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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

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REPORT ON THE BIOASSAY OF 2,4-DINITROTOLUENE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

CONTRIBUTORS: This report presents the results of the bioassay of 2,4-dinitrotoluene conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Bioassay Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. R. W. Fleischman (3), Dr. D. W. Hayden (3), Dr. A. S. Krishna Murthy (3), Dr. A. Russfield (3), and Dr. D. S. Wyand (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5) and Dr. A. Chu (7), using methods selected for the Bioassay Program by Dr. J. J. Gart (8).

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The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (8), Mr. J. Nam (8), Dr. H. M. Pettigrew (8), and Dr. R. E. Tarone (8).

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SUMMARY

A bioassay of practical-grade 2,4-dinitrotoluene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 2,4-Dinitrotoluene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 2,4-dinitrotoluene were 0.02 and 0.008 percent, respectively. For male and female mice, the high and low time-weighted average concentrations were 0.04 and 0.008 percent, respectively. After a 78-week period of compound administration, observation of the rats continued for an additional 26 weeks and observation of the mice continued for 13 additional weeks.

For the chronic rat bioassay, 25 rats of each sex were placed on test as high dose controls, and 50 rats of each sex served as the low dose controls. For the mice, 50 males and 50 females were placed on test as controls for each of the high dose and low dose groups.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

In the male rats, a significantly increased incidence of fibroma of the skin and subcutaneous tissue occurred in both the high and the low dose groups when compared to their respective controls. A statistically significant incidence of fibroadenoma of the mammary gland occurred in the high dose female rats.

Among the mice a variety of tumors was observed but none were considered to be associated with the dietary administration of 2,4-dinitrotoluene.

Under the conditions of this bioassay dietary administration of 2,4-dinitrotoluene to Fischer 344 rats induced benign tumors (i.e., fibroma of the skin and subcutaneous tissue in males and fibroadenoma of the mammary gland in females). No evidence was provided for the carcinogenicity of the compound in B6C3F1 mice of either sex.

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I. INTRODUCTION

2,4-Dinitrotoluene (NCI No. C01945), a precursor in the synthesis of azo dyes, was selected for bioassay by the National Cancer Institute along with other dye intermediates in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic nitro compounds are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry (Wynder et al., 1963). The structural relationship of 2,4-dinitrotoluene to the known carcinogen 2,4-diaminotoluene was also a factor in its selection for testing (Weisburger, 1976; Hiasa, 1970).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1-methyl-2,4-dinitrobenzene.* It is also called 2,4-dinitrotoluol and 2,4-DNT.

Precise production figures for 2,4-dinitrotoluene are not available; however, the U.S. International Trade Commission (1977) reported a combined production of 272,610,000 pounds for the 2,4- and 2,6-dinitrotoluene isomers in 1975.

Aside from its use by the dye manufacturing industry, 2,4-dinitrotoluene is used by the munitions industry as a modifier for smokeless powders and, to a limited extent, as a gelatinizing and waterproofing

*The CAS registry number is 121-14-2.

agent in military and commercial explosive compositions (Institute of Makers of Explosives, 1977). 2,4-Dinitrotoluene experiences widespread application as a chemical intermediate for the production of toluene diisocyanate (TDI) which, in turn, is consumed in the production of flexible polyurethane foams; however, most TDI producers use toluene as the starting material, generating 2,4-dinitrotoluene as a captive intermediate in the process (Urso, 1977).

The risk of exposure to 2,4-dinitrotoluene is greatest for workers in the dye and explosives industries and at chemical plants producing TDI. The general population may also experience exposure as a result of discharge of 2,4-dinitrotoluene into rivers and streams from munitions plants (Simon et al., 1977).

2,4-Dinitrotoluene can cause anemia, methemoglobinemia, cyanosis, and liver damage (Sax, 1975). It is rapidly absorbed through the intact skin; however, toxic levels may also be reached by inhalation or ingestion (Manufacturing Chemists Association, 1966). Studies by Simon et al. (1977), suggest that 2,4-dinitrotoluene is not a potent mutagen in the rat, as determined by dominant lethal mutation experiments.

II. MATERIALS AND METHODS

A. Chemicals

Practical-grade 2,4-dinitrotoluene was purchased from J. T. Baker Chemical Company. Analysis by the manufacturer suggested a purity greater than 95 percent. The observed melting point (67° to 70°C) suggested a compound of relative purity due to the narrow range and its close proximity to the literature value (71°C). Thin-layer chromatography visualized with ultraviolet light indicated at least one impurity. Ultraviolet analysis showed a peak at 250 nm which is consistent with the value reported in the literature (252 nm).

Throughout this report the term 2,4-dinitrotoluene is used to represent this practical-grade material.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals was Wayne Lab-Blox® (Allied Mills, Inc.). 2,4-Dinitrotoluene was weighed under an exhaust hood and ground in a mortar and pestle with an aliquot of ground Wayne Lab-Blox® meal. Once visual homogeneity was attained, the mixture and the remainder of the feed to be administered to the treated animals were placed into a 6 kg capacity Patterson-Kelly twin shell stainless steel V-blender. After blending for 20 minutes, the mixture was sealed in double plastic bags and stored in the dark at 4°C. Mixtures were used for only 1 week.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and both C57BL/6CR and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. High and low dose rats and mice, and high dose control rats were supplied by Charles River Breeding Laboratories, Wilmington, Massachusetts. Low dose control animals were supplied by ARS/Sprague-Dawley, Madison, Wisconsin. The low dose groups were received in shipments separate from their respective controls. Upon arrival, a sample of animals was examined for parasites and other signs of disease. The rats and mice to be tested were quarantined for 2 weeks prior to initiation of the bioassay. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C and a range in relative humidity of 10 to 85 percent. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 11 months of study, high dose rats and their controls

were maintained in galvanized- and stainless-steel wire-mesh cages suspended above newspapers. Low dose and low dose control rats were kept in these cages for the first 13 months of study. Newspapers under the cages were replaced daily and cages and racks washed weekly. For the remainder of the study, all rats were housed in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. Low dose rats and their controls were provided with hardwood chip bedding (Ab-sorb-dri®, Wilner Wood Products Co.) for the first 9 months that they were housed in polycarbonate cages. SAN-I-CEL® corncob bedding (Paxton Processing Co.) was used for these animals for the next 12 months. High dose rats and their controls were provided with SAN-I-CEL® for the first 12 months that they were housed in polycarbonate cages. For the remainder of the study, Bed-o'Cobs® (The Anderson's Cob Division) was provided in all treated and control rat cages. Stainless steel cage racks (Fenco Cage Products) were cleaned once every 2 weeks and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. During the observation period following chemical administration, stainless steel wire bar lids were substituted. Both types of lids were supplied by Lab Products, Inc. Nonwoven fiber filter bonnets were used over cage lids. All mice were housed 10 per cage for the first part of the study. Cage

populations for high dose, high dose control, low dose, and low dose control mice were reduced to five per cage after 12 months, 14 months, 19 months, and 19 months, respectively. Cages, lids, filters, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. Bedding was of the same brands as those used for rats. Ab-sorb-dri® was used for 2 months (high dose), 4 months (high dose controls), and 9 months (low dose and low dose controls) prior to SAN-I-CEL® being used for 12 months. A second type of corncob bedding (Bed-o'Cobs®) was then used for the remainder of the bioassay. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly. Food and water were available ad libitum.

