NATIONAL TOXICOLOGY PROGRAM
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CARCINOGENESIS BIOASSAY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

(CAS NO. 1746-01-6)

IN SWISS-WEBSTER MICE (DERMAL STUDY)

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

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In June 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

(CAS No. 1746-01-6)

in SWISS-WEBSTER MICE

(DERMAL STUDY)

NATIONAL TOXICOLOGY PROGRAM
Research Triangle Park
Box 12233
North Carolina 27709
and
Bethesda, Maryland 20205

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

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Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

TABLE OF CONTENTS

TABLES

FIGURES

APPENDIXES

CONTRIBUTORS

This bioassay was conducted at the Illinois Institute of Technology Research Institute (IITRI), Chicago, Illinois, initially under direct contract to NCI and later under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program. The chronic study began in October 1975 and ended in October 1977.

The project director was Mr. A. Shefner (1); Dr. M. E. King (1) was the principal investigator for this study; and Dr. P. Holmes (1,2) assembled the data. Doses of the test chemical were selected by Dr. O. G. Fitzhugh (3,5). Mr. T. Kruckeberg (1) and Mr. K. Kaltenborn (1) were in charge of animal care.

Necropsies were performed under the direction of Dr. A. R. Roesler (1). Histopathologic evaluations were performed by Dr. W. Richter (1). The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group with final approval by the NCI Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (4). Statistical analyses were performed by Dr. J. R. Joiner (5) and Ms. S. Vatsan (5) using methods selected for the bioassay program by Dr. J. J. Gart (6). Chemicals used in this bioassay were synthesized and analyzed under the direction of Dr. A. Gray (1), with the assistance of Mr. S. Cepa (1) and Mr. V. DaPinto (1). Further chemical analyses were conducted at Midwest Research Institute (7). The results of the chemical analytical work were reviewed by Dr. S. S. Olin (5).

This report was prepared at Tracor Jitco (5) under the direction of Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. R. L. Schueler, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; Dr. W. Theriault and Ms. M. Glasser, technical editors.

The following scientists at NCI/NTP (8) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Larry Hart, Dr. William V. Hartwell (Chemical Manager), Dr. Joseph Haseman, Dr. James E. Huff, Dr. C.W. Jameson, Dr. Y. Jack Lee, Dr. Ernest E. McConnell, Dr. John A. Moore, Dr. Sherman F. Stinson, Dr. Raymond Tennant, and Dr. Jerrold M. Ward.

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(1) IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616.
(2) Stauffer Chemical Company, Richmond Research Center, 1200 South 47th Street, Richmond, California 94804.
(3) Now at 4208 Dresden Street, Kensington, Maryland 20795.
(4) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland 20852.

(5) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland 20852.

(6) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.

(7) Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110.

(8) Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; National Toxicology Program, Research Triangle Park, Box 12233, North Carolina 27709.

SUMMARY

A carcinogenesis bioassay was conducted by applying an acetone suspension of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the clipped backs of 30 male and female Swiss-Webster mice 3 days per week for 99 or 104 weeks. Similar groups were pretreated with 1 application of 50 μg dimethylbenzanthracene (DMBA) in 0.1 ml acetone 1 week before TCDD administration began. Female mice received 0.005 μg TCDD per application, and the male mice received 0.001 μg TCDD. As vehicle controls, 45 mice of each sex received 0.1 ml acetone three times per week. Thirty animals of each sex were used as untreated controls.

Throughout the bioassay, mean body weights of the male and female mice administered TCDD, or TCDD following DMBA, were essentially the same as those of the corresponding vehicle-control group. Mean body weights of dosed and vehicle control groups of the males were less than those of the untreated control group throughout the study; for the females, mean body weights were less than the untreated controls during the first 80 weeks.

In female mice, the incidences of fibrosarcoma in the integumentary system in groups dosed with TCDD were significantly (P=0.007) higher than that in the corresponding controls (2/41, 5%; 8/27, 30%). An increase in the same tumor type, although not statistically significant (P=0.084), was also observed in the male mice (3/42, 7%; 6/28, 21%).

In the DMBA-TCDD experiment, failure to have included groups skin painted with only DMBA precludes interpretation of these results.

Under the conditions of this bioassay, 2,3,7,8-tetrachlorodibenzo-p-dioxin applied to the skin was not carcinogenic for male Swiss-Webster mice (the increase of fibrosarcomas in the integumentary system may have been associated with the skin application of TCDD). TCDD was carcinogenic for female Swiss-Webster mice causing fibrosarcomas in the integumentary system.

PEER-REVIEW PANEL AND COMMENTS

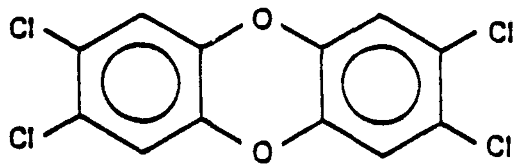
On June 27, 1980, this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Room 1331, Switzer Building, 330 C Street, S.W., Washington, D.C. Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, Thomas Shepard, and Alice Whittemore. Members of the Panel are: Drs. Norman Breslow, Joseph Highland, Charles Irving, Frank Mirer, Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams. Drs. Highland, Schwetz, and Swenberg were unable to attend the review.

Dr. Irving, as the primary reviewer for the report on the bioassay by the dermal route of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), agreed with the conclusion that, under the conditions of the bioassay, TCDD applied to the skin was carcinogenic for female Swiss-Webster mice, inducing increased incidences of fibrosarcomas in the integumentary system. He stated that this was not a well designed study. Criticisms were: (1) a maximal tolerated dose (MTD) was not determined, especially in male mice; (2) the initiation-promotion study with DMBA was poorly designed, as adequate controls, especially positive controls with a known promoter were not included, and the effect of the initiating dose of DMBA by itself was not examined; (3) only one dose/sex was used, and (4) the number of mice (30) in the TCDD exposed groups was considered marginal. Despite these observations, he considered the data to be valid (except for the DMBA groups).

As the secondary reviewer, Dr. Williams agreed with Dr. Irving's conclusions. He urged caution in trying to interpret the findings of an increased incidence of fibrosarcomas in female mice. He opined that the significance of subcutaneous sarcomas in mice is still not clear because of the ease with which this tumor type is induced by implanted inert plastics. Until this phenomenon is better understood mechanistically, an assessment of human risk cannot be made.

Dr. Irving moved that the report on the bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin be accepted provided it is made clear in the summary that the combination DMBA-TCDD experiments were not adequately designed. Dr. Williams seconded the motion and it was approved unanimously.

I. INTRODUCTION



2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Empirical Formula: $C_{12}H_4Cl_4O_2$
Percent by Weight: C 44.7, O 9.95, H 1.25, Cl 44.1
Molecular Weight: 322
Melting Point: 305°C
Decomposition Temperature: >700°C

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD; TCDD; CAS 1746-01-6) occurs as a highly toxic impurity found in herbicides containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5,-T derivatives, as well as in other chemicals synthesized using 2,4,5-trichlorophenol (EHP, 1973; Crossland and Shea, 1973; Rappe, 1978).

The herbicide 2,4,5-T has been marketed in the United States since 1948 (Federal Register, 1978). Production increased sharply between 1960 and 1970, when a 1:1 mixture of 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) was used as a defoliant in Vietnam under the names of "herbicide agent orange, herbicide orange, agent orange, and orange" (Federal Register, 1979). During this 10-year period, about 106 million pounds of 2,4,5-T were sprayed (Federal Register, 1978).

An average of 1.86 ppm TCDD (and as much as 47 ppm in a single sample) was found in surplus herbicide orange preparations stockpiled after the Vietnam war (Firestone, 1978). Commercial trichlorophenols manufactured from 1969 to 1970 contained 0.07 ppm to 6.2 ppm TCDD (Firestone et al., 1972). Woolson et al. (1972) analyzed 42 samples of 2,4,5-T manufactured from 1966 to 1970 and found that 7 contained less than 10 ppm TCDD, 13 of 42 samples contained from 10 ppm to 100 ppm TCDD, and the remaining 22 samples contained less than 0.5 ppm. After the hazardous effects of TCDD had been publicized, manufacturers reduced the TCDD content in products to 0.5 ppm in 1971 (Kearney et al., 1973) and to 0.1 ppm in 1972 (Federal Register, 1978). The 2,4,5-trichlorophenol produced currently in the U.S. reportedly contains on the average 0.026 ppm TCDD (Ramstad et al., 1977).

Since 1974, over 95% of all 2,4,5-T produced in the U.S. has been used on rangelands and pastures for the control of woody plants (Farm Chemicals Handbook, 1977). Limited use on food crops such as rice and blueberries has been permitted (Federal Register, 1978). On February 28, 1979, the Environmental Protection Agency issued a suspension notice regarding the uses of 2,4,5-T on pastures, forests, and rights of way (Federal Register, 1979).

Local populations have been exposed to TCDD as a result of industrial accidents in Germany, The Netherlands, Czechoslovakia, Italy, Great Britain, and the United States, and through the intensive spraying campaigns with Agent Orange in Vietnam (IARC, 1977 and 1978; Crow, 1977; Hay, 1978; Firestone, 1978; Huff et al., 1980).

TCDD undergoes photodecomposition in nonpolar solvents, but not in aqueous solutions or on wet or dry soils (Crosby et al., 1971). Under laboratory conditions, TCDD in thin films of herbicide formulations applied to glass plates, leaves, and soil photodecomposes rapidly; one half of the compound is lost in approximately 6 hours (Crosby and Wong, 1977).

TCDD is apparently not taken up by plant roots or leached into groundwater nor is it found in plants after foliar applications (Kearney et al., 1973). Soil samples treated with 1, 10, or 100 ppm of TCDD and maintained in the laboratory contained as much as 71% of the original material after 1 year (Kearney et al., 1972). Between 1962 and 1970, nearly 350,000 pounds of herbicides were applied by the U.S. military to approximately one square mile at Eglin Air Force Base, Florida. During 1962-1964, a single 92-acre grid had been treated with 87,186 pounds of 2,4,5-T. After 10 years, TCDD could still be recovered from the top 6"- layer of soil (10-710 ppt) and from aquatic silt at the point where eroded soil entered water (10-35 ppt). Livers from captured field mice contained 540-1300 ppt TCDD (Young et al., 1975).

Of the chlorinated dibenzo-p-dioxin isomers tested, TCDD is the most toxic (McConnell and Moore, 1978). Schwetz et al. (1973) found that the acute oral LD_{50} of TCDD for Sherman rats was 22 μg/kg for males and 45 μg/kg for females. The oral LD_{50} is approximately 100 μg/kg for male and female albino Charles River CD rats (Harris et al., 1973), 114 μg/kg for male C57BL/6 mice (Vos et al., 1974), and 190 μg/kg for female Porton rats (Greig et al., 1973). Deaths of the rats and mice in the acute toxicity studies usually occurred several weeks after dosing (Moore, 1978). The mean time until death was 40.4 days in female Porton rats given a single dose of 200 μg/kg TCDD (Greig et al., 1973).

Histopathologic liver changes have been observed 5 weeks after single oral doses as low as 50 μg/kg were administered to male and female CD rats and 1 week after a single dose of 50 μg/kg was administered to female CD-1 mice (Harris et al., 1973). Increased liver weights were found in male Wistar rats 7 days after single intraperitoneal doses of 0.1 μg/kg (Cunningham and Williams, 1972).

Six weekly doses of 0.2 μg/kg administered by gavage produced an increase in lipid accumulation in the liver, while doses of 1, 5, or 25 μg/kg/week for 6 weeks resulted in increased liver weights and decreased thymic weights in male C57BL/6 mice (Vos et al., 1974). The minimal toxic dose in male and female Sprague-Dawley rats was 0.1 μg/kg when administered by gavage five times per week for 13 weeks (Kociba et al., 1976). Liver degeneration and lymphoid depletion of the thymus were detected in animals given 0.1 μg/kg but not in those administered lower doses. Severe liver damage and high mortality occurred among female albino CD rats given daily oral doses of 10 μg/kg for 31 days (Harris et al., 1973). Fewer deaths and slight liver lesions occurred in another group in the same study given 1 μg/kg for 31 days, and no effects on the liver were reported in a group given 0.1 μg/kg for 31 days. In subacute feeding studies, increased liver weights were observed in male and female Sprague-Dawley rats fed 7 or 20 ppb for 42 days (Fries and Marrow, 1975).

Hematologic effects, including an increase in the packed cell volume and erythrocyte count, platelet depression, and leucocytosis, occurred in female CD rats given 10 μg/kg orally for 10 or 14 days (Weissberg and Zinkl, 1973); leucopenia was found in female CD-1 mice given TCDD at 1, 10, or 50 μg/kg; and thrombocytopenia in female CD rats given 0.1, 1, or 10 μg/kg daily for 30 days (Zinkl et al., 1973).

TCDD is eliminated slowly from rats. Twenty-two days after male and female Sprague-Dawley rats were given a single oral dose of 1.0 μg of $[^{14}C]$-TCDD, $[^{14}C]$ activity was detected in the liver and fat (Rose et al., 1976). Piper et al. (1973) reported the half-life of TCDD in male Sprague-Dawley rats to be 17 days.

Van Miller et al. (1976) studied the tissue distribution and excretion of tritiated TCDD in five male Sprague-Dawley rats, three adult female rhesus monkeys, and four male infant rhesus monkeys. All of the animals in each of the three groups received the same intraperitoneal dose (400 μg TCDD/kg in corn oil) and were killed 7 days later. In the rat, 40% of the dose was retained in the liver, whereas less than 10% was retained in the livers of the monkeys. In contrast, a large percentage of the dose in monkeys was located in the skin, muscle, and fat, while similar tissues in the rat contained much lower levels of TCDD.

Results from bacterial mutagenicity tests with TCDD are conflicting (Wassom et al., 1977/1978). However, mutagenicity has been reported among plants and animals administered this chemical. Hussain et al. (1972) and Seiler (1973) reported that TCDD was mutagenic without activation in Salmonella typhimurium TA 1532 but not in Salmonella typhimurium TA 1530. Mercier et al. (1978), however, reported that TCDD was not mutagenic in Salmonella typhimurium TA 1532. These differences may be attributed to solubility problems and treatment protocols. Green (1977) gave 0.25, 0.5, 1.0, 2.0, or 4.0 μg/kg TCDD (dissolved in 1 part acetone: 9 parts corn oil) by gavage to male and female Osborne-Mendel rats twice weekly for 13 weeks and observed an increased incidence of chromosomal breaks in female rats dosed with 4 μg/kg and in males dosed with 2 μg/kg or 4 μg/kg. Jackson (1972) found that TCDD caused chromosomal aberrations in the plant Haemanthus (African blood lily).

Recently, Geiger and Neal (1981) examined the mutagenicity of TCDD (up to 20 μg/plate) using the Salmonella typhimurium histidine auxotrophs TA1535, TA100, TA1538, TA98, and TA1537. TCDD did not show mutagenicity in any of these auxotrophs in the presence of mammalian metabolic activating systems isolated from the livers of Arochlor 1254-treated rats, Arochlor 1254-treated hamsters, or TCDD-treated hamsters. Tests run in the absence of $NADP^+$-dependence metabolic activation failed to reveal any mutagenic activity of TCDD.

Using the sex-linked recessive lethal assay in Drosophila melanogaster, negative results were obtained following intrathoracic injection studies with TCDD (NTP preliminary results).

Treatment with repeated or single doses of as little as 1-10 μg/kg of TCDD increased the frequencies of cleft palate and kidney abnormalities in mice (Courtney and Moore, 1971; Neubert and Dillman, 1972; Neubert et al., 1973; Smith et al., 1976). In rats, embryo-lethal effects occurred under experimental conditions (Sparschu et. al., 1970; Sparschu et al., 1971), and kidney abnormalities (Courtney and Moore, 1971), intestinal hemorrhages, and general edema were produced in fetuses (Khera and Ruddick, 1973). Few follow-up studies of the effects of prenatal exposure on postnatal functions have been published. In mice, fetal kidney abnormalities caused by TCDD progressed to a hydronephrosis during the postnatal period (Moore, et al., 1973). Murray et al. (1979) completed a three generation reproduction study using Sprague-Dawley rats fed TCDD continuously in the diet (at levels of 0, 0.001, 0.01, and 0.1 μg/kg/day); significant decreases for the 0.01 and 0.1 μg/kg groups were observed in fertility, litter size, gestation survival, postnatal survival, and postnatal body weight. No apparent adverse effect on reproduction was seen at the 0.001 μg/kg dose level.

Lamb et al. (1980, 1981, 1981a, 1981b) studied the effects of simulated agent orange (2,4-D; 2,4,5-T; and TCDD) on fertility and reproduction in C57B1/6 male mice. Mating frequency, average fertility, and percent implantation, resorption sites, and fetal malformations were not influenced by the treatment. No significant decrement in fertility or reproduction was observed, nor was any evidence of germ cell toxicity observed. Survival of offspring and neonatal development were apparently unaffected by paternal exposure.

Luster et al. (1980) examined bone marrow, immunologic parameters, and host susceptibility in B6C3F1 mice following pre- and postnatal exposure to TCDD. Mothers were given 0, 1.0, 5.0, and 15.0 μg/kg TCDD/body weight on day 14 of gestation and on days 1, 7, and 14 following birth. Neonatal body, liver, spleen, and thymus weights were decreased in the 5.0 and 15.0 μg/kg groups. RBC counts, hematocrits, and hemoglobin were decreased at the highest TCDD level. Bone marrow toxicity occurred in the 5.0 and 15.0 μg/kg groups, as evidenced by bone-marrow hypocellularity and depressed-colony formation of macrophage-granulocyte progenitor cells and pleuripotent stem cells. Evidence was also presented of a functional depression of the thymus-dependent lymphocyte compartment. Increased susceptibility to either bacterial or syngeneic tumor cell challenge was noted in mice exposed to low levels of TCDD during pre- and postnatal development.

