds.), pp. 273-294. Urban and Schwarzenberg, Baltimore, MD.

Smith, C.G., Almirez, R.G., Berenberg, J., and Asch, R.H. (1983). Tolerance develops to the disruptive effects of delta^9-tetrahydrocannabinol on primate menstrual cycle. *Science* **219**, 1453-1454.

Stefanis, C.N., and Issidorides, M.R. (1976). Cellular effects of chronic cannabis use in man. In Marihuana: *Chemistry, Biochemistry, and Cellular Effects* (G.G. Nahas, W.D.M. Paton, and J.E. Idanpaan- Heikkila, Eds.), p. 533. Springer-Verlag, New York.

Stenchever, M.A., and Allen, M. (1972). The effect of delta-9-tetrahydrocannabinol on the chromosomes of human lymphocytes in culture. *Am. J. Obstet. Gynecol.* **114**, 819-821.

Stenchever, M.A., Kunysz, T.J., and Allen, M.A. (1974). Chromosome breakage in users of marijuana. *Am. J. Obstet. Gynecol.* **118**, 106-113.

Stoeckel, M., Weber, E., Connor, T., and Legator, M.S. (1975). Failure to detect mutagenic effects of delta-9-tetrahydrocannabinol in *in vitro* and *in vivo* studies with mice. *Mutat. Res.* **31**, 313-314.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233.

Szepsenwol, J., Fletcher, J., and Toyo-Goyco, E. (1978). Effects of delta-9-tetrahydrocannabinol in mice. *Fed Proc.* **37**, 450.

Szepsenwol, J., Fletcher, J., Murison, G., and Toyo-Goyco, E. (1980). Carcinogenic effect of delta 9 tetrahydrocannabinol in mice. *Fed Proc.* **40**, 746.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Taylor, F.M., III. (1988). Marijuana as a potential respiratory tract carcinogen: A retrospective analysis of a community hospital population. *South. Med. J.* **81**, 1213-1216.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* **236**, 933-941.

Thompson, G.R., Mason, M.M., Rosenkrantz, H., and Braude, M.C. (1973). Chronic oral toxicity of cannabinoids in rats. *Toxicol. Appl. Pharmacol.* **25**, 373-390.

References

Treinen, K.A., Sneeden, J.L., and Heindel, J.J. (1993). Specific inhibition of FSH-stimulated cAMP accumulation by D^9-tetrahydrocannabinol in cultured rat granulosa cells. *Toxicol.. Appl. Pharmacol*. **118**, 53-57.

Turkanis, S.A., and Karler, R. (1984). Electrophysiological mechanisms of delta-9-tetrahydrocannabinol's convulsant actions. In *The Cannabinoids: Chemical Pharmacologic, and Therapeutic Aspects* (S. Agurell, W.L. Dewey, and R.E. Willette, Eds.), pp. 845-858. Academic Press, New York.

Turturro, A., Duffy, P.H., and Hart, R.W. (1993). Modulation of toxicity by diet and dietary macronutnient restriction. *Mutat. Res*. **295**, 151-164.

Van Went, G.F. (1978). Mutagenicity testing of 3 hallucinogens: LSD, psilocybin and Delta^9-THC using the micronucleus test. *Experientia* **34**, 324-325.

Vollmer, R.R., Cavero, I., Ertel, R.J., Solomon, TA., and Buckley, J.P. (1974). Role of the central autonomic nervous system in the hypotension and bradycardia induced by (-)-delta-9-trans-tetrahydrocannabinol. *J. Pharm. Pharmacol*. **26**, 186-192.

Watanabe, K., Yamamoto, I., Narimatsu, S., and Yoshimura, H. (1984). Is serotonin involved in hypothermic effect and its tolerance development of delta-8-tetra-hydrocannabinol and 11 hydroxy–delta-8- tetrahydrocannabinol in the mouse? *Res. Commun. Subst. Abuse* **5**, 89-101.

Watanabe, K., K.ijima, T., Narimatsu, S., Nishikami, J., Yamamoto, I., and Yoshimura, H. (1990). Comparison of pharmacological effects of tetrahydrocannabinols and their 11-hydroxy metabolites in mice. *Chem. Pharm. Bull*. **38**, 2317-2319.

Watanabe, K., Narimatsu, S., Matsunaga, T., Yamamoto, I., and Yoshimura, H. (1993). A cytochrome P450 isozyme having aldehyde oxygenase activity plays a major role in metabolizing cannabinoids by mouse hepatic microsomes. *Biochem. Pharmacol*. **46**, 405-411.

Well, M.E., Sadler, B.M., Brine, D., Taylor, H., and Perez-Reyes, M. (1984). Metabolism, disposition and pharmacokinetics of delta-9-tetrahydrocannabinol in male and female subjects. In *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects* (S. Agurell, W.L. Dewey, and R.E. Willette, Eds.), pp. 185-210. Academic Press, New York.

Westlake, T.M., Howlett, A.C., Ali, S.F., Paule, M.G., Scallet, A.C., and Slikker, W., Jr. (1991). Chronic exposure to D^9-tetrahydrocannabinol fails to irreversibly alter brain cannabinoid receptors. *Brain Res*. **544**, 145-149.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Wright, P.L., Smith, S.H., Keplinger, M.L., Calandra, J.C., and Braude, M.C. (1976). Reproductive and teratologic studies with delta^9-tetrahydrocanabinol and crude marijuana extract. *Toxicol. Appl. Pharmacol*. **38**, 223-235.

Yagiela, J.A., McCarthy, K.D., and Gibb, J.W. (1974). The effect of hypothermic doses of 1-delta-9- tetrahydrocannabinol on biogenic amine metabolism in selected parts of the rat brain. *Life Sci*. **14**, 2367-2378.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988). Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* **11** (Suppl. 12), 1-158.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four *in vitro* genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zias, J., Stark, H., Seligman, J., Levy, R., Werker, E., Breuer, A., and Mechoulam, R. (1993). Early medical use of cannabis. *Nature* **363**, 215.

Zimmerman, A.M., and Raj, A.Y. (1980). Influence of cannabinoids on somatic cells *in vivo*. *Pharmacology* **21**, 277-287.

Zimmerman, A.M., Zimmerman, S., and Raj, A.Y. (1979). Effects of cannabinoids on spermatogenesis in mice. In *Marihuana: Biological Effects. Analysis, Metabolism, Cellular Responses, Reproduction, and Brain* (G.G. Nahas and W.D.M Paton, Eds.), p. 407. Pergamon Press, Oxford and New York.

Zuardi, A.W., Teixeira, N.A., and Karniol, I.C. (1984). Pharmacological interaction of the effects of delta-9-trans-tetrahydrocannabinol and cannabidiol on serum corticosterone levels in rats. *Arch. Int. Pharmacodyn. Ther.* **269**, 12-19.