Pelleted Wayne Lab-Blox® was supplied to all animals during the quarantine and final observation periods. During the period of chemical administration, all treated animals received dosed Wayne Lab-Blox®. Control animals received untreated meal. Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc.) containing stainless steel baffles were used to distribute powdered feed to low dose rats, their controls, and all mice during the entire study, and to high dose rats and their controls for the first 13 months of chemical administration. High dose rats and their controls were fed from stainless

steel gangstyle feed hoppers (Scientific Cages, Inc.) during the last 5 months of chemical administration. During the observation period, rats were fed pellets on the cage floor and mice were fed pellets from a wire bar hopper incorporated into the cage lid. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

Low dose, low dose control, and high dose rats were housed in a room in which other rats were receiving diets treated with* acetylaminofluorene (53-96-3); dulcin (150-69-6) and L-arginine glutamate (4320-30-3); sodium nitrite (7632-00-0); L-arginine glutamate (4320-30-3); N-butyl urea (592-31-4); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 4-nitroanthranilic acid (619-17-0); 1,5-naphthalenediamine (2243-62-1); N-(1-naphthyl) ethylenediamine dihydrochloride (1465-25-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); aniline hydrochloride (142-04-1); and p-anisidine hydrochloride (20265-97-8). High dose control rats shared a room with other rats receiving diets treated with 5-nitro-o-toluidine (99-55-8); hydrazobenzene (530-50-7); 2-aminoanthraquinone (117-79-3); 3-amino-9-ethylcarbazole hydrochloride; 6-nitrobenzimidazole (94-52-0); 1-nitronaphthalene (86-57-7); 2,4-diaminoanisole sulfate (615-05-4); and APC (8003-03-0).

*CAS registry numbers are given in parentheses.

Low dose, low dose control, and high dose mice were housed in a room in which other mice were receiving diets treated with: 2,5-toluenediamine sulfate (6369-59-1); 2-aminoanthraquinone (117-79-3); 3-amino-4-ethoxyacetanilide (17026-81-2); 3-amino-9-ethylcarbazole hydrochloride; 1-amino-2-methylanthraquinone (82-28-0); 5-nitro-o-anisidine (99-59-2); 1-nitronaphthalene (86-57-7); 4-nitroanthranilic acid (619-17-0); 5-nitroacenaphthene (602-87-9); 2,4-diaminoanisole sulfate (615-05-4); 3-nitro-p-acetophenetide (1777-84-0); and N,N-dimethyl-p-nitrosoaniline (138-89-6). High dose control mice shared a room with other mice receiving diets treated with 2-methyl-1-nitro-anthraquinone (129-15-7); p-cresidine (120-71-8); fenaminosulf (140-56-7); 4-chloro-m-phenylenediamine (5131-60-2); and cinnamyl anthranilate (87-29-6).

E. Selection of Initial Concentrations

Six-week subchronic toxicity studies were conducted with Fischer 344 rats and C57BL/6CR mice in order to determine the high concentrations for administration during the chronic bioassay. Animals of each species were distributed among four groups, each consisting of five males and five females. 2,4-Dinitrotoluene was administered in the feed for 4 weeks. Three animal groups of each species received dietary concentrations of 0.00375, 0.0075, and 0.015 percent. A fourth group of each species served as a control, receiving only the basal laboratory diet. No deaths occurred at any dose level tested.

All animals were sacrificed at the end of the test and gross necropsies were performed.

A dosage inducing no mortality and resulting in a depression in mean group body weight of approximately 15 percent relative to controls was to be selected as the initial high dose. When weight gain criteria were not applicable, mortality data alone were utilized.

The initial high dose selected for administration to rats and mice in the chronic study was 0.008 percent. However, for the reasons indicated below, the initial high doses utilized for rats and mice in the chronic study were 0.02 and 0.04 percent, respectively.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, duration of treated and untreated observation periods, and the time-weighted average concentrations) are summarized in Tables 1 and 2.

The treated and control rats were all approximately 6 weeks old at the time they were placed on test. The initial dietary concentrations of 2,4-dinitrotoluene administered to rats were 0.0075 and 0.00375 percent. After week 19, the higher dose was changed from 0.0075 to 0.008 percent to facilitate dose formulation. The rat group receiving 0.00375 percent was sacrificed after 51 weeks and no histopathologic examinations were performed because the dose levels being used in the chronic bioassay were considered, on the basis of weight depression, to have been too low. A new group, receiving

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2,4-DINITROTOLUENE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,4-DINITRO-TOLUENE (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
LOW DOSE CONTROL	50	0	0	104	0
HIGH DOSE CONTROL	25	0	0	104	0
LOW DOSE	50	0.0075 0.008 0	19 59	 26	0.008
HIGH DOSE	50	0.02 0	78	 26	0.02
FEMALE					
LOW DOSE CONTROL	50	0	0	104	0
HIGH DOSE CONTROL	25	0	0	104	0
LOW DOSE	50	0.0075 0.008 0	19 59	 26	0.008
HIGH DOSE	50	0.02	78	 26	0.02

[a] Time-weighted average concentration = $\frac{\Sigma(\text{concentration} \times \text{weeks received})}{\Sigma(\text{weeks receiving chemical})}$

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2,4-DINITROTOLUENE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,4-DINITRO-TOLUENE (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
LOW DOSE CONTROL	50	0	0	91
HIGH DOSE CONTROL	50	0	0	91
LOW DOSE	50	0.008 0	78	13
HIGH DOSE	50	0.04 0	78	13
FEMALE				
LOW DOSE CONTROL	50	0	0	91
HIGH DOSE CONTROL	50	0	0	91
LOW DOSE	50	0.008 0	78	13
HIGH DOSE	50	0.04 0	78	13

0.02 percent, was started with a new control. Throughout this report the groups initially receiving a concentration of 0.0075 percent and their controls are referred to as the low dose and low dose control groups, respectively, while the groups receiving 0.02 percent and their controls are referred to as the high dose and high dose control groups, respectively. These treated rats were supplied with dosed feed for a total of 78 weeks followed by a 26-week observation period.

The treated and control mice were all approximately 6 weeks old at the time they were placed on test. The initial dietary concentrations of 2,4-dinitrotoluene administered to mice were 0.008 and 0.00375 percent. The mouse groups receiving 0.00375 percent were sacrificed after 29 weeks because the groups receiving 0.008 percent did not demonstrate desired weight depression. A new group, receiving 0.04 percent, was started with a new control group. Throughout this report the groups initially receiving a concentration of 0.008 percent and their controls are referred to as the low dose and low dose control groups, respectively, while the groups receiving 0.04 percent and their controls are referred to as the high dose and high dose control groups, respectively. Treated mice were supplied with dosed feed for a total of 78 weeks, followed by a 13-week observation period.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice

daily for mortality. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, ear, uterus, mammary gland, and ovary.

Tissues for which slides were prepared were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An

occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, 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). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

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 used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported 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 was 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 prior to histologic sampling (e.g., skin or mammary tumors), 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, pp. 48-52) was used to compare the tumor incidence of a control

group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing

these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

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 percent of a large number of identical experiments, the true ratio of the risk in a treated 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