Two reports indicate that chronic administration of low levels of TCDD to rats is associated with an increased incidence of neoplasia (IARC Monographs, 1977; Van Miller et al. 1977; Kociba et al., 1978).

Groups of 10 male Sprague-Dawley rats were fed a diet containing TCDD for 78 weeks in the following amounts (figures in parentheses are approximate weekly doses): 0, 1 ppt (0.0003 μg/kg body weight), 5 ppt (0.001 μg/kg), 50 ppt (0.01 μg/kg), 500 ppt (0.1 μg/kg), 1 ppb (0.4 μg/kg), 5 ppb (2.0 μg/kg), 50 ppb (24 μg/kg), 500 ppb (240 μg/kg), or 1000 ppb (500 μg/kg). The three highest dose levels (50, 500, and 1000 ppb) were toxic and killed all animals by the fourth week. In the six remaining test groups, the overall incidence of neoplasms was 23/60 (38%); none occurred in the 1 ppt group. In the 5 ppt group, 5/10 animals had 6 neoplasms (ear-duct carcinoma, lymphocytic leukemia, adenocarcinoma, malignant histocytoma (with metastases), angiosarcoma, Leydig-cell adenoma); the following groups also showed neoplasms: 50 ppt, 3 observed in 3/10; 500 ppt, 4 in 4/10; 1 ppb, 5 in 4/10; 5 ppb, 10 in 7/10. Neoplasms were not observed in the controls (Van Miller et al., 1977).

Groups of 100 Sprague-Dawley rats (50 males and 50 females) for two years received diets containing 0, 22, 210, or 2,200 ppt, equivalent to 0.0, 0.001, 0.01, and 0.1 μg TCDD/kg/day. Continuous ingestion of 0.001 μg/kg/day did not cause any chemically related changes in tumor incidence or toxicity; feeding with 0.01 μg/kg/day increased the incidence ($P< 0.05$) of hepatocellular hyperplastic nodules (female: 18/50 versus 8/86 controls), focal alveolar hyperplasia in the lungs, and urinary excretion of porphyrins (female). Dietary intake of 0.1 μg/kg/day increased the incidence ($P<0.05$) of hepatocellular carcinomas (female: 11/49 versus 1/86) and squamous-cell carcinomas of the lung (female: 7/49 versus 0/86), hard palate/nasal turbinates (male 4/50 versus 0/85; female: 4/49 versus 0/86), and tongue (male: 3/50 versus 0/85). Also increased in frequency by the 0.1 μg TCDD/kg/day were adenoma of the adrenal cortex (male) and hepatocellular hyperplastic nodules (female). At this dose, the incidence of certain age-related lesions was reduced (males: acinar adenoma of the pancreas; females: granulosal cell neoplasms of the ovary, benign and malignant tumors of the mammary gland, pituitary adenoma, and benign tumors of the uterus). Also, chronic administration of TCDD caused multiple toxicologic effects including increased mortality, decreased body weight gain, slight depression of certain hematologic parameters, increased urinary excretion of porphyrins and δ-aminolevulinic acid, increased serum levels of alkaline phosphatase, glutamyl transferase and serum glutamic pyruvic transaminase, and morphologic changes of the hepatic, lymphoid, respiratory, and vascular tissues of the body (Kociba et al., 1978).

These two reports show that chronic administration of TCDD causes an increased incidence of neoplasms, but not whether this substance acts as an initiator or a promoter. This consideration is particularly important because unequivocal evidence is lacking on whether TCDD is a mutagen or is metabolized to a mutagen.

Toth et al. (1978; 1979) reported on the effects of TCDD (0, 0.007, 0.7, 7.0 μg/kg) administered by gavage to male Swiss/H/Riop mice once per week for one year. Treatment was stopped and the mice were necropsied at natural death (588, 649, 633, 424 days). Total liver tumors (benign and malignant were not reported separately) increased significantly when compared to controls at the 0.7 μg/kg dose level (0, 7/38; 0.007, 13/44; 0.7, 21/44: $P < 0.01$; 7.0, 13/43). In addition, TCDD caused chronic ulcerous skin lesions (0/38, 5/44, 13/44, 25/43) followed by "generalized lethal amyloidosis" (0/38, 5/44, 10/44, 17/43).

Kouri et al. (1978) investigated the co-carcinogenic capacity of TCDD and 3-methylcholanthrene (MCA) in C57BL/6 and DBA/2 mice. TCDD (1 or 100 μg/kg) was administered by either ip or sc injection 48 hours before or at the same time as 150 μg MCA was given sc. Mice were examined weekly for injection-site tumors (fibrosarcomas) and the experiment was terminated after 36 weeks. Because MCA alone induced a high tumor incidence (29/36, 81%) in C57BL/6 mice compared to none with ip TCDD (1 or 100 μg/kg) alone, to that with TCDD given 48 hours previously followed by MCA (16/23, 70% for the 1 μg/kg; 21/25, 84% for the 100 μg/kg), or to the combination (27/27, 100% for the 1 μg/kg; 33/43, 71% for 100 μg/kg), these results must be considered inconclusive. In the genetically "less responsive" DBA/2 mice, sc MCA produced tumors in 1/34, 3%, and 3/30, 10%; ip or sc TCDD alone or given prior to MCA caused no apparent increase in tumor incidence. However, TCDD given simultaneously with sc MCA induced significant increases in fibrosarcomas -- 1 μg/kg sc: 21/98, 21%; 100 μg/kg sc: 46/82, 56%; and 100 μg/kg ip: 17/62, 27%. These data suggest a co-carcinogenic effect of TCDD when given with MCA; but this study may have been compromised because dioxane, a known carcinogen, was used as a solvent for TCDD.

The promoter activity of TCDD for hepatocarcinogenesis was determined in female Charles-River rats partially hepatectomized and exposed to a single dose (10 mg/kg) of N-nitrosodiethylamine (diethylnitrosamine, DEN) (Pitot et al., 1980). Rats receiving only DEN and partial hepatectomy or only TCDD exhibited few enzyme-altered foci and no hepatocellular carcinomas. Partially hepatectomized groups given DEN followed by 0.14 or 1.4 μg/kg TCDD sc once every 2 weeks for seven months developed increased numbers of foci, neoplastic nodules (3/5 low dose and 1/7 high dose), and carcinomas (5/7 high dose). In comparison, phenobarbital was less effective in causing foci, but equal in producing carcinomas (8/10). These studies by Pitot et al. (1980) demonstrate that TCDD is a promoting agent for DEN-initiated hepatocarcinogenesis.

DiGiovanni et al. (1977), using the two-stage mouse skin carcinogenesis model, applied TCDD (2 μg/mouse in 0.2 ml acetone to CD-1 mice alone or 5 minutes before DMBA (2.56 μg/mouse); starting one week later, 12-O-tetradecanoylphorbol-13-acetate (TPA, 5 μg in 0.2 ml acetone) was applied twice weekly for 32 weeks. Results are given as mice with papillomas and papillomas/mouse: TCDD/TPA, 3/21 (14%), 0.1; DMBA/TPA, 12/29 (41%), 1.8; and DMBA/TCDD/TPA, 14/22 (63%), 2.2. These data suggest that TCDD alone may be a weak tumor initiator and that TCDD plus DMBA increases the tumor rate over that of DMBA alone, resembling a co-carcinogenic effect.

Berry and co-workers (1978) obtained negative skin promotion results on female CD-1 mice following an initiation dose of DMBA (200 nmol in 0.2 ml acetone) and subsequent twice weekly applications of TCDD (0.1 μg in acetone) for 30 weeks. Further, TCDD alone did not produce papillomas;

skin rashes were noted. In dose-finding experiments, TCDD increased slightly the incidence of intrafollicular epidermis (at 1 μg/mouse) and caused gastrointestinal damage (a single 2 μg application) resulting in the death of 30 percent of treated animals (numbers of animals not given).

Cohen et al. (1979) reported on an anticarcinogenic effect of TCDD (1 μg in 0.2 ml acetone) when applied to female Sencar mice 72 hours prior to skin painting with benzo(a)pyrene (B(a)P, 100 nmol) or with 7,12-dimethylbenz(a)anthracene (DMBA, 10 nmol) followed 1 week later by twice-weekly (for 15 weeks) 2 μg applications of TPA. TCDD reduced the number of DMBA-induced papillomas (and papillomas per mouse) from 28/28 (9.1) to 3/28 (0.1), and for B(a)P from 24/28 (3.8) to 7/29 (0.3).

DiGiovanni et al. (1979, 1980) investigated the inhibitory ("anticarcinogenic") activity of TCDD on polycyclic hydrocarbon-induced skin tumorigenesis. In a preliminary report, these authors (1979) reported that TCDD inhibited the initiation of skin papillomas by DMBA and B(a)P. The more recent study (1980) analyzed the effect of treating female CD-1 or Sencar mice with single doses of TCDD (1 μg/mouse) 3 days before, 5 minutes prior to, and 1 day after application of four tumor initiators -- DMBA (10 nmol), B(a)P (100 nmol), MCA (100 nmol) (each requiring metabolic activation), and B(a)P-diol-epoxide (200 nmol) (the apparent ultimate carcinogenic form of B(a)P). One week later, TPA (3.4 nmol) was applied twice weekly for 20 weeks. TCDD applied to Sencar mice 3 days prior to DMBA, B(a)P, or MCA markedly inhibited skin papilloma induction (% of control: 2.3, 14, and 43); when given one day after the initiator, TCDD increased the incidence when compared to controls (113%, 125%, 107%). Similar inhibitory effects occurred with B(a)P-diol-epoxide: TCDD 3 days prior, 19% of control; 5 minutes before, 50%; 1 day after, 61%.

These data allow the inference that TCDD possesses a modicum capability to initiate dermal tumors, a marked inhibitory action when applied prior to initiation/promotion, and a moderate tumor promoting index when given after initiation.

Other articles summarizing the carcinogenic activity of TCDD (and dioxins) are available (Berry et al., 1979; EHP, 1973; Huff, et al., 1980; IARC, 1977; Kimbrough, 1979; Kociba et al., 1979; McConnell, 1980).

Other long-term carcinogenesis studies on the "dioxins" that have been completed or are ongoing within the National Toxicology Program are summarized in the following paragraphs.

Dibenzo-p-dioxin, in diets containing 5,000 or 10,000 ppm, was fed to groups of 35 male and female Osborne-Mendel rats for 100-117 weeks and to groups of 50 male and female B6C3F1 mice for 91-97 weeks (NCI/NTP, 1979). No compound-related carcinogenic effects were observed. The 10,000 ppm male rats and the 5,000 and 10,000 ppm female rats had an increased incidence of fatty metamorphosis in the liver.

2,7-Dichlorodibenzo-p-dioxin was added to diets of Osborne-Mendel rats (110-117 weeks) and B6C3F1 mice (91-101 weeks) at levels of 5,000 or 10,000 ppm (NCI/NTP, 1979a). Under these conditions, 2,7-dichlorodibenzo-p-dioxin was considered to be not carcinogenic for male and female Osborne-Mendel rats or female B6C3F1 mice. For male B6C3F1 mice the combined incidences of leukemia (0/50, 3/50, 1/45) and lymphoma (0/50, 4/50, 2/45) show a significantly increased rate for the low-dose groups (0/50 versus 7/50, P=0.006); hepatocellular adenomas were significantly increased in the low- and high-dose groups when compared with the controls (4/49, 15/50: P=0.005, 12/42: P=0.011); and when the hepatocellular carcinomas (4/49, 5/50, 5/42) are added the combined tumor incidences when compared to controls were also significantly increased (8/49, 20/50: P=0.008, 17/40: P=0.010). These data support the conclusion that 2,7-dichlorodibenzo-p-dioxin was carcinogenic for male B6C3F1 mice.

A mixture of hexachlorodibenzo-p-dioxins (31% 1,2,3,6,7,8- and 67% 1,2,3,7,8,9-) was given by gavage twice per week for 104 weeks to Osborne-Mendel rats and B6C3F1 mice (NCI/NTP, 1980a). HCDD doses were 0, 1.25, 2.5, or 5 μg/kg/wk for rats and male mice and 0, 2.5, 5, or 10 μg/kg/wk for female mice. No compound-related tumors were observed in male rats; however, toxic hepatitis was observed in these animals: 0/24, 28/49, 35/50, and 34/48. HCDD induced a significantly increased incidence of neoplastic nodules in female rats when compared to vehicle controls (5/75, 10/50: P=0.026, 12/50: P=0.006, 30/50: P<0.001); hepatocellular carcinoma occurred only in the high-dose group (4/50). Toxic hepatitis was increased significantly in the female rats as well: 0/25, 33/50, 37/50, 44/50. In mice, the incidence of hepatocellular adenomas increased in the high dose groups (male--7/73, 5/50, 9/49, 15/48: P=0.003; female--2/73, 4/48, 4/47, 9/47: P=0.003). The observed incidences of hepatocellular carcinomas were not significantly different from vehicle controls (male: 8/73, 9/50, 5/49, 9/48; female: 1/73, 0/48, 2/47, 2/47). When these liver tumors are combined, dose-related trends remain and significant increases are recorded for the high-dose groups (male: 15/73, 14/50, 14/49, 24/48: P=0.001; female: 3/73, 4/48, 6/47, 10/47: P=0.004).

A separate skin-painting (dermal application) study using the same hexachlorodibenzo-p-dioxin mixture was conducted with Swiss-Webster mice (NCI/NTP, 1980b). No compound-induced carcinogenic effect was observed following 104 weeks exposure to 0.01 μg HCDD (suspended in 0.1 ml acetone) three times per week.

A companion gavage experiment (NTP, 1981) was conducted concurrently with the skin-painting study (the subject of this report). For 104 weeks Osborne-Mendel rats and male B6C3F1 mice received 0, 0.01, 0.05, or 0.5 μg/kg per week in two divided doses, and female mice were given 0, 0.04, 0.2, or 2.0 μg/kg/week. TCDD treatment significantly increased the incidences of follicular-cell thryoid adenomas in male rats (1/69, 1%; 5/48, 10%; 6/50, 12%: P=0.021; 10/50, 20%: $P < 0.001$) and of neoplastic nodules in livers of female rats (5/75, 7%; 1/49, 2%; 3/50, 6%; 12/49, 24%: P=0.006). In mice, TCDD increased the numbers of hepatocellular carcinomas in males (8/73, 11%; 9/49, 18%; 8/49, 16%; 17/50, 34%: P=0.002) and in females (1/73, 1%, 2/50, 4%; 2/48, 4%; 6/47, 13%: P=0.014); total liver tumors (carcinomas and adenomas) were likewise increased (males: 15/73, 21%, 12/49, 24%; 13/49, 27%; 27/50, 54%: $P < 0.001$ and females: 3/73, 4%; 6/50, 12%; 6/48, 13%; 11/47, 23%: P=0.002). Also, female mice had increased incidence of follicular-cell thyroid adenomas (0/69, 0%; 3/50, 6%; 1/47, 2%; 5/46, 11%).

TCDD and other dioxins were selected as a class for testing in the early 1970's following reports that TCDD was a contaminant in 2,4,5-T, which was shown to be teratogenic in rats, and because human exposure to 2,4,5-T (containing dioxin) was widespread.

II. MATERIALS AND METHODS

A. Chemical

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was synthesized in the Chemistry Division of IITRI, Chicago, Illinois, by the condensation of potassium 2,4,5-trichlorophenate in the presence of the Ullman copper catalyst as described in Appendix E. IITRI reported the purity to be 99.4% based on results of gas chromatographic analysis of the chemical. Samples analyzed by Dow Chemical Company, Midland, Michigan, were found to contain less than 1% of two impurities, tentatively identified as a trichlorodibenzo-p-dioxin and a pentachlorodibenzo-p-dioxin. The presence of 0.1% to 0.2% hexachlorodibenzo-p-dioxin was detected by gas chromatography and mass spectrometry (Stehl, 1974).

7,12-Dimethylbenzanthracene (DMBA) (Lot No. 85973) used for pretreatment in certain animal groups was obtained from K & K Laboratories (Cleveland, Ohio). Its purity was not evaluated but was stated by the manufacturer to be at least 95%.

The TCDD was stored at room temperature in brown glass vials in an unlighted glove-box hood and was exposed to light only at 3-month intervals, when samples were removed for preparation of stock suspensions in acetone.

B. Dosage Preparation

Fresh stock suspensions of 10 μg/ml TCDD in acetone (Mallinckrodt Inc., St. Louis, MO) were prepared every 3 months. The 10 μg/ml primary stock solution was further diluted to 0.25 μg TCDD/ml and was used as the skin paint stock solution. At the time of administration of TCDD, the stock solution was shaken well and suitable aliquots were added to additional acetone to give the desired concentrations of the test chemical. Enough DMBA was dissolved in acetone so that the volume applied (100 μl) contained 50 μg DMBA.

The suspensions of the TCDD and the solutions of DMBA in acetone were kept in brown glass bottles with Teflon®-lined caps. The bottles were sealed with tape, triple-bagged in plastic, and continuously stored at 4°C.

The backs of all animals were clipped weekly (on Thursdays), and acetone or acetone suspensions of TCDD and DMBA were applied (on Mondays, Wednesdays, and Fridays) to the clipped areas of vehicle control and test groups of mice with automatic pipettes equipped with disposable tips. Animals were held on an incline with the posterior elevated and test solutions applied to the posterior were allowed to flow towards the head. Fluorescent lighting was used.