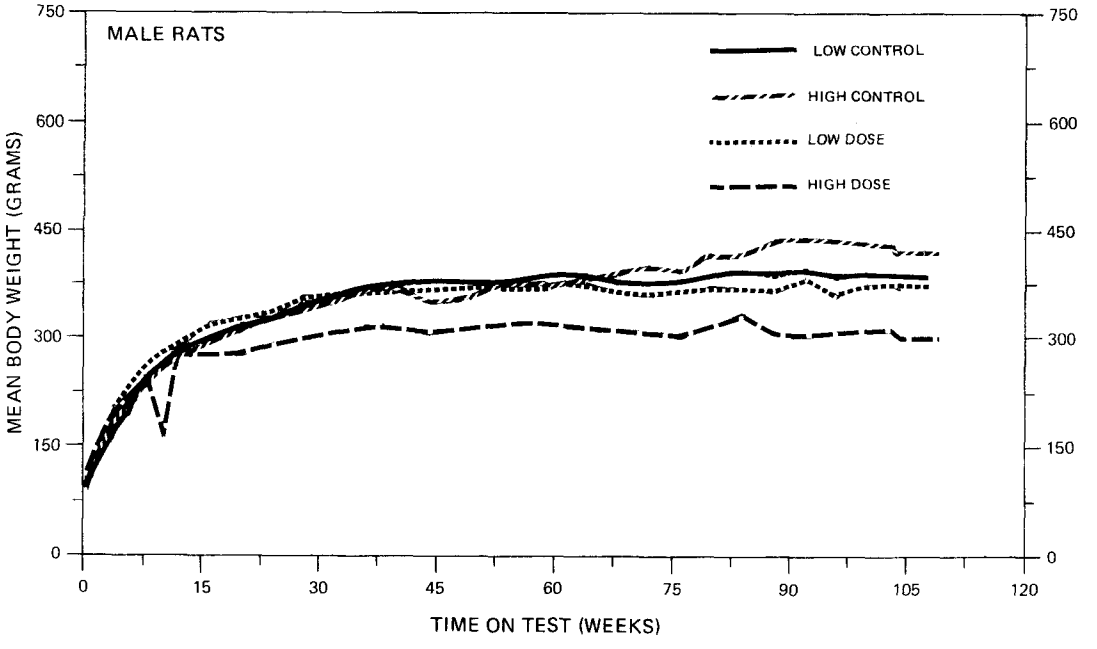
can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the 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.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

In males, mean body weight depression was evident in the high dose group, when compared to the high dose controls, as early as week 16 (Figure 1). At the end of the bioassay these high dose males weighed approximately 25 percent less than their controls. Very slight mean body weight depression was recorded for the low dose males when compared to their controls. The same general weight depression patterns were observed in females. The exceptions were: the unexplained large weight gain and subsequent loss in the low dose females as compared to their controls during weeks 36 to 68; the negative and positive peaks observed in high dose males and females, respectively, in week 10; and a mean group body weight in the high dose females approximately 18 percent less than their controls at the end of the bioassay.

Clinical observations recorded for rats were primarily limited to palpable subcutaneous masses (one low dose female, one low dose control female, and three high dose males) and ulcerative inguinal lesions (one high dose male, one low dose control male, and one low dose control female). The only other clinical signs reported were pale discoloration of the eye in one high dose male and an abscess on the ventral surface in one high dose female.



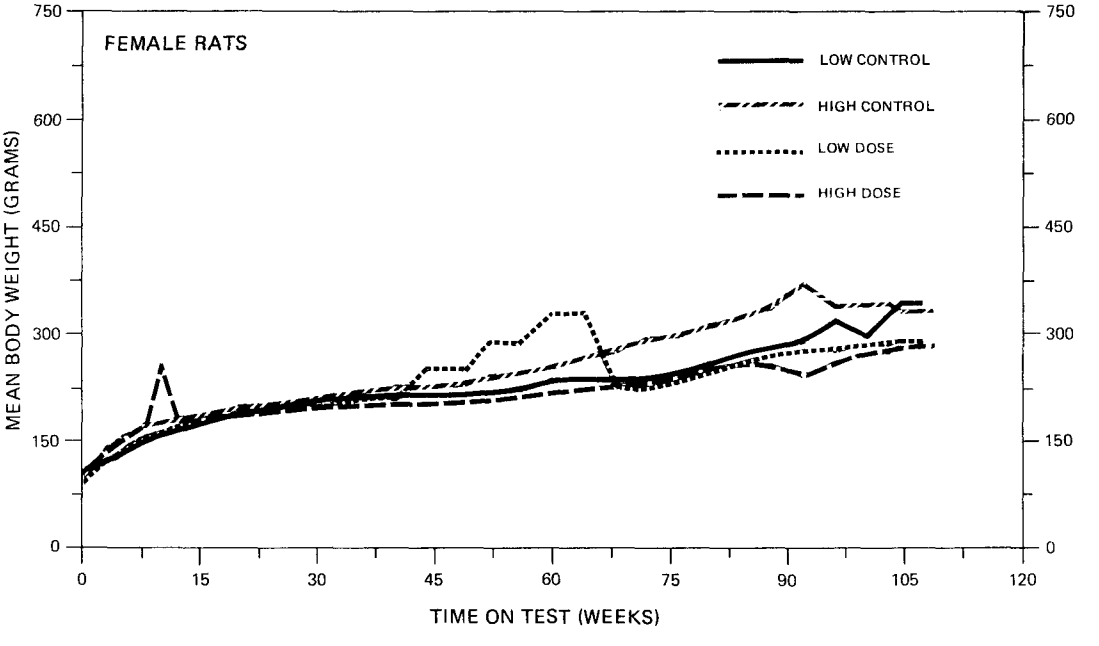


FIGURE 1
GROWTH CURVES FOR 2,4-DINITROTOLUENE CHRONIC STUDY RATS

B. Survival

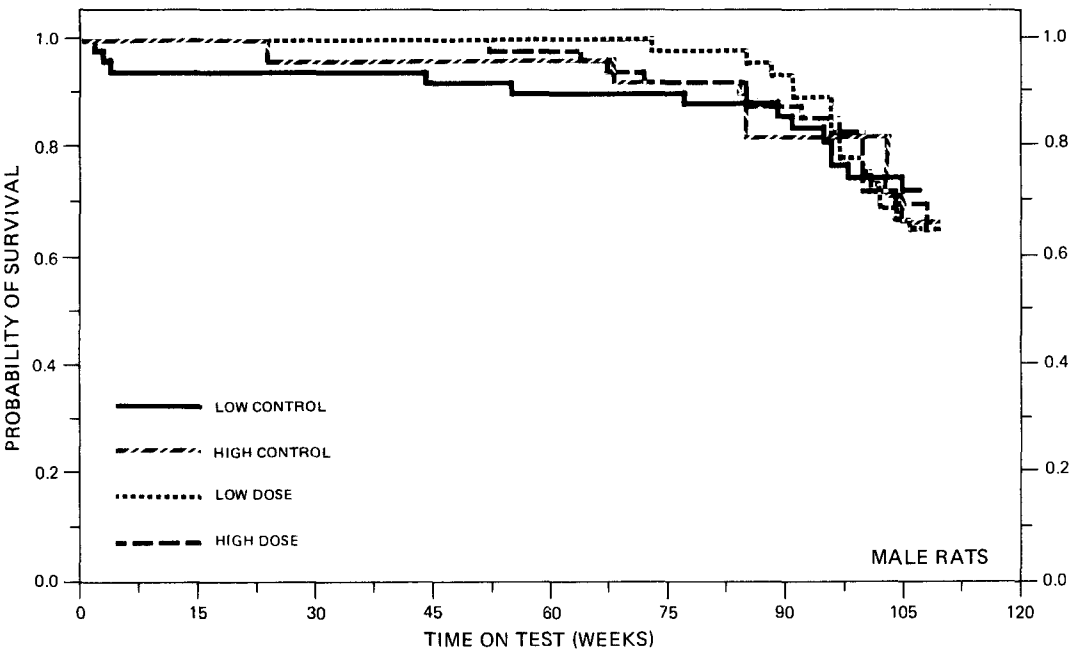
The estimated probabilities of survival for male and female rats in the control and 2,4-dinitrotoluene-treated groups are shown in Figure 2. Because the experiments for the low dose and high dose rats were conducted at different times, each was assigned its own set of controls.

For male rats neither the high dose nor the low dose group experienced a significantly different survival rate from its corresponding control group. Despite the sacrifice of five males from each group in week 78, survival was relatively good: 58 percent (29/50) of the high dose, 58 percent (29/50) of the low dose, 52 percent (13/25) of the high dose control, and 64 percent (32/50) of the low dose control group survived until the end of the study.

For female rats neither the high dose nor the low dose group had a significantly different survival rate from its corresponding control group. Five females were sacrificed from each group in week 78. Survival, however, was relatively good as 52 percent (26/50) of the high dose, 48 percent (12/25) of the high dose control, 62 percent (31/50) of the low dose, and 62 percent (31/50) of the low dose control survived until the end of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are tabulated in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are tabulated in Appendix C (Tables C1 and C2).



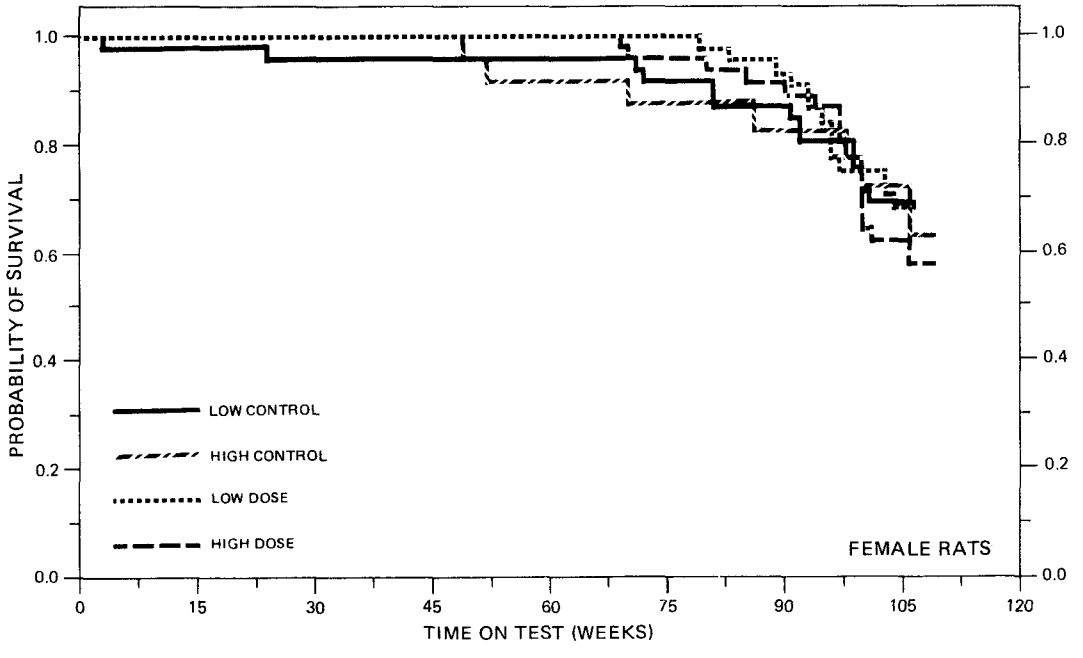


FIGURE 2
SURVIVAL COMPARISONS OF 2,4-DINITROTOLUENE CHRONIC STUDY RATS

There appeared to be an increase in the occurrence of integumentary tumors in low and high dose males. The predominant tumor type was the fibroma (skin/subcutaneous tissue 7/49 low dose, 13/49 high dose), with the sporadic occurrence of squamous-cell papillomas (1/49 low dose), basal-cell carcinomas (1/49 low dose), fibrosarcomas (1/49 low dose; 2/49 high dose), and lipomas (3/49 high dose). None of these integumentary tumor types were observed in control males. Among females, a slight increase in fibromas of the skin was noted among the high dose group. The fibromas were circumscribed, well-differentiated masses composed of mature fibroblasts enmeshed in bundles and whorls of collagen.

In assessment of other organ systems, there was a high incidence of fibroadenomas of the mammary gland in high dose females. The histologic appearance of these tumors was basically similar to those described by Hallowes and Young (1973). However, certain histologic variations were seen in these fibroadenomas which were not noted by Hallowes and Young. Marked variation in the epithelial/stromal ratio of the tumors was noted and many contained large dilated ducts of galactoceles which contained secretion. Within a focal area of a fibroadenoma in one low dose female, transformation to an intraductal carcinoma was noted. In the affected area, cells were more hyperchromatic, piled upon each other, and there were numerous mitotic figures. In one high dose female with multiple mammary tumors, four were fibroadenomas and one was an adenocarcinoma. Lobular hyperplasia of mammary

tissue adjacent to the fibroadenomas frequently occurred and, in some areas, there was marked basophilia of the cytoplasm and hyperchromicity of nuclei in the hyperplastic lobules. In general, the remaining organ systems showed a similar variety and incidence of neoplasms in the chemically treated and control groups, and these were considered to be part of the general background level of neoplasms in Fischer 344 rats.

Certain unusual neoplasms occurred in a low incidence in some treated groups and not in controls. These included one hemangiosarcoma in the subcutis, one hemangiosarcoma of the urinary bladder, and one adenocarcinoma of the prostate gland in the high dose males. However, these are not considered to be related to chemical administration.

The incidence and variety of nonneoplastic, degenerative, proliferative, and inflammatory lesions were similar in control and chemically treated rats.

Based on the increase of fibromas in male rats and fibroadenomas in high dose female rats observed in this histopathologic examination, 2,4-dinitrotoluene appeared to induce benign tumors in the Fischer 344 rat.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis for every type

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2,4-DINITROTOLUENE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/45(0.00)	0/25(0.00)	3/49(0.06)	3/48(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.554	0.321
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	96	108
Subcutaneous Tissue or Skin: Fibroma[b]	0/46(0.00)	0/25(0.00)	7/49(0.14)	13/49(0.27)
P Values[c]	---	---	P = 0.008	P = 0.003
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.827	2.106
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	96	85
Subcutaneous Tissue: Lipoma[b]	0/46(0.00)	0/25(0.00)	0/49(0.00)	3/49(0.06)
P Values[c]	---	---	---	N.S.
Relative Risk (Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	N.S.
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	---	---	---	108

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	1/45(0.02)	2/25(0.08)	3/45(0.07)	3/48(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	3.000	0.781
Lower Limit	---	---	0.252	0.097
Upper Limit	---	---	153.830	8.952
Weeks to First Observed Tumor	105	109	102	78
Hematopoietic System: Leukemia[b]	3/46(0.07)	4/25(0.16)	4/49(0.08)	3/49(0.06)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.252	0.383
Lower Limit	---	---	0.224	0.061
Upper Limit	---	---	8.138	2.111
Weeks to First Observed Tumor	105	85	78	64
Pituitary: Adenoma NOS or Basophil Adenoma[b]	9/44(0.20)	3/21(0.14)	5/44(0.11)	0/35(0.00)
P Values[c]	---	---	N.S.	P = 0.048(N)
Relative Risk (Control)[d]	---	---	0.556	0.000
Lower Limit	---	---	0.159	0.000
Upper Limit	---	---	1.689	0.979
Weeks to First Observed Tumor	105	78	78	---

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	6/45(0.13)	2/25(0.08)	3/46(0.07)	3/45(0.07)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.489	0.833
Lower Limit	---	---	0.084	0.104
Upper Limit	---	---	2.140	9.528
Weeks to First Observed Tumor	96	109	106	108
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/42(0.06)	0/23(0.00)	3/41(0.07)	5/47(0.11)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.537	Infinite
Lower Limit	---	---	0.186	0.637
Upper Limit	---	---	17.606	Infinite
Weeks to First Observed Tumor	105	---	96	108
Testis: Interstitial-Cell Tumor[b]	44/45(0.98)	19/24(0.79)	43/46(0.93)	46/49(0.94)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.956	1.186
Lower Limit	---	---	0.914	0.963
Upper Limit	---	---	1.058	1.411
Weeks to First Observed Tumor	77	78	78	72

TABLE 3 (Concluded)