To determine the accuracy of the concentration of the TCDD in the stock suspensions in acetone, IITRI analyzed samples when the stocks were freshly prepared and at the end of the 3-month periods of use (Appendix F). The mean concentration of 18 samples containing a theoretical level of 0.25 μg/ml was 0.27±0.05 μg/ml. The range was 0.17 to 0.34 μg/ml.

C. Animals

Male and female Swiss-Webster mice, obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, were used in subchronic and chronic studies. The animals used in the chronic studies were approximately 4 weeks old when received and were isolated for 2 weeks before the start of the bioassay. Those animals with no visible signs of disease were then earmarked for individual identification and assigned to dosed or control groups according to a table of random numbers. Because of animal supply limitations, multiple shipments of mice received within a 2-week period were used. The mice from each shipment were evenly distributed among all test and control groups and all were approximately the same age when placed on study.

D. Animal Maintenance

The temperature in rooms where mice were housed was maintained at 20° to 22°C and the relative humidity at 40% to 50% with 15 changes of room air per hour. Negative air pressure relative to the hallways was maintained in the animal rooms. Exhaust air from the animal rooms and hoods was passed through HEPA filters before being released into the exterior atmosphere. Fluorescent lighting was provided 12 hours each day.

Mice were housed 10 per cage in clear polystyrene cages (Table 1) covered with a special tight-fitting polystyrene lid adapted to hold two metal filter housings and a water bottle. Filter housings containing FG 50 filters at the point of air entry and exit were attached to each cage, and the exit filters were joined to a manifold that led through a large vertical pipe at the end of the rack and then through a flexible hose to the HEPA filter exhaust system. This arrangement of individually vented cages provided a constant flow of air that was filtered as it entered and left the cages to prevent the release of the test chemical from the cages into the room.

Because of the possible toxicity of the test chemical for laboratory personnel, the cages (including lids) housing the animals treated dermally with TCDD were used only once and were discarded every week. The used cages and lids were triple-sealed in plastic bags and incinerated, as was all waste material from the animal rooms and the hoods. The glass water bottles and stainless steel sipper tubes from the used cages were rinsed in the same room with the organic solvent chlorothene N.U.® (Table 1) to dilute out any dioxin present and were then sanitized at 82°C in an automatic washer. The polycarbonate cages housing the control animals were recycled three times and incinerated. The corresponding water bottles and sipper tubes were not rinsed in chlorothene before washing.

Disposable clothing was worn by all personnel and, after use, was incinerated by the procedure used for the cages and other waste material. Respirators were also worn in the animal rooms. All dosing of animals was carried out in hoods.

Animals were fed Wayne® Lab Blox and cages were cleaned and provided with fresh hardwood chip bedding and food once per week (Table 1). Wayne® Lab Blox and tap water were available ad libitum.

For the chronic study, dosed groups of mice were housed in one room, and vehicle-control groups were housed in a separate room. Untreated control groups, serving as room environmental-control groups, were housed in each of these rooms.

Table 1. Specifications and Sources of Materials Used for Animal Maintenance

Item	Specifications	Manufacturer or Supplier
Cages	19"x10.5"x8"	Maryland Plastics Federalsburg, MD
Chlorothene N. U.®	A formulation of 1,1,1-trichloroethane	Central Solvents Chicago, IL
Feed	Wayne® Lab Blox, Pellets	Allied Mills Inc. Chicago, IL
Bedding	Absorb-Dri® hardwood chips	Lab Products, Inc. Garfield, NJ

E. Subchronic Studies

In a preliminary subchronic dermal application study, 2.5 to 80 μg TCDD per week was applied to female Swiss albino mice and the mice were then observed for an additional 35 weeks. Four animals administered 80 μg and one administered 40 μg died during the first 2 weeks. No dose-related effects on weight gain were observed.

In subchronic dermal application studies conducted to determine the dose to be used in the chronic studies, TCDD was applied to 10 mice of each sex three times weekly for 13 weeks at doses of 0.005 (males only), 0.010, 0.050, 0.100, 0.625, 1.25, 2.5, 5, or 10 μg. The mice were observed daily for deaths. At the end of the study, necropsies and histologic examinations of tissues were performed on eight male mice administered 0.005 μg, nine females administered 0.01 μg, and two or three males or females in each of the remaining dosed groups. Except for the male groups administered 0.005 to 0.05 μg and the females given 0.01 to 0.10 μg, necropsies were performed only on those animals that died before termination of the study.

Mortality and the incidences of histopathologic change for the dosed groups are given in Table 2.

The mortality rates indicate that the male mice were more susceptible than the female mice to dermal applications of TCDD. Histopathologic changes occurred in male mice at doses of TCDD that were lower than those inducing such changes in the females. The lethal doses in male mice caused marked effects on the lymphoid and hematopoietic tissues as well as on the liver and lung. Respiratory tract toxicity included bronchiolar adenomatoid changes with hyperplasia and squamous metaplasia. Significant pulmonary pathologic changes were not noted in the females. Liver damage in dosed groups of both the males and the females included diffuse fatty change, hepatocellular necrosis, and, secondarily, ascites.

Estimates of maximum tolerated doses could not be made for either sex. Liver damage was found in six of eight male mice given the lowest dose (0.005 μg) and in females given the lowest dose (0.01 μg), and threshold liver damage was observed in two additional female mice given the lowest dose.

Table 2. Doses, Mortality, and Histopathologic Changes in TCDD Subchronic Dermal Application Studies in Mice

Dose(a)	Mortality (Percent)		Incidence of Histopathologic Change											
			Lymph Node		Spleen		Thymus		Bone Marrow		Lung		Liver	
(µg)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
10	100	100	2/2	0/2	2/2	2/2	1/2	1/2	1/2	1/2	2/2	0/2	2/2	2/2
5	100	80	1/3	0/2	3/3	2/2	1/3	0/2	3/3	0/2	2/3	0/2	3/3	2/2
2.5	100	90	0/2	1/2	2/2	2/2	2/2	0/2	1/2	0/2	2/2	0/2	2/2	2/2
1.25	90	70	0/2	1/2	2/2	2/2	1/2	2/2	2/2	0/2	2/2	0/2	2/2	2/2
0.625	50	50	1/2	1/2	2/2	2/2	-	1/2	2/2	1/2	1/2	0/2	2/2	2/2
0.1	30	0	2/2	0/2	1/2	0/2	-	0/2	2/2	0/2	1/2	0/2	2/2	2/2(b)
0.05	10	10	-	0/3	2/2	0/3	-	1/3	0/2	0/3	1/2	0/3	2/2	2/3
0.01	0	0	0/2	0/9	0/2	0/9	0/2	0/9	0/2	0/9	0/2	0/9	1/2	5/9(c)
0.005	0	-	0/8	-	1/8	-	0/8	-	0/8	-	0/8	-	6/8	-

(a) Dose per animal, administered 3 times per week for 13 weeks.
(b) The two females examined for liver changes had only threshold changes.
(c) Three of the females examined for liver changes had only threshold changes.

The doses selected for the mice in the chronic study were 0.001 μg per application for males and 0.005 μg per application for females, the doses to be applied three times per week on alternate days. These doses corresponded to approximately 0.15 μg/kg/wk and 0.75 μg/kg/wk, respectively, based on an average mouse weight of 20 μg and on the use of three applications of TCDD per week.

F. Chronic Studies

Mice receiving TCDD, with or without pretreatment with DMBA, were housed in one room with untreated control group No. 2. Vehicle controls were housed in a second room with untreated control group No. 1. The vehicle-control groups of each sex were shared with a dermal study of HCDD (a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins) which was housed in a third room. Thirty mice of either sex were treated with TCDD alone and an additional 30 mice of either sex were given one application of 50 μg DMBA 1 week before the initiation of the TCDD applications.

The test groups, doses administered, and durations of the chronic dermal application studies are shown in Table 3.

G. Clinical Examinations and Pathology

Animals were observed twice daily for mortality. Body weights were recorded every 2 weeks for the first 12 weeks and every month thereafter. Moribund animals and those that survived to the termination of the study were killed using sodium pentobarbital and necropsied.

Gross and microscopic examinations were performed on major tissues and major organs and on all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues and organs were taken at necropsy: skin, mandibular lymph node, salivary gland, mammary gland, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, colon, liver, gall bladder, pancreas, spleen, kidney, adrenal, ovary or

Table 3. Test Groups, Doses, and Times on Study of Mice in the TCDD Chronic Dermal Application Studies

Test Group(a)	Initial No. of Animals(b)	Room	TCDD Dose(c) (μg/application)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
MALE					
Untreated-Control No. 1	15	1C9	0	0	104
Untreated-Control No. 2	15	1A6	0	0	104
Vehicle-Control (d)	45	1C9	0	0	104
Dosed	30	1A6	0.001	99	0
Dosed plus DMBA	30	1A6	0.001(e)	104	0
FEMALE					
Untreated-Control No. 1	15	1C9	0	0	104
Untreated-Control No. 2	15	1A6	0	0	104
Vehicle-Control(d)	45	1C9	0	0	104
Dosed	30	1A6	0.005	104	0
Dosed plus DMBA	30	1A6	0.005(e)	104	0

(a) All animals were approximately 6 weeks of age when placed on study.
(b) Mice from multiple shipments covering a 2-week period were evenly distributed among all test and control groups.
(c) The TCDD was administered 3 times per week in 0.1 ml acetone.
(d) Vehicle controls received 0.1 ml acetone 3 times per week.
(e) Each animal in this group was administered 50 μg of DMBA in one application 1 week prior to the initiation of dermal applications of TCDD.

testis, nasal cavity, brain, pituitary, spinal cord, skeletal muscle, sciatic nerve, and all tissue masses.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Data on this experiment have been recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

Life table methods were used to analyze the weeks of death of animals with histologically observed tumors under the principles described by Saffiotti et al. (1972).

III. RESULTS

A. Body Weights and Clinical Signs

Mean body weights of the male or female groups of mice administered TCDD, or TCDD following DMBA, were essentially the same as those of corresponding vehicle-control groups throughout the bioassay (Figure 1). Among males, mean body weights of dosed and vehicle control groups were less than that of the untreated controls throughout the study, and with females were less than mean body weights of untreated controls during the first 80 weeks. No other clinical signs were observed.

B. Survival

Estimates of the probabilities of survival for male and female mice administered TCDD, or TCDD following DMBA, by dermal application at the doses of this bioassay, together with those of the controls, are shown by the Kaplan and Meier curves in Figure 2. Five study groups were used for each sex: a group administered TCDD alone, a group administered TCDD following DMBA, a vehicle-control group, and two untreated-control groups. In the survival graphs, the two untreated-control groups were combined into one group. The results of the Cox test comparing the survival of the group administered TCDD alone with that of the pooled untreated-control group, or with that of the group administered TCDD following DMBA, are significant (P=0.005 and P=0.032, respectively) in male mice due to shortened survival in the group dosed with TCDD alone. In females, the Cox test comparing the survival of the group administered TCDD alone with that of the vehicle-control group indicates significantly shorter survival (P=0.031) in the group dosed with TCDD alone than in the vehicle control group. The Cox test of the TCDD and the vehicle control groups indicates that there were no significant differences in final survival rates in male mice for the entire test period. In females, the group administered TCDD following DMBA has a survival comparable with each control group and with the group administered TCDD alone.

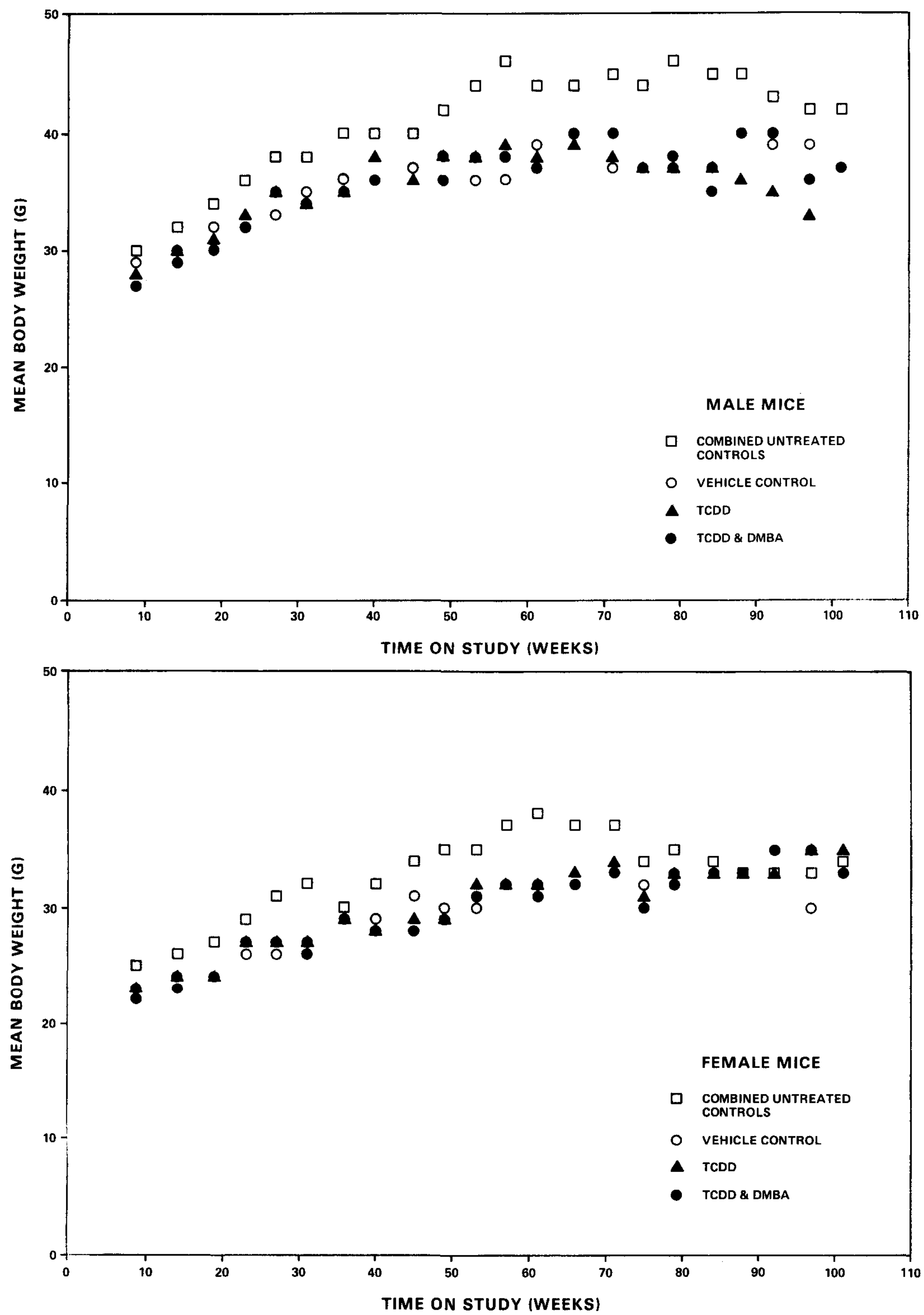


Figure 1. Growth Curves for Mice Administered TCDD or TCDD Following DMBA by Dermal Application

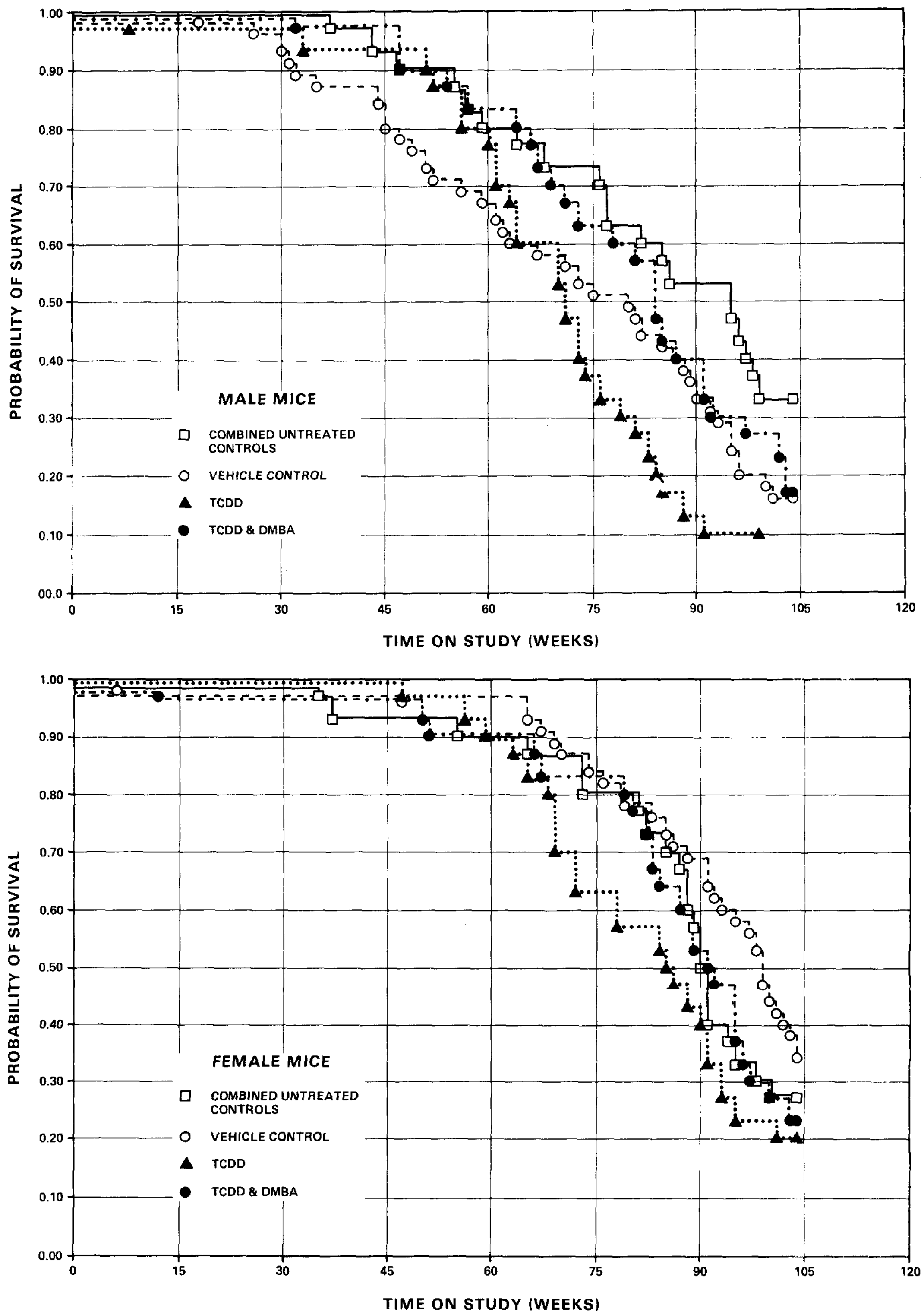


Figure 2. Survival Curves for Mice Administered TCDD or TCDD Following DMBA by Dermal Application

In male mice, 10/30 (33%) of the combined untreated-control group, 7/45 (16%) of the vehicle-control group, 3/30 (10%) of the group dosed with TCDD alone, and 5/30 (17%) of the group dosed with TCDD following DMBA lived to the end of the study. At week 52 of the study, 27/30 (90%) of the pooled untreated-control group, 33/45 (73%) of the vehicle-control group, and 27/30 (90%) of the group dosed with TCDD alone or TCDD following DMBA were living. In females, 8/30 (27%) of the combined untreated-control group, 16/45 (36%) of the vehicle-control group, 6/30 (20%) of the group dosed with TCDD alone, and 7/30 (23%) of the group dosed with TCDD following DMBA lived to the end of the study at week 104. At week 52, 28/30 (93%) of the combined untreated-control group, 43/45 (96%) of the vehicle-control group, 29/30 (97%) of the group dosed with TCDD alone, and 27/30 (90%) of the group dosed with TCDD following DMBA were alive.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendixes A and C, Tables A1, A2, C1, and C2; findings on nonneoplastic lesions are summarized in Appendixes B and D, Tables B1, B2, D1, and D2. Groups of animals receiving the test chemical or the vehicle control and untreated controls are tabulated and summarized separately.

The only significant finding was skin tumors located on the back or adjacent areas. Most of the skin tumors were fibrosarcomas (Table 4) with only an occasional fibroma, myxoma, or keratoacanthoma. Few epithelial skin tumors were found. Fibrosarcomas of the integumentary system occurred in females in the following incidences: vehicle controls, 2/41 (5%); TCDD following DMBA, 8/29 (28%); and TCDD alone, 8/27 (30%).

A variety of nonneoplastic lesions were seen. A few appeared to be related to chemical exposure. These included inflammatory hepatic lesions and hepatic cytomegaly in female mice, but no inflammatory or hyperplastic lesions were found in the epidermis at the site of TCDD or DMBA applications.

In summary, according to the histopathologic evidence, incidence of skin tumors was increased at the site of application of TCDD in both sexes. DMBA pretreatment had no effect upon the carcinogenicity of TCDD. Acetone as a vehicle had no unusual or obvious influence upon skin tumor production

Table 4. Locations of Integumentary Fibrosarcomas in Female Mice

Group	Animal Number	Tumor Site
Untreated Control	76-2069	Right hind leg
Vehicle Control	76-1979	Right hind leg
	76-1978	Right lateral side of head, involving eye
TCDD Following DBMA	76-2186	Right rear leg; left front quarter
	76-2173	Right femur, extending to left femur
	76-2182	Dorsal side, anterior
	76-2169	Right lateral surface
	76-2178	Left lateral side near head
	76-2185	Right lateral side adjacent to front leg; dorsal surface above backbone; left lateral side near rib cage
	76-2179	Right lateral side
	76-2175	Right lateral side of head
TCDD Alone	76-1886	Right ventral, surrounding foreleg
	76-1867	Right lateral side, anterior to hind leg
	76-1882	Ventral side
	76-1878	Ventral side, posterior
	76-1887	Rectal area
	76-1881	Dorsal posterior into left leg and tail
	76-1890	Anterior left lateral side into rib cage
	76-1891	Mid lateral, dorsal skin

in this study; there was no evidence to suggest that TCDD was a systemic tumorigen when applied to the backs of mice as was done in this study.

D. Statistical Analyses of Results

Tables 5 and 6 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more than one group. Since the doses in the two dosed groups of each sex did not differ in TCDD alone, no trend analysis was made. There were no differences between the two untreated-control groups of either sex in the incidence of animals with tumors at any site.

Regarding the incidences of animals with tumors, there were no significant differences between the vehicle-control groups and the pool of their respective untreated-control groups or between those of the TCDD groups and the groups administered TCDD following DMBA in either sex, except that the incidence of male mice with hemangiosarcomas is higher (P=0.048) in the TCDD group than in the group administered TCDD following DMBA.

In female mice, the incidence of animals with fibrosarcoma in the integumentary system is significantly higher in the TCDD group and TCDD group previously treated with DMBA (P=0.007 and P=0.010, respectively). Table 7 shows the weeks to death of mice with histologically observed skin tumors of all types. In male mice, although the incidence for this tumor is not significant when the Fisher exact tests are applied, life table analyses (which are sensitive to decreased time-to-tumor (latency) as well as to increased tumor incidence) indicate a significant (P=0.007) effect in the TCDD group relative to the vehicle control group. When life table analyses were applied to female mice, the results were significant (P=0.001).

Significant results are not observed for any other type of tumor in either sex.

Statistical analysis indicates that, in female mice, there is an association between dermal application of TCDD, or of TCDD following DMBA, and the development of fibrosarcoma of the integumentary system. Fibrosarcoma was observed to develop earlier in the TCDD group of male mice than in the vehicle control group.

Table 5. Analyses of the Incidence of Primary Tumors in Male Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

Topography: Morphology	Vehicle Control	TCDD	TCDD plus DMBA
Integumentary System: Fibrosarcoma (b)	3/42 (7)	6/28 (21)	5/30 (17)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		3.000	2.333
Lower Limit		0.699	0.491
Upper Limit		16.947	13.870
Weeks to First Observed Tumor	87	71	67
Lung: Alveolar/Bronchiolar Adenoma (b)	6/41 (15)	1/28 (4)	5/29 (17)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.244	1.178
Lower Limit		0.005	0.311
Upper Limit		1.845	4.148
Weeks to First Observed Tumor	71	99	54
Lung: Aveolar/Bronchiolar Carcinoma (b)	1/41 (2)	1/28 (4)	2/29 (7)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		1.464	2.828
Lower Limit		0.019	0.154
Upper Limit		110.830	160.860
Weeks to First Observed Tumor	81	51	84
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	7/41 (17)	2/28 (7)	6/29 (21)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.418	1.212
Lower Limit		0.045	0.372
Upper Limit		1.990	3.729
Weeks to First Observed Tumor	71	51	54

Table 5. Analyses of the Incidence of Primary Tumors in Male Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Vehicle Control	TCDD	TCDD plus DMBA
Hematopoietic System: Lymphoma or Leukemia (b)	4/42 (10)	2/28 (7)	5/30 (17)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.750	1.750
Lower Limit		0.071	0.409
Upper Limit		4.815	8.048
Weeks to First Observed Tumor	17	33	47
All Sites: Hemangiosarcoma (b)	1/42 (2)	4/28 (14)	0/30 (0)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		6.000	0.000
Lower Limit		0.633	0.000
Upper Limit		283.461	25.791
Weeks to First Observed Tumor	96	83	--

(a) One dosed group received 0.001 μg TCDD in 0.1 ml acetone. A second dosed group received 0.001 μg TCDD in 0.1 ml acetone after pretreatment with 50 μg DMBA.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the dosed group is the probability level for the Fisher exact test for the comparison of the dosed group with the vehicle control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) The 95% confidence interval of the relative risk between the dosed group and the control group.

Table 6. Analyses of the Incidence of Primary Tumors in Female Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

Topography: Morphology	Vehicle Control	TCDD	TCDD plus DMBA
Integumentary System: Fibrosarcoma (b)	2/41 (5)	8/27 (30)	8/29 (28)
P Values (c)		P=0.007	P=0.010
Relative Risk (Vehicle Control) (d)		6.074	5.655
Lower Limit		1.331	1.235
Upper Limit		54.061	50.697
Weeks to First Observed Tumor	98	56	66
Lung: Alveolar/Bronchiolar Adenoma (b)	4/41 (10)	1/25 (4)	3/28 (11)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.410	1.098
Lower Limit		0.009	0.172
Upper Limit		3.806	5.939
Weeks to First Observed Tumor	79	91	89
Lung: Alveolar/Bronchiolar Carcinoma (b)	5/41 (12)	1/25 (4)	3/28 (11)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.328	0.879
Lower Limit		0.007	0.146
Upper Limit		2.678	4.101
Weeks to First Observed Tumor	74	69	95
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	8/41 (20)	2/25 (8)	6/28 (21)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.410	1.098
Lower Limit		0.045	0.349
Upper Limit		1.837	3.168
Weeks to First Observed Tumor	74	69	89

Table 6. Analyses of the Incidence of Primary Tumors in Female Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Vehicle Control	TCDD	TCDD plus DMBA
Hematopoietic System: Lymphoma (b)	14/41 (34)	10/27 (37)	8/29 (28)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		1.085	0.808
Lower Limit		0.502	0.337
Upper Limit		2.186	1.762
Weeks to First Observed Tumor	69	63	51
All Sites: Hemangioma (b)	2/41 (5)	0/27 (0)	1/29 (3)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.000	0.707
Lower Limit		0.000	0.012
Upper Limit		5.027	12.847
Weeks to First Observed Tumor	104	--	84
All Sites: Hemangioma or Hemangiosarcoma (b)	3/41 (7)	0/27 (0)	1/29 (3)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		0.000	0.471
Lower Limit		0.000	0.009
Upper Limit		2.468	5.487
Weeks to First Observed Tumor	104	--	84
Urinary Bladder: Leiomyosarcoma, Invasive (b)	0/30 (0)	0/27 (0)	2/27 (7)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		--	Infinite
Lower Limit		--	0.335
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	83

Table 6. Analyses of the Incidence of Primary Tumors in Female Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Vehicle Control	TCDD	TCDD plus DMBA
Vagina: Leiomyosarcoma, Invasive (b)	0/41 (0)	0/27 (0)	2/29 (7)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		--	Infinite
Lower Limit		--	0.421
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	86
Uterus: Leiomyoma (b)	0/36 (0)	2/25 (8)	1/29 (3)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		Infinite	Infinite
Lower Limit		0.431	0.067
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	93	92
Uterus: Leiomyosarcoma (b)	0/36 (0)	0/25 (0)	2/29 (7)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		--	Infinite
Lower Limit		--	0.371
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	83
Ovary: Leiomyosarcoma, Invasive (b)	0/33 (0)	0/22 (0)	2/29 (7)
P Values (c)		N.S.	N.S.
Relative Risk (Vehicle Control) (d)		--	Infinite
Lower Limit		--	0.342
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	83

Table 6. Analyses of the Incidence of Primary Tumors in Female Mice Administered TCDD or TCDD Following DMBA by Dermal Application (a)

(continued)

(a) One dosed group received 0.005 μg TCDD in 0.1 ml acetone. A second dosed group received 0.005 g TCDD in 0.1 ml acetone after pretreatment with 50 μg DMBA.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the dosed group is the probability level for the Fisher exact test for the comparison of the dosed group with the vehicle control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) The 95% confidence interval of the relative risk between the dosed group and the control group.

Table 7. Weeks to Death of Mice in the TCDD Studies with Histologically Confirmed Tumors of the Integumentary System

Group	Type of Tumor(a)			
	Fibroma	Fibrosarcoma	Sebaceous Adenoma	Kerato-acanthoma
Male Mice				
Untreated Controls	64			
Vehicle Controls	45	87,95,103	45	
TCDD		71,73,76,79 83,84		
TCDD + DMBA	66	67,84,91, 102,103		
Female Mice				
Untreated Controls		90		
Vehicle Controls		98,100		
TCDD	69	56,78,84,88 91,93,102,102		
TCDD + DMBA		66,82,83,87 89,95,97,100		104

(a) Entries are based on the week in which an animal died and the designated type of tumor found upon histopathologic examination.

IV. DISCUSSION

Throughout the bioassay, mean body weights of mice in the male or female groups administered TCDD or TCDD following DMBA were essentially the same as those of corresponding vehicle-control groups. Mean body weights of dosed and vehicle control groups were less than those of the untreated male controls throughout the study and were less than female untreated controls for the first 80 weeks.

Acetone as a vehicle had no obvious influence upon skin tumor production, but an increased incidence of pyelonephritis was observed in male mice exposed to acetone alone or in combination with TCDD.

In female mice, the incidence of fibrosarcoma in the integumentary system in TCDD and TCDD following DMBA-dose groups was significantly higher than that in the controls ($P=0.007$ and $P=0.010$, respectively). There was an increased incidence of fibrosarcomas in the integumentary system in male mice administered TCDD, but the results of the Fisher exact test were not statistically significant. However, the fibrosarcomas appeared significantly earlier in the TCDD-dosed male mice than in the vehicle controls. In addition, the dose administered to males was 20% of that administered to female mice. Dose-response data were not available as single doses of TCDD were used in each sex. The effects of DMBA alone were not determined in this study.

No statistically significant differences in tumor incidences were measured between animals administered TCDD alone and those pretreated with DMBA prior to TCDD administration, but the incidence of male mice with hemangiosarcoma was higher ($P=0.048$) in the TCDD group than in the group administered TCDD following DMBA. The significance of these observations cannot be evaluated due to the failure to include groups treated only with DMBA.

Deviations in the conduct of the study from accepted protocol for skin painting studies may have limited its effectiveness. Applying solutions of test chemicals in volumes sufficient to flow from the clipped area hindered assessing topical effects. However, the occurrence of tumors in the dermal layer suggest possible chemical penetration through the epidermis or entry via hair follicles or glands. The lack of early observations on occurrence of tissue masses among living animals precludes evaluating time to onset of tumor. Nevertheless, the time of tumor detection among dead or moribund animals with tumors is significantly earlier among treated animals than in vehicle or matched controls.

No reports of skin painting bioassays of TCDD of greater than 32 weeks duration were found in the literature.

A feeding study by Kociba et al. (1978) reported positive results in which groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing TCDD at concentrations of about 0.022 ppb, 0.210 ppb, or 2.2 ppb for 2 years. The control groups consisted of 86 animals. The incidences of females with hepatocellular carcinomas were: control 1/86, low-dose 0/50, mid-dose 2/50, and high-dose 11/49. Hepatocellular hyperplastic nodules occurred at increased incidences in the females receiving either the mid or high doses (control 8/86, low-dose 3/50, mid-dose 18/50, high-dose 23/50). The incidences of the liver tumors in the dosed groups of male rats were not significant. Squamous-cell carcinomas of the lung, hard palate/nasal turbinates, or tongue occurred at increased incidences in both the male and female rats administered the TCDD.

When male or female DBA/2 mice were administered TCDD by subcutaneous injection in a single dose of 100 μg/kg body weight and a simultaneous, unstated dose of methylcholanthrene (MCA) and then observed for 36 weeks, the incidence of skin tumors that developed was greater than that induced by the administration of MCA alone (Kouri et al., 1978). However, these results are compromised by the use of dioxane as a solvent for TCDD. One per cent dioxane in drinking water has previously been found to be carcinogenic for Sprague-Dawley rats (Argus et al., 1973,), Sherman rats (Kociba et al., 1974), and Osborne-Mendel and B6C3F1 mice (NCI, 1978).

A bioassay of TCDD administered by gavage was run concurrently with the present study (NTP, 1981). Under the conditions of that bioassay, TCDD was carcinogenic for Osborne-Mendel rats, causing increased incidences of follicular-cell thyroid tumors in males and liver tumors in females. TCDD was also carcinogenic for B6C3F1 mice, causing increased incidences of liver tumors in males and females and of follicular-cell thyroid tumors in females.

V. CONCLUSION

Under the conditions of this bioassay, 2,3,7,8-tetrachlorodibenzo-p-dioxin applied to the skin was not carcinogenic for male Swiss-Webster mice (the increase of fibrosarcomas in the integumentary system may have been associated with the skin application of TCDD). TCDD was carcinogenic for female Swiss-Webster mice causing fibrosarcomas in the integumentary system.