[a]Treated groups received time-weighted average concentrations of 0.008 or 0.020 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH 2,4-DINITROTOLUENE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	1/47(0.02)	0/23(0.00)	0/49(0.00)	1/50(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.000	Infinite
Lower Limit	---	---	0.000	0.025
Upper Limit	---	---	17.891	Infinite
Weeks to First Observed Tumor	107	---	---	109
Subcutaneous Tissue: Fibroma[b]	0/48(0.00)	0/23(0.00)	0/49(0.00)	3/50(0.06)
P Values[c]	---	---	---	N.S.
Relative Risk (Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.285
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	---	---	---	100
Mammary Gland: Fibroadenoma[b]	9/48(0.19)	4/23(0.17)	12/49(0.24)	23/50(0.46)
P Values[c]	---	---	N.S.	P = 0.016
Relative Risk (Control)[d]	---	---	1.306	2.645
Lower Limit	---	---	0.559	1.062
Upper Limit	---	---	3.183	9.435
Weeks to First Observed Tumor	92	109	83	69

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia[b]	2/48(0.04)	2/23(0.09)	2/49(0.04)	4/50(0.08)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.980	0.920
Lower Limit	---	---	0.074	0.145
Upper Limit	---	---	13.043	9.724
Weeks to First Observed Tumor	105	106	107	93
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	19/46(0.41)	8/21(0.38)	22/45(0.49)	14/40(0.35)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.184	0.919
Lower Limit	---	---	0.718	0.446
Upper Limit	---	---	1.955	2.154
Weeks to First Observed Tumor	71	109	78	78
Adrenal: Pheochromocytoma[b]	2/47(0.04)	2/23(0.09)	2/49(0.04)	0/50(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.959	0.000
Lower Limit	---	---	0.072	0.000
Upper Limit	---	---	12.769	3.177
Weeks to First Observed Tumor	105	109	78	---

TABLE 4 (Concluded)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	2/45(0.04)	3/21(0.14)	2/45(0.04)	6/48(0.13)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.000	0.875
Lower Limit	---	---	0.075	0.213
Upper Limit	---	---	13.270	5.047
Weeks to First Observed Tumor	105	109	94	100
Uterus: Endometrial Stromal Polyp[b]	15/46(0.33)	6/23(0.26)	14/47(0.30)	11/49(0.22)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.914	0.861
Lower Limit	---	---	0.464	0.343
Upper Limit	---	---	1.789	2.539
Weeks to First Observed Tumor	78	86	91	78

[a]Treated groups received time-weighted average concentrations of 0.008 or 0.020 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

of tumor that was observed in more than 5 percent of any of the 2,4-dinitrotoluene-dosed groups of either sex is included. The Cochran-Armitage test was not used in these analyses since the low dose and the low dose control were started at a different time from the high dose and the high dose control.

For male rats the Fisher exact test showed that the high dose group had a significantly ($P = 0.003$) higher incidence of fibromas of the subcutaneous tissue and skin than the high dose control. For the comparison of low dose to low dose control the Fisher exact test was also significant ($P = 0.008$). In the historical data compiled by this laboratory for the NCI Bioassay Program, 23/584 (3 percent) of the untreated male Fischer 344 rats had fibromas of the subcutaneous tissue or skin.

Based on these results the statistical conclusion is that the administration of 2,4-dinitrotoluene to male Fischer 344 rats was associated with the increased incidence of fibromas of the subcutaneous tissue or skin.

In females the Fisher exact test indicated a significant ($P = 0.016$) increase in fibroadenomas of the mammary gland in the high dose compared to the high dose control. The historical data indicated 115/585 (20 percent) untreated female Fischer 344 rats had a mammary fibroadenoma.

Based upon these results the statistical conclusion is that the administration of 2,4-dinitrotoluene to the high dose female Fischer

344 rats was associated with the increased incidence of fibroadenomas of the mammary gland.

The possibility of a negative association between administration and incidence was noted for pituitary adenomas in male rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

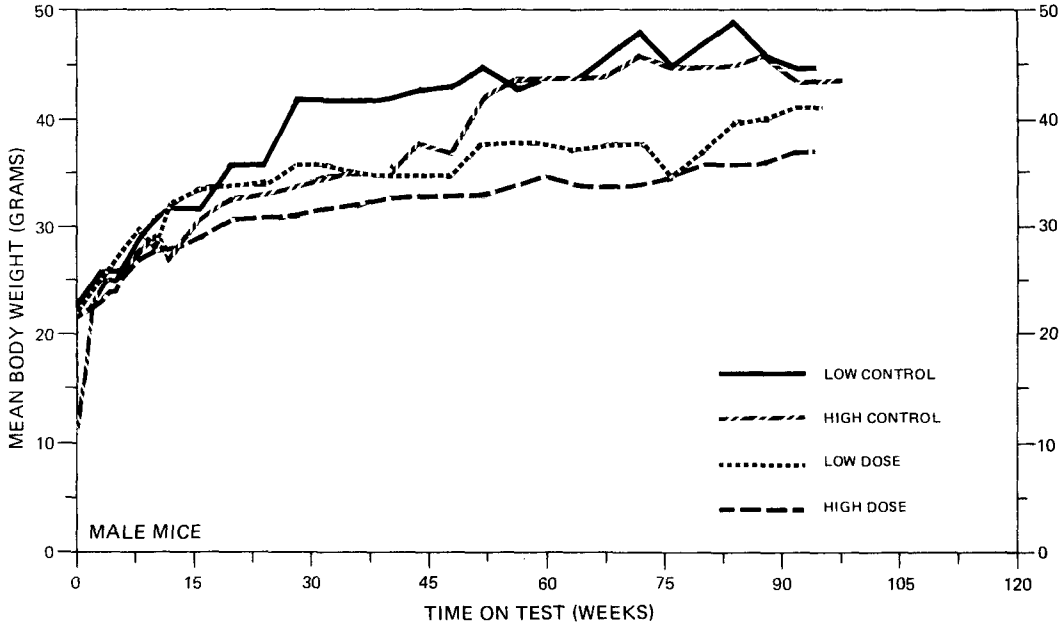
When compared with their respective controls, high and low dose mice of both sexes exhibited mean body weight depression by week 30 (Figure 3). Approximate weight gain, expressed as a percentage of the weight gained by their respective control groups at the end of the bioassay, was 91 percent for low dose males, 82 percent for high dose males, 89 percent for low dose females, and 76 percent for high dose females.

No clinical observations were reported for any treated or control mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2,4-dinitrotoluene-treated groups are shown in Figure 4. Because the low and high dose groups were tested at different times, each was assigned its own control group.

The statistical tests did not indicate a significant positive relationship between dosage and mortality for either sex. In male mice survival was high despite the sacrifice of five high dose and five high dose control males in week 78 and of five of the low dose control males in week 79. Seventy-eight percent of the high dose, 74 percent of the high dose control, 90 percent of the low dose, and 82 percent of the low dose control males survived until the end of the test.