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Appendix A

Summary of the Incidence of Neoplasms in Mice Administered TCDD by Dermal Application

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED TCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	14	42	28
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	42	28
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(14)	(42)	(28)
FIBROMA		1 (7%)		
FIBROSARCOMA			1 (2%)	
*SUBCUT TISSUE	(14)	(14)	(42)	(28)
SEBACEOUS ADENOMA			1 (2%)	
FIBROMA			1 (2%)	
FIBROSARCOMA			2 (5%)	6 (21%)
RESPIRATORY SYSTEM				
#LUNG	(13)	(14)	(41)	(28)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (8%)	2 (14%)	6 (15%)	1 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (15%)		1 (2%)	1 (4%)
CORTICAL CARCINOMA, METASTATIC	1 (8%)			
FIBROSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(14)	(42)	(28)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)		1 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)		2 (5%)	1 (4%)
GRANULOCYTIC LEUKEMIA			1 (2%)	
#BONE MARROW	(14)	(14)	(40)	(25)
FIBROSARCOMA, INVASIVE				1 (4%)
CIRCULATORY SYSTEM				
#HEART	(13)	(14)	(42)	(28)
HEMANGIOSARCOMA	1 (8%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LIVER	(14)	(14)	(42)	(27)
HEMANGIOMA		1 (7%)		
HEMANGIOSARCOMA	1 (7%)		1 (2%)	4 (15%)
DIGESTIVE SYSTEM				
#PAROTID GLAND	(12)	(13)	(36)	(26)
ADENOMA, NOS				1 (4%)
#LIVER	(14)	(14)	(42)	(27)
HEPATOCELLULAR ADENOMA			1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (7%)			
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(14)	(40)	(23)
CORTICAL CARCINOMA	1 (9%)			
#THYROID	(13)	(13)	(35)	(26)
ADENOMA, NOS	1 (8%)			
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(14)	(14)	(42)	(28)
PAPILLARY ADENOMA		1 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(14)	(14)	(42)	(28)
FIBROSARCOMA, INVASIVE				1 (4%)
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(14)	(42)	(28)
FIBROSARCOMA			1 (2%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATH[a]	7	12	30	23
MORIBUND SACRIFICE	1		8	4
SCHEDULED SACRIFICE	5		4	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	2	3	3	3
ANIMAL MISSING				

[a] INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	6	15	13
TOTAL PRIMARY TUMORS	9	6	19	15
TOTAL ANIMALS WITH BENIGN TUMORS	2	5	8	2
TOTAL BENIGN TUMORS	2	5	9	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	1	8	12
TOTAL MALIGNANT TUMORS	7	1	10	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2	1
TOTAL SECONDARY TUMORS	1		2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED TCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	12	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	12	41	27
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(12)	(41)	(27)
FIBROMA				1 (4%)
FIBROSARCOMA		1 (8%)	2 (5%)	8 (30%)
MYXOMA				1 (4%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(12)	(41)	(25)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	2 (17%)	4 (10%)	1 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (13%)		5 (12%)	1 (4%)
FIBROSARCOMA, METASTATIC			1 (2%)	4 (16%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(12)	(41)	(27)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (27%)	4 (33%)	9 (22%)	7 (26%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (13%)		4 (10%)	2 (7%)
#BONE MARROW	(13)	(12)	(37)	(25)
FIBROMA			1 (3%)	
#SPLEEN	(15)	(12)	(40)	(24)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
FIBROMA			1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (4%)
#LYMPH NODE	(12)	(9)	(30)	(20)
FIBROSARCOMA, METASTATIC				1 (5%)
#CERVICAL LYMPH NODE	(12)	(9)	(30)	(20)
FIBROSARCOMA, METASTATIC				1 (5%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#PANCREATIC L.NODE	(12)	(9)	(30)	(20)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	
#THYMUS	(15)	(11)	(39)	(22)
THYMOMA, MALIGNANT			1 (3%)	
FIBROSARCOMA, METASTATIC				1 (5%)
CIRCULATORY SYSTEM				
#HEART/ATRIUM	(15)	(12)	(40)	(27)
FIBROSARCOMA, METASTATIC				1 (4%)
#LIVER	(15)	(12)	(41)	(27)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
#UTERUS	(15)	(12)	(36)	(25)
HEMANGIOMA			1 (3%)	
#OVARY	(14)	(10)	(33)	(22)
HEMANGIOMA			1 (3%)	
DIGESTIVE SYSTEM				
#PAROTID GLAND	(15)	(11)	(36)	(22)
MIXED TUMOR, BENIGN			1 (3%)	
#LIVER	(15)	(12)	(41)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
#PANCREAS	(15)	(12)	(40)	(26)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
LIPOMA			1 (3%)	
#PANCREATIC DUCT	(15)	(12)	(40)	(26)
SARCOMA, NOS			1 (3%)	
#GASTRIC SEROSA	(15)	(12)	(41)	(25)
SARCOMA, NOS			1 (2%)	
URINARY SYSTEM				
*GENITOURINARY TRACT	(15)	(12)	(41)	(27)
FIBROSARCOMA			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(11)	(41)	(23)
CORTICAL ADENOMA	1 (7%)			
REPRODUCTIVE SYSTEM				
#UTERUS	(15)	(12)	(36)	(25)
ADENOCARCINOMA, NOS			1 (3%)	
LEIOMYOMA	2 (13%)			2 (8%)
ENDOMETRIAL STROMAL POLYP				1 (4%)
#CERVIX UTERI	(15)	(12)	(36)	(25)
LEIOMYOMA	1 (7%)			
#OVARY	(14)	(10)	(33)	(22)
LUTEOMA	1 (7%)			
GRANULOSA-CELL TUMOR			1 (3%)	1 (5%)
LIPOMA			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEAL CAVITY	(15)	(12)	(41)	(27)
FIBROSARCOMA, METASTATIC				1 (4%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(12)	(41)	(27)
THYMOMA, METASTATIC			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
FIBROSARCOMA				1 (4%)
FIBROSARCOMA, METASTATIC				1 (4%)
PLEURAL CAVITY				
FIBROSARCOMA, METASTATIC				1 (4%)

ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATH@	8	11	28	24
MORIBUND SACRIFICE	1	2	1	
SCHEDULED SACRIFICE	3		15	3
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	3	2	1	3
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	25	18
TOTAL PRIMARY TUMORS	15	7	39	27
TOTAL ANIMALS WITH BENIGN TUMORS	6	2	10	6
TOTAL BENIGN TUMORS	6	2	11	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	5	23	16
TOTAL MALIGNANT TUMORS	9	5	27	20
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	5
TOTAL SECONDARY TUMORS			6	11
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1	1
TOTAL UNCERTAIN TUMORS			1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

Appendix B

Summary of the Incidence of Nonneoplastic Lesions in Mice Administered TCDD by Dermal Application