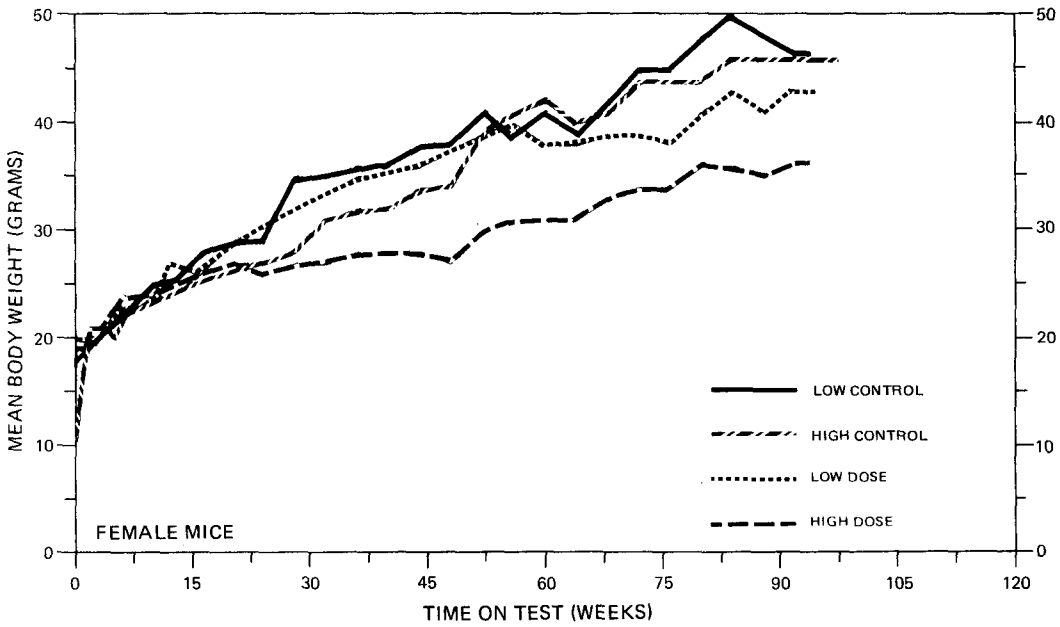


FIGURE 3
GROWTH CURVES FOR 2,4-DINITROTOLUENE CHRONIC STUDY MICE

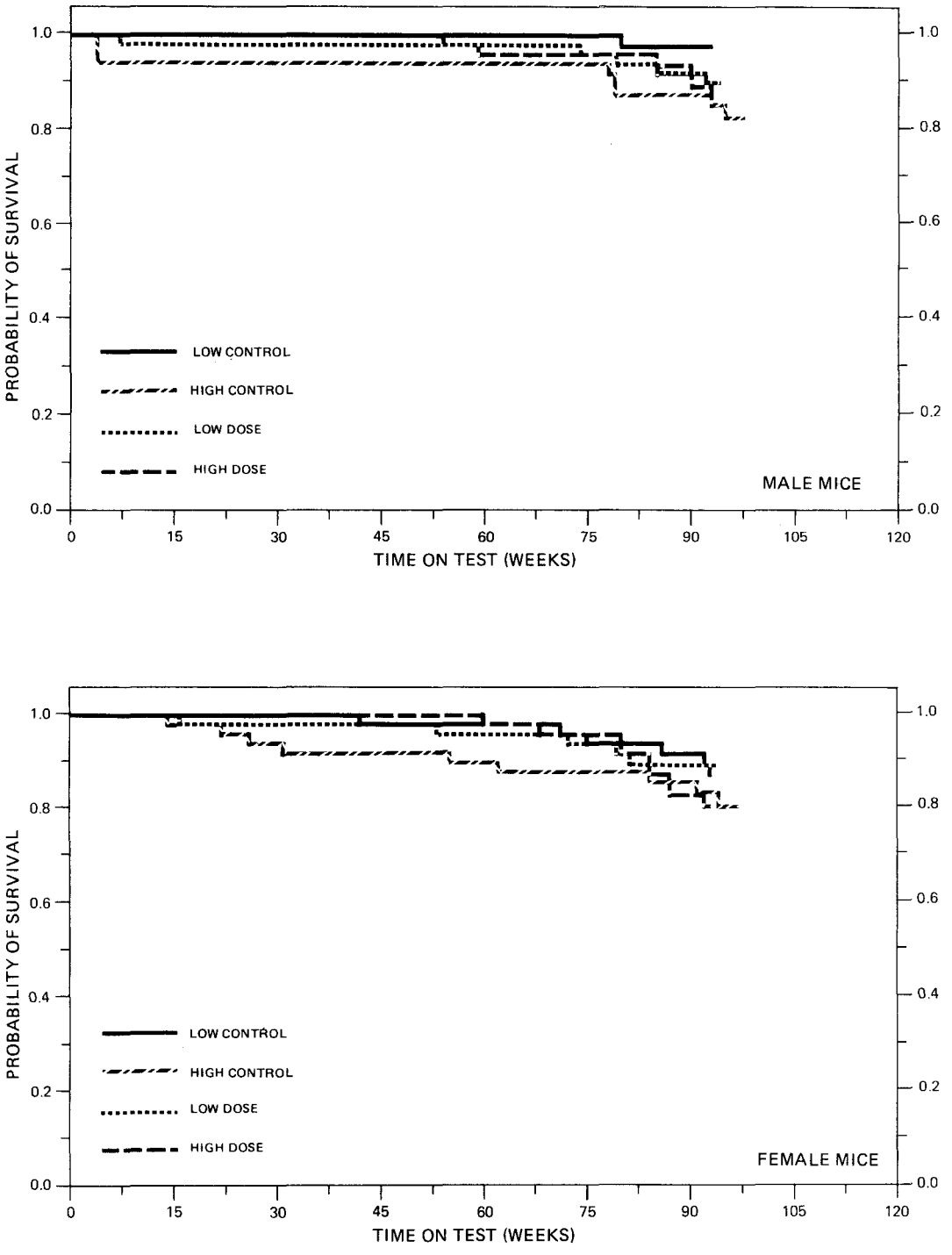


FIGURE 4
SURVIVAL COMPARISONS OF 2,4-DINITROTOLUENE CHRONIC STUDY MICE

In females five of the high dose treated and five of the high dose control mice were sacrificed in week 78, as well as five low dose controls in week 79. Seventy-two percent of the high dose, 70 percent of the high dose control, 84 percent of the low dose, and 78 percent of the low dose control survived until the end of the study.

Thus in both sexes survival was adequate for meaningful statistical analyses.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

There appeared to be no increase in the incidence of neoplasms in the treated mice compared with their corresponding control groups. With few exceptions, the same variety of neoplasms occurred in the chemically treated and control groups. This spectrum of neoplasms was similar to that expected in untreated B6C3F1 mice.

The incidence and variety of nonneoplastic, degenerative, proliferative, and inflammatory lesions was similar in the control and chemically treated mice.

This histopathologic examination of B6C3F1 mice treated with 2,4-dinitrotoluene provided no evidence of carcinogenicity.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis for every type

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 2,4-DINITROTOLUENE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	12/46(0.26)	10/45(0.22)	6/47(0.13)	9/48(0.19)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.489	0.844
Lower Limit	---	---	0.165	0.335
Upper Limit	---	---	1.281	2.095
Weeks to First Observed Tumor	93	93	92	59
Hematopoietic System: Malignant Lymphoma[b]	2/46(0.04)	2/46(0.04)	1/48(0.02)	3/49(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.479	1.408
Lower Limit	---	---	0.008	0.169
Upper Limit	---	---	8.888	16.250
Weeks to First Observed Tumor	93	97	93	78
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/46(0.04)	4/45(0.09)	3/48(0.06)	1/48(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.437	0.234
Lower Limit	---	---	0.173	0.005
Upper Limit	---	---	16.575	2.254
Weeks to First Observed Tumor	93	97	93	93

TABLE 5 (Concluded)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	7/46(0.15)	11/45(0.24)	3/48(0.06)	2/48(0.04)
P Values[c]	---	---	N.S.	P = 0.005(N)
Relative Risk (Control)[d]	---	---	0.411	0.170
Lower Limit	---	---	0.072	0.019
Upper Limit	---	---	1.679	0.725
Weeks to First Observed Tumor	79	78	93	93
Hematopoietic System: Hemangiosarcoma or Hemangioma[b]	0/46(0.00)	0/46(0.00)	3/48(0.06)	0/49(0.00)
P Values[c]	---	---	N.S.	---
Relative Risk (Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.578	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	93	---

[a]Treated groups received time-weighted average concentrations of 0.008 or 0.040 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2,4-DINITROTOLUENE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	4/46(0.09)	4/45(0.09)	1/46(0.02)	1/50(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.250	0.225
Lower Limit	---	---	0.005	0.005
Upper Limit	---	---	2.401	2.167
Weeks to First Observed Tumor	93	78	94	93
Hematopoietic System: Malignant Lymphoma[b]	5/46(0.11)	11/46(0.24)	4/46(0.09)	7/50(0.14)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.800	0.586
Lower Limit	---	---	0.169	0.211
Upper Limit	---	---	3.480	1.509
Weeks to First Observed Tumor	79	94	94	68
Lung: Alveolar/Bronchiolar Adenoma[b]	0/46(0.00)	1/45(0.02)	3/46(0.07)	0/48(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite	0.000
Lower Limit	---	---	0.603	0.000
Upper Limit	---	---	Infinite	17.480
Weeks to First Observed Tumor	---	97	84	---

TABLE 6 (Concluded)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Papilloma[b]	0/45(0.00)	3/42(0.07)	0/44(0.00)	0/41(0.00)
P Values[c]	---	---	---	N.S.
Relative Risk (Control)[d]	---	---	---	0.000
Lower Limit	---	---	---	0.000
Upper Limit	---	---	---	1.692
Weeks to First Observed Tumor	---	97	---	---
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	4/37(0.11)	6/37(0.16)	3/38(0.08)	0/34(0.00)
P Values[c]	---	---	N.S.	P = 0.017(N)
Relative Risk (Control)[d]	---	---	0.730	0.000
Lower Limit	---	---	0.114	0.000
Upper Limit	---	---	4.022	0.670
Weeks to First Observed Tumor	93	97	94	---

[a]Treated groups received time-weighted average concentrations of 0.008 or 0.040 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

of tumor that was observed in more than 5 percent of any of the 2,4-dinitrotoluene-dosed groups of either sex is included.

There were no tumors in either sex having a statistically significant positive association between chemical administration and incidence. As such, there was no convincing evidence of carcinogenicity in B6C3F1 mice at the dose levels used in this experiment.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 2,4-dinitrotoluene that could not be established under the conditions of this test.

The possibility of a negative association between administration and incidence was observed for pituitary adenomas in female mice and for alveolar/bronchiolar neoplasms in male mice.

V. DISCUSSION

Under the conditions of this bioassay, dietary administration of 2,4-dinitrotoluene was associated with increased incidences of fibromas of the subcutaneous tissue and skin in male rats and fibroadenomas of the mammary gland in female rats, but there were no increased incidences of tumors in treated mice of either sex when compared to controls. No significant association was demonstrated between chemical administration and mortality in either species.

In male rats integumentary tumors (i.e., fibromas) were the only neoplasms observed at statistically significant incidences. The incidences of skin and subcutaneous tissue fibromas were 0/46, 0/25, 7/49 (14 percent), and 13/49 (27 percent) in low dose control, high dose control, low dose, and high dose males, respectively. The only group among the female rats exhibiting these tumors was the high dose (3/50 or 6 percent). Statistical analyses of the incidences of these subcutaneous fibromas indicated a significant positive increase in incidence for the high dose males compared to the high dose control males.

There were certain unusual neoplasms (i.e., hemangiosarcoma in the subcutis, hemangiosarcoma of the urinary bladder, and prostate gland adenocarcinoma) that occurred at low incidences in high dose but not low dose or control male rats. These tumors were not considered to be related to chemical administration.

In female rats the only neoplasm observed at a significantly increased incidence was fibroadenoma of the mammary gland. This

tumor occurred at incidences of 9/48 (19 percent), 4/23 (17 percent), 12/49 (24 percent), and 23/50 (46 percent) in the low dose control, high dose control, low dose, and high dose groups, respectively. The comparison of the high dose group to its control group indicated a statistically significant increase in incidence in the dosed females.

There were no neoplasms occurring at statistically significant incidences in mice of either sex.

Under the conditions of this bioassay dietary administration of 2,4-dinitrotoluene to Fischer 344 rats induced benign tumors (i.e., skin and subcutaneous tissue fibromas in male Fischer 344 rats and mammary fibroadenomas in female Fischer 344 rats). No evidence was provided for the carcinogenicity of the compound in B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2,4-DINITROTOLUENE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTRCL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
ANIMALS INITIALLY IN STUDY	50	25	50	50
ANIMALS NECROPSIED	46	25	49	49
ANIMALS EXAMINED HISTOPATHOIOGICALLY **	45	25	49	49
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(25)	(49)	(49)
SQUAMOUS CELL PAPILLOMA			1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (2%)			
BASAL-CELL CARCINOMA			1 (2%)	
FIBROMA			2 (4%)	
*SUBCUT TISSUE	(46)	(25)	(49)	(49)
FIBROMA			5 (10%)	13 (27%)
FIBROSARCOMA			1 (2%)	2 (4%)
LIPOMA				3 (6%)
ANGIOSARCOMA				1 (2%)
RESPIRATORY SYSTEM				
#TRACHEA	(45)	(11)	(44)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)			
#LUNG	(45)	(25)	(48)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	2 (8%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (4%)		
PHEOCHROMOCYTOMA, METASTATIC		1 (4%)		
FIBROSARCOMA, METASTATIC				1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(46)	(25)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)			
LEUKEMIA,NOS			1 (2%)	
UNDIFFERENTIATED LEUKEMIA		2 (8%)		
MYELOMONOCYTIC LEUKEMIA	1 (2%)		1 (2%)	3 (6%)
LYMPHOCYTIC LEUKEMIA	2 (4%)	2 (8%)		
GRANULOCYTIC LEUKEMIA			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
#SPLEEN	(45)	(25)	(48)	(49)
LEIOMYOMA			1 (2%)	
MYELOMONOCYTIC LEUKEMIA			1 (2%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(43)	(24)	(43)	(48)
FIBROSARCOMA, METASTATIC				1 (2%)
#LIVER	(45)	(25)	(49)	(48)
HEPATOCELLULAR CARCINOMA			3 (6%)	3 (6%)
#STOMACH	(45)	(24)	(45)	(49)
SQUAMOUS CELL PAPILLOMA		1 (4%)		
BASAL-CELL CARCINOMA		1 (4%)		
#DUODENUM	(45)	(24)	(45)	(49)
ADENOCARCINOMA, NOS				1 (2%)
#ILEUM	(45)	(24)	(45)	(49)
CYSTADENOCARCINOMA, NOS			1 (2%)	
URINARY SYSTEM				
#URINARY BLADDER	(44)	(23)	(44)	(49)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)	
HEMANGIOSARCOMA				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(21)	(44)	(35)
CARCINOMA, NOS			2 (5%)	
ADENOMA, NOS	9 (20%)	1 (5%)	5 (11%)	
BASOPHIL ADENOMA		2 (10%)		
#ADRENAL	(45)	(25)	(46)	(45)
CORTICAL ADENOMA	1 (2%)			

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

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
PHEOCHROMOCYTOMA	6 (13%)	2 (8%)	3 (7%)	3 (7%)
PHEOCHROMOCYTOMA, MALIGNANT		2 (8%)		
#ADRENAL MEDULLA	(45)	(25)	(46)	(45)
GANGLIONEUROMA			1 (2%)	
#THYROID	(42)	(23)	(41)	(47)
FOLLICULAR-CELL CARCINOMA				2 (4%)
C-CELL ADENOMA	2 (5%)		3 (7%)	1 (2%)
C-CELL CARCINOMA	1 (2%)			4 (9%)
#PARATHYROID	(32)	(15)	(27)	(30)
ADENOMA, NOS				1 (3%)
#PANCREATIC ISLETS	(45)	(25)	(45)	(48)
ISLET-CELL ADENOMA	1 (2%)	2 (8%)	3 (7%)	2 (4%)
ISLET-CELL CARCINOMA				1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(46)	(25)	(49)	(49)
ADENOCARCINOMA, NOS				1 (2%)
FIBROADENOMA		1 (4%)	1 (2%)	
*PREPUTIAL GLAND	(46)	(25)	(49)	(49)
CARCINOMA, NOS		1 (4%)		1 (2%)
ADENOMA, NOS	1 (2%)	1 (4%)		
#PROSTATE	(45)	(23)	(45)	(48)
ADENOCARCINOMA, NOS				1 (2%)
#TESTIS	(45)	(24)	(46)	(49)
INTERSTITIAL-CELL TUMOR	44 (98%)	19 (79%)	43 (93%)	46 (94%)
NERVOUS SYSTEM				
#BRAIN	(45)	(25)	(45)	(49)
GLIOMA, NOS			1 (2%)	
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(46)	(25)	(49)	(49)
ADENOMA, NOS			1 (2%)	