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED TCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	14	42	28
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	42	28
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(14)	(42)	(28)
EDEMA, NOS			1 (2%)	
INFLAMMATION, NOS			2 (5%)	
ULCER, NOS			3 (7%)	1 (4%)
ULCER, FOCAL				1 (4%)
ULCER, ACUTE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC	1 (7%)			
INFLAMMATION PROLIFERATIVE			1 (2%)	
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, PAPILLARY			1 (2%)	
HYPERKERATOSIS	1 (7%)		3 (7%)	
ACANTHOSIS			1 (2%)	
*SUBCUT TISSUE	(14)	(14)	(42)	(28)
EPIDERMAL INCLUSION CYST			1 (2%)	
ULCER, NOS			1 (2%)	2 (7%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (4%)
INFLAMMATION, GRANULOMATOUS				1 (4%)
GRANULATION, TISSUE			1 (2%)	
NECROSIS, NOS			2 (5%)	1 (4%)
RESPIRATORY SYSTEM				
#TRACHEA	(13)	(13)	(36)	(27)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
#LUNG/BRONCHUS	(13)	(14)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (8%)	2 (14%)	1 (2%)	
#LUNG/BRONCHIOLE	(13)	(14)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	6 (46%)	7 (50%)	13 (32%)	9 (32%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HYPERPLASIA, EPITHELIAL			1 (2%)	
#LUNG	(13)	(14)	(41)	(28)
ATELECTASIS		1 (7%)	2 (5%)	1 (4%)
CONGESTION, NOS		3 (21%)	3 (7%)	
INFLAMMATION, FOCAL			1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (8%)	1 (7%)	3 (7%)	7 (25%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
EMPYEMA			1 (2%)	
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
NECROSIS, NOS			1 (2%)	
ALVEOLAR MACROPHAGES			1 (2%)	
HEMATOPOIETIC SYSTEM				
#SPLEEN	(13)	(14)	(40)	(28)
AMYLOID, NOS		1 (7%)		
HYPOPLASIA, NOS	1 (8%)	1 (7%)	5 (13%)	1 (4%)
ATROPHY, NOS				2 (7%)
HYPERPLASIA, NOS	2 (15%)	1 (7%)	4 (10%)	3 (11%)
HEMATOPOIESIS		3 (21%)	5 (13%)	
#SPLENIC FOLLICLES	(13)	(14)	(40)	(28)
HYPOPLASIA, NOS		1 (7%)		1 (4%)
HYPERPLASIA, NOS		1 (7%)	2 (5%)	6 (21%)
#SPLENIC RED PULP	(13)	(14)	(40)	(28)
HYPOPLASIA, NOS			2 (5%)	
MEGAKARYOCYTOSIS			1 (3%)	
HEMATOPOIESIS				4 (14%)
GRANULOPOIESIS			1 (3%)	
#LYMPH NODE	(8)	(11)	(25)	(13)
CONGESTION, NOS		1 (9%)		
HYPERPLASIA, NOS			2 (8%)	2 (15%)
HYPERPLASIA, LYMPHOID		1 (9%)		
#CERVICAL LYMPH NODE	(8)	(11)	(25)	(13)
HYPERPLASIA, NOS	4 (50%)	7 (64%)	10 (40%)	3 (23%)
#TRACHEAL LYMPH NODE	(8)	(11)	(25)	(13)
HYPERPLASIA, NOS	1 (13%)			
#PANCREATIC L.NODE	(8)	(11)	(25)	(13)
ABSCESS, NOS				1 (8%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NECROSIS, NOS				1 (8%)
NECROSIS, FOCAL				1 (8%)
HYPERPLASIA, NOS				1 (8%)
#LUMBAR LYMPH NODE	(8)	(11)	(25)	(13)
HYPERPLASIA, LYMPHOID			1 (4%)	
#MESENTERIC L. NODE	(8)	(11)	(25)	(13)
ABSCESS, NOS			1 (4%)	
INFLAMMATION PROLIFERATIVE			1 (4%)	
NECROSIS, CASEOUS			1 (4%)	
#RENAL LYMPH NODE	(8)	(11)	(25)	(13)
HYPERPLASIA, NOS	1 (13%)			
#LUNG/BRONCHIOLE	(13)	(14)	(41)	(28)
HYPERPLASIA, LYMPHOID			1 (2%)	
#LIVER	(14)	(14)	(42)	(27)
HEMATOPOIESIS			1 (2%)	
#KIDNEY	(14)	(14)	(42)	(28)
HYPERPLASIA, LYMPHOID			1 (2%)	1 (4%)
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(8)	(11)	(25)	(13)
LYMPHANGIECTASIS			1 (4%)	
#MESENTERIC L. NODE	(8)	(11)	(25)	(13)
LYMPHANGIECTASIS	1 (13%)			
#HEART	(13)	(14)	(42)	(28)
MINERALIZATION	1 (8%)			
#HEART/ATRIUM	(13)	(14)	(42)	(28)
THROMBOSIS, NOS		1 (7%)		
THROMBUS, ORGANIZED				1 (4%)
#MYOCARDIUM	(13)	(14)	(42)	(28)
INFLAMMATION, CHRONIC FOCAL			3 (7%)	
#ENDOCARDIUM	(13)	(14)	(42)	(28)
INFLAMMATION PROLIFERATIVE	1 (8%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HYPERPLASIA, NOS	1 (8%)			
*RENAL ARTERY	(14)	(14)	(42)	(28)
ARTERIOSCLEROSIS, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#PAROTID GLAND	(12)	(13)	(36)	(26)
INFLAMMATION, NOS		1 (8%)	3 (8%)	1 (4%)
INFLAMMATION, FOCAL	1 (8%)			
NECROSIS, FOCAL			1 (3%)	
#LIVER	(14)	(14)	(42)	(27)
CONGESTION, NOS	1 (7%)			
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	1 (4%)
INFLAMMATION, ACUTE		1 (7%)		1 (4%)
INFLAMMATION, ACUTE DIFFUSE			1 (2%)	
CIRRHOSIS, NOS	1 (7%)			
DEGENERATION, HYDROPIC			1 (2%)	
NECROSIS, NOS	1 (7%)	1 (7%)	1 (2%)	1 (4%)
NECROSIS, FOCAL			1 (2%)	1 (4%)
NECROSIS, COAGULATIVE				1 (4%)
AMYLOID, NOS		1 (7%)		
LIPOIDOSIS	1 (7%)			
HEPATOCYTOMEGALY	1 (7%)		2 (5%)	
#PORTAL TRACT	(14)	(14)	(42)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	1 (4%)
#LIVER/CENTRILOBULAR	(14)	(14)	(42)	(27)
METAMORPHOSIS FATTY	1 (7%)			
HEPATOCYTOMEGALY	5 (36%)	2 (14%)	1 (2%)	2 (7%)
#LIVER/PERIPORTAL	(14)	(14)	(42)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			2 (5%)	1 (4%)
#LIVER/HEPATOCYTES	(14)	(14)	(42)	(27)
CLOUDY SWELLING	1 (7%)			
#BILE DUCT	(14)	(14)	(42)	(27)
HYPERPLASIA, FOCAL		1 (7%)		
#STOMACH	(14)	(14)	(42)	(28)
HYPERKERATOSIS			3 (7%)	1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#DUODENUM	(11)	(12)	(34)	(22)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (9%)			
URINARY SYSTEM				
*GENITOURINARY TRACT	(14)	(14)	(42)	(28)
RETENTION OF CONTENT				1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
#KIDNEY	(14)	(14)	(42)	(28)
HYDRONEPHROSIS	1 (7%)		1 (2%)	1 (4%)
GLOMERULONEPHRITIS, NOS				1 (4%)
PYELONEPHRITIS, NOS			5 (12%)	1 (4%)
PYELONEPHRITIS, FOCAL				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	8 (57%)	10 (71%)	20 (48%)	9 (32%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)	
GLOMERULONEPHRITIS, ACUTE				3 (11%)
ABSCESS, NOS				1 (4%)
GLOMERULONEPHRITIS, SUBACUTE				1 (4%)
INFLAMMATION, CHRONIC			1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	9 (64%)	5 (36%)	6 (14%)	10 (36%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	
#KIDNEY/GLOMERULUS	(14)	(14)	(42)	(28)
AMYLOIDOSIS			1 (2%)	
#KIDNEY/TUBULE	(14)	(14)	(42)	(28)
CALCULUS, NOS			1 (2%)	
CALCIFICATION, NOS			1 (2%)	
#KIDNEY/PELVIS	(14)	(14)	(42)	(28)
ABSCESS, NOS			1 (2%)	
METAPLASIA, SQUAMOUS			1 (2%)	
#URINARY BLADDER	(13)	(13)	(39)	(25)
CAST, NOS			1 (3%)	
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, CHRONIC	4 (31%)	2 (15%)	3 (8%)	4 (16%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
INFLAMMATION PROLIFERATIVE			3 (8%)	
HYPERPLASIA, EPITHELIAL			1 (3%)	4 (16%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
POLYPOID HYPERPLASIA			1 (3%)	
*URETHRA	(14)	(14)	(42)	(28)
HYPERPLASIA, EPITHELIAL				1 (4%)
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(14)	(40)	(23)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (3%)	
DEGENERATION PIGMENTARY	5 (45%)	4 (29%)	4 (10%)	
AMYLOIDOSIS			1 (3%)	
LIPOIDOSIS	1 (9%)			
#ADRENAL CORTEX	(11)	(14)	(40)	(23)
HAMARTOMA		1 (7%)		
#ZONA GLOMERULOSA	(11)	(14)	(40)	(23)
METAPLASIA, NOS	5 (45%)	7 (50%)	6 (15%)	5 (22%)
#ZONA FASCICULATA	(11)	(14)	(40)	(23)
HYPERTROPHY, FOCAL	1 (9%)			
#ADRENAL MEDULLA	(11)	(14)	(40)	(23)
CONGESTION, NOS		2 (14%)	1 (3%)	
#PANCREATIC ISLETS	(13)	(14)	(42)	(24)
HYPERPLASIA, NOS		3 (21%)		
REPRODUCTIVE SYSTEM				
*GENITAL SYSTEM	(14)	(14)	(42)	(28)
RETENTION OF CONTENT	1 (7%)		2 (5%)	1 (4%)
INFLAMMATION, SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (4%)
PLASMA-CELL INFILTRATE			1 (2%)	
*BULBOURETHRAL GLAND	(14)	(14)	(42)	(28)
INFLAMMATION, ACUTE SUPPURATIVE		1 (7%)	1 (2%)	
POLYPOID HYPERPLASIA			1 (2%)	
POLYP				1 (4%)
*PREPUTIAL GLAND	(14)	(14)	(42)	(28)
EPIDERMAL INCLUSION CYST			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
GRANULATION, TISSUE			1 (2%)	
#PROSTATE	(13)	(14)	(39)	(27)
RETENTION OF CONTENT	9 (69%)	9 (64%)	18 (46%)	16 (59%)
INFLAMMATION, ACUTE		1 (7%)		1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	1 (4%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (5%)	
*SEMINAL VESICLE	(14)	(14)	(42)	(28)
DILATATION, NOS	2 (14%)			
RETENTION OF CONTENT	9 (64%)	9 (64%)	18 (43%)	15 (54%)
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (4%)
INFLAMMATION, ACUTE NECROTIZING				1 (4%)
INFLAMMATION, CHRONIC	1 (7%)			
*COAGULATING GLAND	(14)	(14)	(42)	(28)
INFLAMMATION, ACUTE NECROTIZING				1 (4%)
#TESTIS	(14)	(13)	(41)	(26)
MINERALIZATION	3 (21%)			1 (4%)
DEGENERATION, NOS			1 (2%)	
NECROSIS, FOCAL			1 (2%)	
NECROSIS, CASEOUS			1 (2%)	
ATROPHY, NOS	4 (29%)	3 (23%)	5 (12%)	5 (19%)
ATROPHY, FOCAL	1 (7%)			
SPERMATOGENIC ARREST	4 (29%)	6 (46%)	7 (17%)	2 (8%)
HYPOSPERMATOGENESIS		1 (8%)		5 (19%)
HYPERPLASIA, INTERSTITIAL CELL		1 (8%)	1 (2%)	
#TESTIS/TUBULE	(14)	(13)	(41)	(26)
DEGENERATION, NOS			1 (2%)	
*EPIDIDYMIS	(14)	(14)	(42)	(28)
SPERMATOCELE		1 (7%)		1 (4%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
ASPERMATOGENESIS	3 (21%)	3 (21%)	3 (7%)	8 (29%)
HYPOSPERMATOGENESIS	2 (14%)	5 (36%)	4 (10%)	
*VAS DEFERENS	(14)	(14)	(42)	(28)
HYPERPLASIA, EPITHELIAL				1 (4%)
HYPERPLASIA, PAPILLARY				1 (4%)
POLYPOID HYPERPLASIA				1 (4%)
NERVOUS SYSTEM				
#BRAIN	(14)	(14)	(41)	(27)
INFLAMMATION, FOCAL		1 (7%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*PECTORALIS MAJOR MUS	(14)	(14)	(42)	(28)
EPIDERMAL INCLUSION CYST		1 (7%)		
BODY CAVITIES				
*PERITONEUM	(14)	(14)	(42)	(28)
INFLAMMATION PROLIFERATIVE			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(14)	(42)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)	1 (7%)		
ADIPOSE TISSUE				
PIGMENTATION, NOS				2
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				2
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/HISTO PERF			1	
AUTOLYSIS/NO NECROPSY	1	1	3	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED TCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	12	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	12	41	27
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(12)	(41)	(27)
INFLAMMATION, NOS				1 (4%)
ULCER, NOS				3 (11%)
INFLAMMATION, CHRONIC		3 (25%)		1 (4%)
NECROSIS, NOS				2 (7%)
HYPERPLASIA, FOCAL	1 (7%)			
HYPERKERATOSIS	1 (7%)		1 (2%)	2 (7%)
VERRUCA		3 (25%)		
*SUBCUT TISSUE	(15)	(12)	(41)	(27)
ULCER, NOS			1 (2%)	6 (22%)
PUS				1 (4%)
ABSCESS, NOS			1 (2%)	
NECROSIS, NOS		1 (8%)	1 (2%)	4 (15%)
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(11)	(40)	(25)
POLYP				1 (4%)
#LUNG/BRONCHUS	(15)	(12)	(41)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		2 (5%)	1 (4%)
#LUNG/BRONCHIOLE	(15)	(12)	(41)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (27%)	3 (25%)	22 (54%)	11 (44%)
#LUNG	(15)	(12)	(41)	(25)
ATELECTASIS			1 (2%)	
CONGESTION, NOS	2 (13%)	1 (8%)		
INFLAMMATION, NOS				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (8%)		1 (4%)
INFLAMMATION, INTERSTITIAL	3 (20%)		5 (12%)	7 (28%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
BRONCHOPNEUMONIA ACUTE SUPPURATI		1 (8%)		
HYPERPLASIA, ADENOMATOUS	1 (7%)		2 (5%)	
HEMATOPOIETIC SYSTEM				
*MAMMARY GLAND	(15)	(12)	(41)	(27)
ADENOSIS	2 (13%)		2 (5%)	4 (15%)
#BONE MARROW	(13)	(12)	(37)	(25)
FIBROUS OSTEODYSTROPHY	3 (23%)	1 (8%)	7 (19%)	3 (12%)
HYPERPLASIA, NOS			1 (3%)	
RETICULOCYTOSIS	1 (8%)			
HYPERPLASIA, HEMATOPOIETIC				1 (4%)
#SPLEEN	(15)	(12)	(40)	(24)
CYST, NOS			1 (3%)	
HEMORRHAGIC CYST		1 (8%)		
HYPOPLASIA, NOS	1 (7%)	1 (8%)	3 (8%)	
ATROPHY, NOS				1 (4%)
HYPERPLASIA, NODULAR			1 (3%)	
HYPERPLASIA, NOS	3 (20%)	1 (8%)		1 (4%)
MEGAKARYOCYTOSIS			1 (3%)	
HYPERPLASIA, GRANULOCYTIC				1 (4%)
HYPERPLASIA, LYMPHOID	1 (7%)			
HEMATOPOIESIS			2 (5%)	1 (4%)
#SPLENIC FOLLICLES	(15)	(12)	(40)	(24)
HYPERPLASIA, NODULAR				1 (4%)
HYPERPLASIA, NOS	2 (13%)	2 (17%)	4 (10%)	4 (17%)
#SPLENIC RED PULP	(15)	(12)	(40)	(24)
AMYLOIDOSIS				1 (4%)
HYPOPLASIA, NOS	1 (7%)		1 (3%)	
HYPERPLASIA, HEMATOPOIETIC		2 (17%)		
HYPERPLASIA, GRANULOCYTIC				1 (4%)
HEMATOPOIESIS	2 (13%)		1 (3%)	1 (4%)
ERYTHROPOIESIS				1 (4%)
#LYMPH NODE	(12)	(9)	(30)	(20)
NECROSIS, NOS				1 (5%)
HYPERPLASIA, NOS	2 (17%)	3 (33%)	6 (20%)	3 (15%)
#CERVICAL LYMPH NODE	(12)	(9)	(30)	(20)
ABSCESS, NOS		1 (11%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HYPERPLASIA, NOS	1 (8%)	1 (11%)	17 (57%)	6 (30%)
#PANCREATIC L.NODE	(12)	(9)	(30)	(20)
PIGMENTATION, NOS			1 (3%)	
HYPERPLASIA, NOS			1 (3%)	2 (10%)
#LUNG/BRONCHIOLE	(15)	(12)	(41)	(25)
HYPERPLASIA, LYMPHOID			1 (2%)	
#PAROTID GLAND	(15)	(11)	(36)	(22)
FIBROSING ADENOSIS			1 (3%)	
#LIVER	(15)	(12)	(41)	(27)
HEMATOPOIESIS	1 (7%)			
#LIVER/PERIPORTAL	(15)	(12)	(41)	(27)
HYPERPLASIA, LYMPHOID				1 (4%)
#ADRENAL	(15)	(11)	(41)	(23)
MYELOPOIESIS			1 (2%)	
#THYMUS	(15)	(11)	(39)	(22)
HYPERPLASIA, NOS		1 (9%)	2 (5%)	
#THYMIC MEDULLA	(15)	(11)	(39)	(22)
HYPERPLASIA, NOS	2 (13%)			
CIRCULATORY SYSTEM				
#BONE MARROW	(13)	(12)	(37)	(25)
PERIARTERITIS				1 (4%)
#SPLEEN	(15)	(12)	(40)	(24)
PERIARTERITIS		1 (8%)		
#PANCREATIC L.NODE	(12)	(9)	(30)	(20)
LYMPHANGIECTASIS			1 (3%)	2 (10%)
#MESENTERIC L. NODE	(12)	(9)	(30)	(20)
LYMPHANGIECTASIS			2 (7%)	
#LUNG	(15)	(12)	(41)	(25)
EMBOLISM, NOS		1 (8%)		
#HEART	(15)	(12)	(40)	(27)
MINERALIZATION				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ENDOCARDIOSIS	2 (13%)			
#HEART/ATRIUM	(15)	(12)	(40)	(27)
THROMBOSIS, NOS	1 (7%)		2 (5%)	
#MYOCARDIUM	(15)	(12)	(40)	(27)
INFLAMMATION, ACUTE SUPPURATIVE		2 (17%)		
INFLAMMATION, CHRONIC FOCAL	1 (7%)			
NECROSIS, NOS		1 (8%)		
*CENTRAL VEINS/LIVER	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
#UTERUS	(15)	(12)	(36)	(25)
PERIARTERITIS				2 (8%)
#OVARY	(14)	(10)	(33)	(22)
PERIARTERITIS				1 (5%)
#THYROID	(15)	(11)	(39)	(22)
PERIARTERITIS				1 (5%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(11)	(36)	(22)
INFLAMMATION, NOS			1 (3%)	
#PAROTID GLAND	(15)	(11)	(36)	(22)
INFLAMMATION, NOS		1 (9%)	1 (3%)	
#LIVER	(15)	(12)	(41)	(27)
CONGESTION, NOS		1 (8%)		
INFLAMMATION, NOS	1 (7%)			1 (4%)
INFLAMMATION, FOCAL			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	1 (4%)
INFLAMMATION, ACUTE				1 (4%)
INFLAMMATION, ACUTE DIFFUSE		1 (8%)		2 (7%)
NECROSIS, FOCAL		1 (8%)		
NECROSIS, COAGULATIVE	1 (7%)		1 (2%)	2 (7%)
AMYLOIDOSIS			1 (2%)	
PIGMENTATION, NOS		1 (8%)		
FOCAL CELLULAR CHANGE	1 (7%)			
HEPATOCYTOMEGALY				2 (7%)
ANGIECTASIS		1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LIVER/CENTRILOBULAR	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
HEPATITIS, TOXIC				1 (4%)
DEGENERATION, HYDROPIC		1 (8%)		
NECROSIS, NOS	1 (7%)			
#LIVER/PERIPORTAL	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)	1 (8%)		2 (7%)
HYPERPLASIA, NOS	1 (7%)			
#LIVER/KUPFFER CELL	(15)	(12)	(41)	(27)
HYPERPLASIA, NOS			1 (2%)	
*GALLBLADDER	(15)	(12)	(41)	(27)
CALCULUS, NOS		1 (8%)		
INFLAMMATION, CHRONIC	1 (7%)	1 (8%)		
#BILE DUCT	(15)	(12)	(41)	(27)
HYPERPLASIA, NOS	2 (13%)			
#PANCREAS	(15)	(12)	(40)	(26)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (3%)	
HYPERPLASIA, NOS	1 (7%)			
#STOMACH	(15)	(12)	(41)	(25)
INFLAMMATION, ACUTE	1 (7%)			
HYPERKERATOSIS			2 (5%)	1 (4%)
#SMALL INTESTINE	(13)	(11)	(40)	(24)
ABSCESS, NOS				1 (4%)
NECROSIS, NOS				1 (4%)
URINARY SYSTEM				
#KIDNEY	(15)	(12)	(41)	(27)
HYDRONEPHROSIS	1 (7%)	1 (8%)	1 (2%)	1 (4%)
CONGESTION, NOS		1 (8%)		
GLOMERULONEPHRITIS, NOS	1 (7%)	1 (8%)		
PYELONEPHRITIS, NOS			1 (2%)	1 (4%)
PYELONEPHRITIS, FOCAL				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	5 (33%)	7 (58%)	26 (63%)	8 (30%)
GLOMERULONEPHRITIS, ACUTE			1 (2%)	2 (7%)
ABSCESS, NOS				1 (4%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
GLOMERULONEPHRITIS, SUBACUTE		1 (8%)		1 (4%)
INFLAMMATION, CHRONIC	2 (13%)		1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	10 (67%)	4 (33%)	16 (39%)	13 (48%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS	2 (13%)		2 (5%)	1 (4%)
INFARCT, HEALED			1 (2%)	
#KIDNEY/GLOMERULUS	(15)	(12)	(41)	(27)
DEGENERATION, HYALINE		1 (8%)		
AMYLOIDOSIS			1 (2%)	
#KIDNEY/PELVIS	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
#URINARY BLADDER	(13)	(10)	(30)	(20)
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (5%)
INFLAMMATION, CHRONIC	4 (31%)	2 (20%)	10 (33%)	8 (40%)
INFLAMMATION, CHRONIC FOCAL			2 (7%)	
INFLAMMATION PROLIFERATIVE				1 (5%)
ATROPHY, NOS			1 (3%)	
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(11)	(41)	(23)
CONGESTION, NOS			2 (5%)	
EDEMA, NOS				2 (9%)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
DEGENERATION PIGMENTARY	9 (60%)	8 (73%)	22 (54%)	12 (52%)
AMYLOID, NOS				1 (4%)
AMYLOIDOSIS		1 (9%)		
#ADRENAL CORTEX	(15)	(11)	(41)	(23)
HAMARTOMA	1 (7%)		1 (2%)	1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (2%)	
#ZONA GLOMERULOSA	(15)	(11)	(41)	(23)
METAPLASIA, NOS	6 (40%)	7 (64%)	20 (49%)	14 (61%)
#THYROID	(15)	(11)	(39)	(22)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)	
#THYROID FOLLICLE	(15)	(11)	(39)	(22)
GOITER COLLOID			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(12)	(41)	(27)
DILATATION/DUCTS	3 (20%)		3 (7%)	4 (15%)
INFLAMMATION PROLIFERATIVE				1 (4%)
*VAGINA	(15)	(12)	(41)	(27)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
#UTERUS	(15)	(12)	(36)	(25)
MINERALIZATION			1 (3%)	
HEMORRHAGE			1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
ABSCESS, NOS			1 (3%)	
NECROSIS, NOS			1 (3%)	
NECROSIS, FOCAL				1 (4%)
#UTERUS/ENDOMETRIUM	(15)	(12)	(36)	(25)
HYPERPLASIA, NOS	1 (7%)	1 (8%)	8 (22%)	10 (40%)
HYPERPLASIA, CYSTIC	12 (80%)	8 (67%)	22 (61%)	13 (52%)
#OVARY/OVIDUCT	(15)	(12)	(36)	(25)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
#OVARY	(14)	(10)	(33)	(22)
CYST, NOS	1 (7%)		4 (12%)	1 (5%)
ATRESIA	10 (71%)	8 (80%)	20 (61%)	14 (64%)
HEMORRHAGE		1 (10%)		
HEMORRHAGIC CYST	1 (7%)		2 (6%)	1 (5%)
ABSCESS, NOS		1 (10%)		
AMYLOIDOSIS		1 (10%)		
CHOLESTEROL DEPOSIT		1 (10%)		
GROWTH, RETARDATION				1 (5%)
ATROPHY, NOS	8 (57%)	5 (50%)	25 (76%)	9 (41%)
ATROPHY, CYSTIC	4 (29%)	3 (30%)	1 (3%)	8 (36%)
LUTEINIZATION	2 (14%)		2 (6%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(15)	(12)	(41)	(27)
ABSCESS, NOS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
BODY CAVITIES				
*ABDOMINAL WALL	(15)	(12)	(41)	(27)
HERNIA, NOS			1 (2%)	
*PERITONEUM	(15)	(12)	(41)	(27)
INFLAMMATION, NOS				2 (7%)
*PLEURA	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(12)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (20%)		1 (2%)	1 (4%)
BACTERIAL SEPTICEMIA		1 (8%)		2 (7%)
AMYLOIDOSIS	1 (7%)	1 (8%)	3 (7%)	
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				3
SPECIAL MORPHOLOGY SUMMARY				
AUTOLYSIS/NO NECROPSY		3	4	3

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Appendix C

Summary of the Incidence of Neoplasms in Mice Administered TCDD plus DMBA by Dermal Application

TABLE C1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED TCDD FOLLOWING DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	14	42	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	42	30
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(14)	(42)	(30)
FIBROMA		1 (7%)		1 (3%)
FIBROSARCOMA			1 (2%)	
*SUBCUT TISSUE	(14)	(14)	(42)	(30)
SEBACEOUS ADENOMA			1 (2%)	
FIBROMA			1 (2%)	
FIBROSARCOMA			2 (5%)	5 (17%)
RESPIRATORY SYSTEM				
#LUNG	(13)	(14)	(41)	(29)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (8%)	2 (14%)	6 (15%)	5 (17%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (15%)		1 (2%)	2 (7%)
CORTICAL CARCINOMA, METASTATIC	1 (8%)			
FIBROSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(14)	(42)	(30)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)		3 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)		2 (5%)	1 (3%)
GRANULOCYTIC LEUKEMIA			1 (2%)	
#PULMONARY LYMPH NODE	(8)	(11)	(25)	(19)
ALVEOLAR/BRONCHIOLAR CA, METASTA				1 (5%)
#MESENTERIC L. NODE	(8)	(11)	(25)	(19)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (5%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#BRACHIAL LYMPH NODE	(8)	(11)	(25)	(19)
FIBROSARCOMA, METASTATIC				1 (5%)
#THYMUS	(11)	(13)	(30)	(24)
FIBROSARCOMA, METASTATIC				1 (4%)
CIRCULATORY SYSTEM				
#HEART	(13)	(14)	(42)	(30)
HEMANGIOSARCOMA	1 (8%)			
#LIVER	(14)	(14)	(42)	(29)
HEMANGIOMA		1 (7%)		
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(42)	(29)
HEPATOCELLULAR ADENOMA			1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (7%)			
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(14)	(40)	(29)
CORTICAL CARCINOMA	1 (9%)			
#THYROID	(13)	(13)	(35)	(25)
ADENOMA, NOS	1 (8%)			
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(14)	(14)	(42)	(30)
PAPILLARY ADENOMA		1 (7%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(14)	(14)	(42)	(30)
LIPOMA				1 (3%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(14)	(42)	(30)
FIBROSARCOMA			1 (2%)	1 (3%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATH@	7	12	30	21
MORIBUND SACRIFICE	1		8	4
SCHEDULED SACRIFICE	5		4	3
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	2	3	3	2
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	6	15	16
TOTAL PRIMARY TUMORS	9	6	19	20
TOTAL ANIMALS WITH BENIGN TUMORS	2	5	8	7
TOTAL BENIGN TUMORS	2	5	9	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	1	8	11
TOTAL MALIGNANT TUMORS	7	1	10	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2	3
TOTAL SECONDARY TUMORS	1		2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE C2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED TCDD FOLLOWING DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	12	41	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	12	41	29
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(12)	(41)	(29)
KERATOACANTHOMA				1 (3%)
FIBROSARCOMA				2 (7%)
*SUBCUT TISSUE	(15)	(12)	(41)	(29)
FIBROSARCOMA		1 (8%)	2 (5%)	6 (21%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(12)	(41)	(28)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	2 (17%)	4 (10%)	3 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (13%)		5 (12%)	3 (11%)
FIBROSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(12)	(41)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (27%)	4 (33%)	9 (22%)	5 (17%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (13%)		4 (10%)	1 (3%)
#BONE MARROW	(13)	(12)	(37)	(28)
FIBROMA			1 (3%)	
#SPLEEN	(15)	(12)	(40)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
FIBROMA			1 (3%)	
#LYMPH NODE	(12)	(9)	(30)	(25)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (4%)
#LYMPH NODE OF THORAX	(12)	(9)	(30)	(25)
ALVEOLAR/BRONCHIOLAR CA, INVASIV				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#TRACHEAL LYMPH NODE	(12)	(9)	(30)	(25)
ALVEOLAR/BRONCHIOLAR CA, METASTA				1 (4%)
#PANCREATIC L.NODE	(12)	(9)	(30)	(25)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	
#THYMUS	(15)	(11)	(39)	(28)
ALVEOLAR/BRONCHIOLAR CA, METASTA				1 (4%)
THYMOMA, MALIGNANT			1 (3%)	1 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (4%)
CIRCULATORY SYSTEM				
#LIVER	(15)	(12)	(41)	(28)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
#UTERUS	(15)	(12)	(36)	(29)
HEMANGIOMA			1 (3%)	
#OVARY	(14)	(10)	(33)	(29)
HEMANGIOMA			1 (3%)	1 (3%)
DIGESTIVE SYSTEM				
#PAROTID GLAND	(15)	(11)	(36)	(27)
MIXED TUMOR, BENIGN			1 (3%)	
#LIVER	(15)	(12)	(41)	(28)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
HEPATOCELLULAR CARCINOMA				1 (4%)
#PANCREAS	(15)	(12)	(40)	(28)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
LIPOMA			1 (3%)	
#PANCREATIC DUCT	(15)	(12)	(40)	(28)
SARCOMA, NOS			1 (3%)	
#GASTRIC SEROSA	(15)	(12)	(41)	(29)
SARCOMA, NOS			1 (2%)	
URINARY SYSTEM				
*GENITOURINARY TRACT	(15)	(12)	(41)	(29)
FIBROSARCOMA			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#URINARY BLADDER	(13)	(10)	(30)	(27)
LEIOMYOSARCOMA, INVASIVE				2 (7%)
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(11)	(41)	(25)
CORTICAL ADENOMA	1 (7%)			
REPRODUCTIVE SYSTEM				
*VAGINA	(15)	(12)	(41)	(29)
LEIOMYOSARCOMA, INVASIVE				2 (7%)
#UTERUS	(15)	(12)	(36)	(29)
ADENOCARCINOMA, NOS			1 (3%)	
LEIOMYOMA	2 (13%)			1 (3%)
LEIOMYOSARCOMA				2 (7%)
#CERVIX UTERI	(15)	(12)	(36)	(29)
LEIOMYOMA	1 (7%)			1 (3%)
#OVARY	(14)	(10)	(33)	(29)
PAPILLARY ADENOMA				1 (3%)
LUTEOMA	1 (7%)			
GRANULOSA-CELL TUMOR			1 (3%)	
LIPOMA			1 (3%)	
LEIOMYOSARCOMA, INVASIVE				2 (7%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*COCCYX	(15)	(12)	(41)	(29)
FIBROSARCOMA, METASTATIC				1 (3%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(12)	(41)	(29)
THYMOMA, METASTATIC			1 (2%)	1 (3%)
FIBROSARCOMA, METASTATIC				1 (3%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATHa	8	11	28	19
MORIBUND SACRIFICE	1	2	1	4
SCHEDULED SACRIFICE	3		15	4
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	3	2	1	3
ANIMAL MISSING				

a INCLUDES AUTOLYZED ANIMALS

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	25	25
TOTAL PRIMARY TUMORS	15	7	39	31
TOTAL ANIMALS WITH BENIGN TUMORS	6	2	10	8
TOTAL BENIGN TUMORS	6	2	11	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	5	23	21
TOTAL MALIGNANT TUMORS	9	5	27	23
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	6
TOTAL SECONDARY TUMORS			6	12
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1	
TOTAL UNCERTAIN TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

Appendix D

Summary of the Incidence of Nonneoplastic Lesions
in Mice Administered TCDD plus DMBA
by Dermal Application

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED TCDD FOLLOWING DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	14	42	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	14	42	30
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(14)	(42)	(30)
EPIDERMAL INCLUSION CYST				1 (3%)
EDEMA, NOS			1 (2%)	1 (3%)
INFLAMMATION, NOS			2 (5%)	
ULCER, NOS			3 (7%)	2 (7%)
ULCER, ACUTE			1 (2%)	
INFLAMMATION, ACUTE DIFFUSE				1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (3%)
ABSCESS, NOS				2 (7%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC	1 (7%)			4 (13%)
INFLAMMATION PROLIFERATIVE			1 (2%)	
FIBROSIS				1 (3%)
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, PAPILLARY			1 (2%)	
HYPERKERATOSIS	1 (7%)		3 (7%)	1 (3%)
ACANTHOSIS			1 (2%)	1 (3%)
*SUBCUT TISSUE	(14)	(14)	(42)	(30)
EPIDERMAL INCLUSION CYST			1 (2%)	
ULCER, NOS			1 (2%)	2 (7%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (3%)
ABSCESS, NOS				2 (7%)
GRANULATION, TISSUE			1 (2%)	
NECROSIS, NOS			2 (5%)	
NECROSIS, FOCAL				1 (3%)
RESPIRATORY SYSTEM				
#TRACHEA	(13)	(13)	(36)	(28)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LUNG/BRONCHUS	(13)	(14)	(41)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (8%)	2 (14%)	1 (2%)	
#LUNG/BRONCHIOLE	(13)	(14)	(41)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR	6 (46%)	7 (50%)	13 (32%)	6 (21%)
HYPERPLASIA, EPITHELIAL			1 (2%)	
#LUNG	(13)	(14)	(41)	(29)
ATELECTASIS		1 (7%)	2 (5%)	2 (7%)
CONGESTION, NOS		3 (21%)	3 (7%)	
INFLAMMATION, FOCAL			1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (8%)	1 (7%)	3 (7%)	7 (24%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
EMPYEMA			1 (2%)	
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE FOCAL				1 (3%)
INFLAMMATION, ACUTE DIFFUSE				1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
NECROSIS, NOS			1 (2%)	
ALVEOLAR MACROPHAGES			1 (2%)	
HEMATOPOIETIC SYSTEM				
#SPLEEN	(13)	(14)	(40)	(29)
AMYLOID, NOS		1 (7%)		
HYPOPLASIA, NOS	1 (8%)	1 (7%)	5 (13%)	1 (3%)
ATROPHY, NOS				2 (7%)
HYPERPLASIA, NOS	2 (15%)	1 (7%)	4 (10%)	2 (7%)
HYPERPLASIA, RETICULUM CELL				1 (3%)
HYPERPLASIA, LYMPHOID				1 (3%)
HEMATOPOIESIS		3 (21%)	5 (13%)	1 (3%)
#SPLENIC FOLLICLES	(13)	(14)	(40)	(29)
HYPOPLASIA, NOS		1 (7%)		1 (3%)
HYPERPLASIA, NOS		1 (7%)	2 (5%)	4 (14%)
#SPLENIC RED PULP	(13)	(14)	(40)	(29)
HYPOPLASIA, NOS			2 (5%)	
MEGAKARYOCYTOSIS			1 (3%)	
HEMATOPOIESIS				4 (14%)
ERYTHROPOIESIS				2 (7%)
GRANULOPOIESIS			1 (3%)	
#LYMPH NODE	(8)	(11)	(25)	(19)
MINERALIZATION				1 (5%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
CONGESTION, NOS		1 (9%)		
ABSCESS, NOS				1 (5%)
NECROSIS, NOS				1 (5%)
HYPERPLASIA, NOS			2 (8%)	2 (11%)
HYPERPLASIA, LYMPHOID		1 (9%)		1 (5%)
#CERVICAL LYMPH NODE	(8)	(11)	(25)	(19)
ATROPHY, FOCAL				1 (5%)
HYPERPLASIA, NOS	4 (50%)	7 (64%)	10 (40%)	8 (42%)
#TRACHEAL LYMPH NODE	(8)	(11)	(25)	(19)
HYPERPLASIA, NOS	1 (13%)			
#LUMBAR LYMPH NODE	(8)	(11)	(25)	(19)
CYST, NOS				1 (5%)
HYPERPLASIA, LYMPHOID			1 (4%)	
#MESENTERIC L. NODE	(8)	(11)	(25)	(19)
ABSCESS, NOS			1 (4%)	
INFLAMMATION PROLIFERATIVE			1 (4%)	
NECROSIS, CASEOUS			1 (4%)	
#RENAL LYMPH NODE	(8)	(11)	(25)	(19)
HYPERPLASIA, NOS	1 (13%)			
#LUNG/BRONCHIOLE	(13)	(14)	(41)	(29)
HYPERPLASIA, LYMPHOID			1 (2%)	
#LIVER	(14)	(14)	(42)	(29)
HEMATOPOIESIS			1 (2%)	
ERYTHROPOIESIS				1 (3%)
GRANULOPOIESIS				1 (3%)
#KIDNEY	(14)	(14)	(42)	(28)
HYPERPLASIA, LYMPHOID			1 (2%)	
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(8)	(11)	(25)	(19)
LYMPHANGIECTASIS			1 (4%)	
#MESENTERIC L. NODE	(8)	(11)	(25)	(19)
LYMPHANGIECTASIS	1 (13%)			2 (11%)
*VERTEBRAL COLUMN	(14)	(14)	(42)	(30)
PERIARTERITIS				1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#HEART	(13)	(14)	(42)	(30)
MINERALIZATION	1 (8%)			
#BASE OF HEART	(13)	(14)	(42)	(30)
INFLAMMATION, ACUTE SUPPURATIVE				1 (3%)
#HEART/ATRIUM	(13)	(14)	(42)	(30)
THROMBOSIS, NOS		1 (7%)		
#MYOCARDIUM	(13)	(14)	(42)	(30)
INFLAMMATION, ACUTE FOCAL				1 (3%)
INFLAMMATION, CHRONIC FOCAL			3 (7%)	1 (3%)
#ENDOCARDIUM	(13)	(14)	(42)	(30)
INFLAMMATION PROLIFERATIVE	1 (8%)			
HYPERPLASIA, NOS	1 (8%)			
*RENAL ARTERY	(14)	(14)	(42)	(30)
ARTERIOSCLEROSIS, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(12)	(13)	(36)	(27)
ATROPHY, CYSTIC				1 (4%)
#PAROTID GLAND	(12)	(13)	(36)	(27)
INFLAMMATION, NOS		1 (8%)	3 (8%)	
INFLAMMATION, FOCAL	1 (8%)			1 (4%)
NECROSIS, FOCAL			1 (3%)	
HYPERPLASIA, NOS				1 (4%)
#LIVER	(14)	(14)	(42)	(29)
CONGESTION, NOS	1 (7%)			
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE DIFFUSE			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC				1 (3%)
CIRRHOSIS, NOS	1 (7%)			
DEGENERATION, HYDROPIC			1 (2%)	
NECROSIS, NOS	1 (7%)	1 (7%)	1 (2%)	
NECROSIS, FOCAL			1 (2%)	
AMYLOID, NOS		1 (7%)		
LIPOIDOSIS	1 (7%)			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HEPATOCYTOMEGALY	1 (7%)		2 (5%)	
HYPERPLASTIC NODULE				1 (3%)
#PORTAL TRACT	(14)	(14)	(42)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
#LIVER/CENTRILOBULAR	(14)	(14)	(42)	(29)
HEPATITIS, TOXIC				3 (10%)
DEGENERATION, HYDROPIC				1 (3%)
NECROSIS, FOCAL				1 (3%)
METAMORPHOSIS FATTY	1 (7%)			
HEPATOCYTOMEGALY	5 (36%)	2 (14%)	1 (2%)	2 (7%)
#LIVER/PERIPORTAL	(14)	(14)	(42)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR			2 (5%)	
#LIVER/KUPFFER CELL	(14)	(14)	(42)	(29)
PIGMENTATION, NOS				1 (3%)
#LIVER/HEPATOCYTES	(14)	(14)	(42)	(29)
CLOUDY SWELLING	1 (7%)			
#BILE DUCT	(14)	(14)	(42)	(29)
HYPERPLASIA, FOCAL		1 (7%)		
#STOMACH	(14)	(14)	(42)	(26)
HYPERKERATOSIS			3 (7%)	
#DUODENUM	(11)	(12)	(34)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (9%)			
INFLAMMATION, ACUTE				1 (4%)
#DUODENAL SUBSEROSA	(11)	(12)	(34)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
#COLON	(11)	(13)	(36)	(25)
HYPERPLASIA, FOCAL				1 (4%)
URINARY SYSTEM				
*GENITOURINARY TRACT	(14)	(14)	(42)	(30)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
#KIDNEY	(14)	(14)	(42)	(28)
HYDRONEPHROSIS	1 (7%)		1 (2%)	3 (11%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
GLOMERULONEPHRITIS, NOS				2 (7%)
PYELONEPHRITIS, NOS			5 (12%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	8 (57%)	10 (71%)	20 (48%)	12 (43%)
INFLAMMATION, SUPPURATIVE				1 (4%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)	
PYELONEPHRITIS, ACUTE				1 (4%)
INFLAMMATION, CHRONIC			1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	9 (64%)	5 (36%)	6 (14%)	11 (39%)
PLASMA-CELL INFILTRATE				1 (4%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	
#KIDNEY/GLOMERULUS	(14)	(14)	(42)	(28)
AMYLOIDOSIS			1 (2%)	
#KIDNEY/TUBULE	(14)	(14)	(42)	(28)
CALCULUS, NOS			1 (2%)	
CALCIFICATION, NOS			1 (2%)	
#KIDNEY/PELVIS	(14)	(14)	(42)	(28)
ABSCESS, NOS			1 (2%)	
METAPLASIA, SQUAMOUS			1 (2%)	
#URINARY BLADDER	(13)	(13)	(39)	(25)
CAST, NOS			1 (3%)	
INFLAMMATION, ACUTE			1 (3%)	1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)
INFLAMMATION ACUTE AND CHRONIC				1 (4%)
INFLAMMATION, CHRONIC	4 (31%)	2 (15%)	3 (8%)	7 (28%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
INFLAMMATION PROLIFERATIVE			3 (8%)	1 (4%)
HYPERPLASIA, EPITHELIAL			1 (3%)	2 (8%)
POLYPOID HYPERPLASIA			1 (3%)	1 (4%)
METAPLASIA, SQUAMOUS				1 (4%)
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(14)	(40)	(29)
CONGESTION, NOS				1 (3%)
EDEMA, NOS				1 (3%)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (3%)	1 (3%)
DEGENERATION PIGMENTARY	5 (45%)	4 (29%)	4 (10%)	4 (14%)
AMYLOIDOSIS			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
LIPOIDOSIS	1 (9%)			
#ADRENAL CORTEX	(11)	(14)	(40)	(29)
HAMARTOMA		1 (7%)		
HYPERPLASIA, NOS				1 (3%)
#ZONA GLOMERULOSA	(11)	(14)	(40)	(29)
METAPLASIA, NOS	5 (45%)	7 (50%)	6 (15%)	7 (24%)
#ZONA FASCICULATA	(11)	(14)	(40)	(29)
HYPERTROPHY, FOCAL	1 (9%)			
#ADRENAL MEDULLA	(11)	(14)	(40)	(29)
CONGESTION, NOS		2 (14%)	1 (3%)	
#PANCREATIC ISLETS	(13)	(14)	(42)	(30)
HYPERPLASIA, NOS		3 (21%)		
REPRODUCTIVE SYSTEM				
*GENITAL SYSTEM	(14)	(14)	(42)	(30)
RETENTION OF CONTENT	1 (7%)		2 (5%)	1 (3%)
INFLAMMATION; SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
PLASMA-CELL INFILTRATE			1 (2%)	
*BULBOURETHRAL GLAND	(14)	(14)	(42)	(30)
INFLAMMATION, ACUTE SUPPURATIVE		1 (7%)	1 (2%)	4 (13%)
POLYPOID HYPERPLASIA			1 (2%)	
*PREPUTIAL GLAND	(14)	(14)	(42)	(30)
EPIDERMAL INCLUSION CYST			1 (2%)	
GRANULATION, TISSUE			1 (2%)	
#PROSTATE	(13)	(14)	(39)	(29)
RETENTION OF CONTENT	9 (69%)	9 (64%)	18 (46%)	17 (59%)
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	4 (14%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (5%)	
*SEMINAL VESICLE	(14)	(14)	(42)	(30)
DILATATION, NOS	2 (14%)			
RETENTION OF CONTENT	9 (64%)	9 (64%)	18 (43%)	17 (57%)
INFLAMMATION, ACUTE		1 (7%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	4 (13%)
INFLAMMATION, CHRONIC	1 (7%)			
INFLAMMATION PROLIFERATIVE				1 (3%)
#TESTIS	(14)	(13)	(41)	(28)
MINERALIZATION	3 (21%)			
DEGENERATION, NOS			1 (2%)	
NECROSIS, FOCAL			1 (2%)	
NECROSIS, CASEOUS			1 (2%)	
PIGMENTATION, NOS				1 (4%)
ATROPHY, NOS	4 (29%)	3 (23%)	5 (12%)	7 (25%)
ATROPHY, FOCAL	1 (7%)			
SPERMATOGENIC ARREST	4 (29%)	6 (46%)	7 (17%)	8 (29%)
HYPOSPERMATOGENESIS		1 (8%)		
HYPERPLASIA, INTERSTITIAL CELL		1 (8%)	1 (2%)	4 (14%)
#TESTIS/TUBULE	(14)	(13)	(41)	(28)
DEGENERATION, NOS			1 (2%)	
*EPIDIDYMIS	(14)	(14)	(42)	(30)
SPERMATOCELE		1 (7%)		
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
ASPERMATOGENESIS	3 (21%)	3 (21%)	3 (7%)	7 (23%)
HYPOSPERMATOGENESIS	2 (14%)	5 (36%)	4 (10%)	4 (13%)
*VAS DEFERENS	(14)	(14)	(42)	(30)
HYPERPLASIA, EPITHELIAL				1 (3%)
NERVOUS SYSTEM				
#BRAIN	(14)	(14)	(41)	(28)
INFLAMMATION, FOCAL		1 (7%)		
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*VERTEBRAL COLUMN	(14)	(14)	(42)	(30)
ABSCESS, NOS				1 (3%)
DEGENERATION, NOS				1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NECROSIS, NOS				1 (3%)
MALACIA				1 (3%)
*SKELETAL MUSCLE	(14)	(14)	(42)	(30)
ABSCESS, NOS				1 (3%)
*PECTORALIS MAJOR MUS	(14)	(14)	(42)	(30)
EPIDERMAL INCLUSION CYST		1 (7%)		
BODY CAVITIES				
*PERITONEUM	(14)	(14)	(42)	(30)
INFLAMMATION PROLIFERATIVE			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(14)	(42)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)	1 (7%)		2 (7%)
CONNECTIVE TISSUE				
INFLAMMATION, ACUTE SUPPURATIVE				1
INFLAMMATION PROLIFERATIVE				2
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/HISTO PERF			1	1
AUTOLYSIS/NO NECROPSY	1	1	3	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED TCDD FOLLOWING DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	12	41	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	12	41	29
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(12)	(41)	(29)
ULCER, NOS				1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE				1 (3%)
INFLAMMATION, CHRONIC		3 (25%)		2 (7%)
HYPERPLASIA, NOS				2 (7%)
HYPERPLASIA, FOCAL	1 (7%)			
HYPERKERATOSIS	1 (7%)		1 (2%)	1 (3%)
VERRUCA		3 (25%)		1 (3%)
*SUBCUT TISSUE	(15)	(12)	(41)	(29)
ULCER, NOS			1 (2%)	
PUS				1 (3%)
ABSCESS, NOS			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC				1 (3%)
NECROSIS, NOS		1 (8%)	1 (2%)	4 (14%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(15)	(12)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		2 (5%)	
#LUNG/BRONCHIOLE	(15)	(12)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (27%)	3 (25%)	22 (54%)	11 (39%)
#LUNG	(15)	(12)	(41)	(28)
ATELECTASIS			1 (2%)	1 (4%)
CONGESTION, NOS	2 (13%)	1 (8%)		2 (7%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (8%)		2 (7%)
INFLAMMATION, INTERSTITIAL	3 (20%)		5 (12%)	3 (11%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
BRONCHOPNEUMONIA ACUTE SUPPURATI		1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
PNEUMONIA, CHRONIC MURINE				1 (4%)
HYPERPLASIA, ADENOMATOUS	1 (7%)		2 (5%)	2 (7%)
HEMATOPOIETIC SYSTEM				
*MAMMARY GLAND	(15)	(12)	(41)	(29)
ADENOSIS	2 (13%)		2 (5%)	1 (3%)
#BONE MARROW	(13)	(12)	(37)	(28)
FIBROUS OSTEODYSTROPHY	3 (23%)	1 (8%)	7 (19%)	6 (21%)
HYPERPLASIA, NOS			1 (3%)	
RETICULOCYTOSIS	1 (8%)			
#SPLEEN	(15)	(12)	(40)	(27)
CYST, NOS			1 (3%)	
HEMORRHAGIC CYST		1 (8%)		
HYPOPLASIA, NOS	1 (7%)	1 (8%)	3 (8%)	2 (7%)
HYPERPLASIA, NODULAR			1 (3%)	
HYPERPLASIA, NOS	3 (20%)	1 (8%)		1 (4%)
MEGAKARYOCYTOSIS			1 (3%)	
HYPERPLASIA, LYMPHOID	1 (7%)			2 (7%)
HEMATOPOIESIS			2 (5%)	5 (19%)
#SPLENIC FOLLICLES	(15)	(12)	(40)	(27)
HYPOPLASIA, NOS				1 (4%)
HYPERPLASIA, NODULAR				1 (4%)
HYPERPLASIA, NOS	2 (13%)	2 (17%)	4 (10%)	2 (7%)
#SPLENIC RED PULP	(15)	(12)	(40)	(27)
HYPOPLASIA, NOS	1 (7%)		1 (3%)	
HYPERPLASIA, HEMATOPOIETIC		2 (17%)		
HEMATOPOIESIS	2 (13%)		1 (3%)	6 (22%)
#LYMPH NODE	(12)	(9)	(30)	(25)
HYPERPLASIA, NOS	2 (17%)	3 (33%)	6 (20%)	2 (8%)
#CERVICAL LYMPH NODE	(12)	(9)	(30)	(25)
ABSCESS, NOS		1 (11%)		
HYPERPLASIA, NODULAR				1 (4%)
HYPERPLASIA, NOS	1 (8%)	1 (11%)	17 (57%)	15 (60%)
#LYMPH NODE OF THORAX	(12)	(9)	(30)	(25)
HYPERPLASIA, LYMPHOID				1 (4%)
#PANCREATIC L.NODE	(12)	(9)	(30)	(25)
PIGMENTATION, NOS			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HYPERPLASIA, NOS			1 (3%)	
#LUNG/BRONCHIOLE	(15)	(12)	(41)	(28)
HYPERPLASIA, LYMPHOID			1 (2%)	1 (4%)
#PAROTID GLAND	(15)	(11)	(36)	(27)
FIBROSING ADENOSIS			1 (3%)	
#LIVER	(15)	(12)	(41)	(28)
HEMATOPOIESIS	1 (7%)			1 (4%)
#ADRENAL	(15)	(11)	(41)	(25)
MYELOPOIESIS			1 (2%)	
#ADRENAL CORTEX	(15)	(11)	(41)	(25)
HYPERPLASIA, GRANULOCYTIC				1 (4%)
#THYMUS	(15)	(11)	(39)	(28)
CYST, NOS				1 (4%)
NECROSIS, CENTRAL				1 (4%)
HYPERPLASIA, NOS		1 (9%)	2 (5%)	
HYPERPLASIA, LYMPHOID				1 (4%)
#THYMIC MEDULLA	(15)	(11)	(39)	(28)
HYPERPLASIA, NOS	2 (13%)			1 (4%)
CIRCULATORY SYSTEM				
#SPLEEN	(15)	(12)	(40)	(27)
PERIARTERITIS		1 (8%)		
#PANCREATIC L.NODE	(12)	(9)	(30)	(25)
LYMPHANGIECTASIS			1 (3%)	1 (4%)
#MESENTERIC L. NODE	(12)	(9)	(30)	(25)
LYMPHANGIECTASIS			2 (7%)	
#LUNG/BRONCHUS	(15)	(12)	(41)	(28)
EMBOLISM, NOS				1 (4%)
#LUNG	(15)	(12)	(41)	(28)
EMBOLISM, NOS		1 (8%)		
#HEART	(15)	(12)	(40)	(28)
ENDOCARDIOSIS	2 (13%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#HEART/ATRIUM	(15)	(12)	(40)	(28)
THROMBOSIS, NOS	1 (7%)		2 (5%)	
#MYOCARDIUM	(15)	(12)	(40)	(28)
INFLAMMATION, ACUTE SUPPURATIVE		2 (17%)		
INFLAMMATION, CHRONIC FOCAL	1 (7%)			
NECROSIS, NOS		1 (8%)		
*CENTRAL VEINS/LIVER	(15)	(12)	(41)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
#OVARY	(14)	(10)	(33)	(29)
THROMBOSIS, NOS				1 (3%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(11)	(36)	(27)
INFLAMMATION, NOS			1 (3%)	
#PAROTID GLAND	(15)	(11)	(36)	(27)
INFLAMMATION, NOS		1 (9%)	1 (3%)	3 (11%)
INFLAMMATION, FOCAL				2 (7%)
INFLAMMATION, CHRONIC				1 (4%)
#LIVER	(15)	(12)	(41)	(28)
CONGESTION, NOS		1 (8%)		
INFLAMMATION, NOS	1 (7%)			
INFLAMMATION, FOCAL			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	1 (4%)
INFLAMMATION, ACUTE DIFFUSE		1 (8%)		1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)
CIRRHOSIS, BILIARY				1 (4%)
NECROSIS, FOCAL		1 (8%)		
NECROSIS, COAGULATIVE	1 (7%)		1 (2%)	1 (4%)
AMYLOIDOSIS			1 (2%)	
PIGMENTATION, NOS		1 (8%)		
FOCAL CELLULAR CHANGE	1 (7%)			
HEPATOCYTOMEGALY				1 (4%)
ANGIECTASIS		1 (8%)		
#LIVER/CENTRILOBULAR	(15)	(12)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
DEGENERATION, HYDROPIC		1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NECROSIS, NOS	1 (7%)			
HEPATOCYTOMEGALY				5 (18%)
#LIVER/PERIPORTAL	(15)	(12)	(41)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)	1 (8%)		
HYPERPLASIA, NOS	1 (7%)			
#LIVER/KUPFFER CELL	(15)	(12)	(41)	(28)
HYPERPLASIA, NOS			1 (2%)	
*GALLBLADDER	(15)	(12)	(41)	(29)
CALCULUS, NOS		1 (8%)		
INFLAMMATION, CHRONIC	1 (7%)	1 (8%)		
#BILE DUCT	(15)	(12)	(41)	(28)
HYPERPLASIA, NOS	2 (13%)			
#PANCREAS	(15)	(12)	(40)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (3%)	
HYPERPLASIA, NOS	1 (7%)			
#STOMACH	(15)	(12)	(41)	(29)
INFLAMMATION, ACUTE	1 (7%)			
HYPERKERATOSIS			2 (5%)	
#DUODENUM	(13)	(11)	(40)	(27)
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)
URINARY SYSTEM				
#KIDNEY	(15)	(12)	(41)	(28)
HYDRONEPHROSIS	1 (7%)	1 (8%)	1 (2%)	1 (4%)
CONGESTION, NOS		1 (8%)		
GLOMERULONEPHRITIS, NOS	1 (7%)	1 (8%)		
PYELONEPHRITIS, NOS			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	5 (33%)	7 (58%)	26 (63%)	14 (50%)
GLOMERULONEPHRITIS, ACUTE			1 (2%)	
GLOMERULONEPHRITIS, SUBACUTE		1 (8%)		
INFLAMMATION, CHRONIC	2 (13%)		1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	10 (67%)	4 (33%)	16 (39%)	17 (61%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS	2 (13%)		2 (5%)	1 (4%)
INFARCT, HEALED			1 (2%)	
#KIDNEY/GLOMERULUS	(15)	(12)	(41)	(28)
DEGENERATION, HYALINE		1 (8%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
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TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
AMYLOIDOSIS			1 (2%)	
#URINARY BLADDER	(13)	(10)	(30)	(27)
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, CHRONIC	4 (31%)	2 (20%)	10 (33%)	11 (41%)
INFLAMMATION, CHRONIC FOCAL			2 (7%)	
ATROPHY, NOS			1 (3%)	
METAPLASIA, SQUAMOUS				2 (7%)
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(11)	(41)	(25)
CONGESTION, NOS			2 (5%)	
EDEMA, NOS				2 (8%)
DEGENERATION PIGMENTARY	9 (60%)	8 (73%)	22 (54%)	17 (68%)
AMYLOIDOSIS		1 (9%)		
#ADRENAL CORTEX	(15)	(11)	(41)	(25)
HAMARTOMA	1 (7%)		1 (2%)	1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (2%)	
#ZONA GLOMERULOSA	(15)	(11)	(41)	(25)
METAPLASIA, NOS	6 (40%)	7 (64%)	20 (49%)	11 (44%)
#THYROID	(15)	(11)	(39)	(25)
AMYLOID, NOS				1 (4%)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)	
#THYROID FOLLICLE	(15)	(11)	(39)	(25)
GOITER COLLOID			1 (3%)	
#PARATHYROID	(6)	(6)	(15)	(10)
AMYLOID, NOS				1 (10%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(12)	(41)	(29)
DILATATION/DUCTS	3 (20%)		3 (7%)	1 (3%)
*VAGINA	(15)	(12)	(41)	(29)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
#UTERUS	(15)	(12)	(36)	(29)
MINERALIZATION			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
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TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HEMORRHAGE			1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
ABSCESS, NOS			1 (3%)	
NECROSIS, NOS			1 (3%)	1 (3%)
#UTERUS/ENDOMETRIUM	(15)	(12)	(36)	(29)
HYPERPLASIA, NOS	1 (7%)	1 (8%)	8 (22%)	6 (21%)
HYPERPLASIA, CYSTIC	12 (80%)	8 (67%)	22 (61%)	21 (72%)
#OVARY/OVIDUCT	(15)	(12)	(36)	(29)
INFLAMMATION, ACUTE SUPPURATIVE		1 (8%)		
HYPERPLASIA, PAPILLARY				1 (3%)
#OVARY	(14)	(10)	(33)	(29)
CYST, NOS	1 (7%)		4 (12%)	
ATRESIA	10 (71%)	8 (80%)	20 (61%)	15 (52%)
HEMORRHAGE		1 (10%)		1 (3%)
HEMORRHAGIC CYST	1 (7%)		2 (6%)	1 (3%)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (3%)
ABSCESS, NOS		1 (10%)		
NECROSIS, FOCAL				1 (3%)
AMYLOIDOSIS		1 (10%)		
CHOLESTEROL DEPOSIT		1 (10%)		
ATROPHY, NOS	8 (57%)	5 (50%)	25 (76%)	22 (76%)
ATROPHY, CYSTIC	4 (29%)	3 (30%)	1 (3%)	2 (7%)
LUTEINIZATION	2 (14%)		2 (6%)	2 (7%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(15)	(12)	(41)	(29)
ABSCESS, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(15)	(12)	(41)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
BODY CAVITIES				
*ABDOMINAL WALL	(15)	(12)	(41)	(29)
HERNIA, NOS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(12)	(41)	(29)
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (20%)		1 (2%)	2 (7%)
BACTERIAL SEPTICEMIA		1 (8%)		
AMYLOIDOSIS	1 (7%)	1 (8%)	3 (7%)	1 (3%)
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				1
SPECIAL MORPHOLOGY SUMMARY				
AUTOLYSIS/NO NECROPSY		3	4	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
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Appendix E

Preparation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin

Appendix E

Preparation of 2,3,7,8,-Tetrachlorodibenzo-p-dioxin

This compound was prepared by the condensation of potassium 2,4,5,trichlorophenate in the presence of the Ullmann copper catalyst. Prior to the reaction, the trichlorphenol was sublimed and recrystallized from petroleum ether (b.p. 60^{o}-$70^{o}C$). The phenol was then converted to its potassium salt by treatment with potassium hydroxide in toluene. Water was removed by azeotropic distillation on a Buchi apparatus and the salt residue was treated with additional toluene, then evaporated to dryness.

A 50-g sample of dry potassium 2,4,5,-trichlorophenate was dissolved in 150 ml of bis(ethoxyethyl)ether (BEEE) containing 200 mg of Ullmann copper catalyst that had previously been washed with acetone and stored under ethylene diacetate. A lower boiling solvent fraction was removed by distillation and the mixture was refluxed with stirring in an oil bath set at 210^{o} to $215^{o}C$. The reaction was allowed to proceed for a minimum of 24 hours, since longer reaction times increased the conversion.

A dark brown residue was obtained when the BEEE solvent was removed by distillation at atmospheric pressure. The residue was treated with 200 ml of o-dichlorobenzene and heated to $170^{o}C$. The resulting solution was filtered hot through fluted filter paper and an additional 100 ml of hot o-dichlorobenzene was used to wash the reaction flask and filter. The solvent was removed by filtration after cooling to room temperature. The product was washed with 200 ml of 1% sodium methylate in methanol and 200 ml of chloroform and was then recrystallized from o-dichlorobenzene.

Appendix F

Quarterly Analyses of Stock Solutions

Appendix F

Quarterly Analyses of Stock Solutions

Stock solutions in acetone were analyzed at the beginning and at the end of each quarter by the IITRI Chemistry Division. The method of analysis consisted of adding an internal standard (pentachlorodibenzo-p-dioxin, PCDD) to samples so that the internal standard concentration was approximately the same as that of the sample being analyzed. The solution containing the sample and standard was then injected onto an electron capture-gas chromatography system. The column was a 2 m x 1/8 in. Dexsil 300 with a N_2/CH_4 carrier gas flow rate of 50 ml/minute and an oven temperature of 275°C. Quantitation was achieved by manually measuring the area under the resultant peaks with a planimeter. These values were then multiplied by the attenuation of the gas chromatography electrometer and compared with standard curves for internal standards and test compounds. The standard curve was represented by a third order polynomial equation fitting response to amounts. The amount of dioxin in the test sample was corrected for injection errors and any loss on the column by the value obtained for the internal standard.

The theoretical concentration for the stock solution was 0.25 μg TCDD per milliliter of acetone. The actual concentration as measured by the previously discussed method varied from 0.22 to 0.28 μg/ml.

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