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

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
*EAR CANAL	(46)	(25)	(49)	(49)
SQUAMOUS CELL CARCINOMA		1 (4%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*BODY CAVITIES	(46)	(25)	(49)	(49)
MESOTHELIOMA, NOS	3 (7%)		1 (2%)	1 (2%)
MESOTHELIOMA, MALIGNANT				1 (2%)
*MEDIASTINUM	(46)	(25)	(49)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (4%)		
*PLEURA	(46)	(25)	(49)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (4%)		
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	25	50	50
NATURAL DEATH@	7	3	6	5
MORIBUND SACRIFICE	6	4	10	11
SCHEDULED SACRIFICE	18	5	5	5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	19	13	29	29
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	44	22	47	48
TOTAL PRIMARY TUMORS	75	41	84	92
TOTAL ANIMALS WITH BENIGN TUMORS	44	20	45	46
TOTAL BENIGN TUMORS	65	31	70	69
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	9	12	18
TOTAL MALIGNANT TUMORS	7	10	13	22
TOTAL ANIMALS WITH SECONDARY TUMORS#		2		1
TOTAL SECONDARY TUMORS		3		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3		1	1
TOTAL UNCERTAIN TUMORS	3		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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS
TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
ANIMALS INITIALLY IN STUDY	50	25	50	50
ANIMALS NECROPSIED	48	23	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	23	49	50
INTEGUMENTARY SYSTEM				
*SKIN	(48)	(23)	(49)	(50)
SEBACEOUS ADENOCARCINOMA		1 (4%)		
FIBROMA	1 (2%)			
FIBROSARCOMA	1 (2%)			
FIBROADENOMA	1 (2%)			
*SUBCUT TISSUE	(48)	(23)	(49)	(50)
SQUAMOUS CELL CARCINOMA				1 (2%)
FIBROMA				3 (6%)
RESPIRATORY SYSTEM				
#LUNG	(47)	(23)	(49)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)			
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (4%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(48)	(23)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)			
LEUKEMIA,NOS			1 (2%)	
UNDIFFERENTIATED LEUKEMIA		2 (9%)		
MYELOMONOCYTIC LEUKEMIA	1 (2%)		1 (2%)	4 (8%)
*SUBCUT TISSUE	(48)	(23)	(49)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#SPLEEN	(47)	(23)	(49)	(50)
MYELOMONOCYTIC LEUKEMIA	1 (2%)			
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
DIGESTIVE SYSTEM				
#LIVER	(47)	(23)	(49)	(50)
NEOPLASTIC NODULE		2 (9%)		1 (2%)
HEPATOCELLULAR CARCINOMA	1 (2%)			1 (2%)
#STOMACH	(46)	(23)	(49)	(48)
SQUAMOUS CELL PAPILLOMA			1 (2%)	
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(46)	(21)	(45)	(40)
CARCINOMA,NOS	1 (2%)		2 (4%)	1 (3%)
ADENOMA, NOS	19 (41%)	1 (5%)	22 (49%)	14 (35%)
CHROMOPHOBE ADENOMA		7 (33%)		
#ADRENAL	(47)	(23)	(49)	(50)
CORTICAL ADENOMA	1 (2%)			1 (2%)
PHEOCHROMOCYTOMA	2 (4%)	2 (9%)	2 (4%)	
PHEOCHROMOCYTOMA, MALIGNANT		1 (4%)		
#THYROID	(45)	(21)	(45)	(48)
ADENOMA, NOS			1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (5%)		
C-CELL ADENOMA	2 (4%)	2 (10%)	2 (4%)	2 (4%)
C-CELL CARCINOMA		1 (5%)		4 (8%)
#PANCREATIC ISLETS	(46)	(22)	(47)	(48)
ISLET-CELL ADENOMA	2 (4%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(23)	(49)	(50)
ADENOCARCINOMA, NOS		2 (9%)		1 (2%)
PAPILLARY CYSTADENOMA, NOS	2 (4%)			
PAPILLARY CYSTADENOCARCINOMA,NOS			1 (2%)	
INTRADUCTAL CARCINOMA			1 (2%)	
INFILTRATING DUCT CARCINOMA		1 (4%)		

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

TABLE A2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
FIBROADENOMA	9 (19%)	4 (17%)	12 (24%)	23 (46%)
*CLITORAL GLAND	(48)	(23)	(49)	(50)
SQUAMOUS CELL PAPILLOMA				1 (2%)
*VAGINA	(48)	(23)	(49)	(50)
FIBROSARCOMA	1 (2%)			
LYMPHANGIOSARCOMA	1 (2%)			
#UTERUS	(46)	(23)	(47)	(49)
ENDOMETRIAL STROMAL POLYP	15 (33%)	6 (26%)	14 (30%)	11 (22%)
#UTERUS/ENDOMETRIUM	(46)	(23)	(47)	(49)
CARCINOMA,NOS	1 (2%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(48)	(23)	(49)	(50)
FIBROSARCOMA	1 (2%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
SITE UNKNOWN				
ADENOCARCINOMA, NOS			1	

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

TABLE A2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	25	50	50
NATURAL DEATH@	6	3	4	7
MORIBUND SACRIFICE	8	5	10	12
SCHEDULED SACRIFICE	17	5	5	5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	19	12	31	26
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	38	19	41	40
TOTAL PRIMARY TUMORS	66	34	62	69
TOTAL ANIMALS WITH BENIGN TUMORS	36	18	40	39
TOTAL BENIGN TUMORS	54	23	54	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	8	8	11
TOTAL MALIGNANT TUMORS	12	9	8	12
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		2		1
TOTAL UNCERTAIN TUMORS		2		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 NEOPLASMS IN MICE TREATED WITH 2,4-DINITROTOLUENE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	46	46	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	46	45	48	49
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(46)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(46)	(45)	(48)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (11%)	7 (16%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	4 (9%)	3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(46)	(46)	(48)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)	1 (2%)
#SPLEEN	(46)	(45)	(47)	(44)
HEMANGIOMA			1 (2%)	
HEMANGIOSARCOMA			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		
#MANDIBULAR L. NODE	(34)	(35)	(40)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)		
#PEYERS PATCH	(46)	(43)	(45)	(45)
MALIGNANT LYMPHOMA, NOS				1 (2%)
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
DIGESTIVE SYSTEM				
#LIVER	(46)	(45)	(47)	(48)
HEPATOCELLULAR CARCINOMA	12 (26%)	10 (22%)	6 (13%)	9 (19%)
HEMANGIOMA			1 (2%)	
#STOMACH	(45)	(42)	(45)	(45)
SQUAMOUS CELL PAPILLOMA		1 (2%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(39)	(36)	(33)	(42)
ADENOMA, NOS	1 (3%)			
#THYROID	(44)	(40)	(42)	(41)
ADENOMA, NOS			1 (2%)	
ADENOCARCINOMA, NOS	2 (5%)			
REPRODUCTIVE SYSTEM				
#TESTIS	(46)	(45)	(47)	(47)
SEMINOMA/DYSGERMINOMA	1 (2%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(46)	(46)	(48)	(49)
PAPILLARY CYSTADENOMA, NOS	1 (2%)			
*EAR CANAL	(46)	(46)	(48)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)		
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE B1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	1	7	5	6
MORIBUND SACRIFICE		1		
SCHEDULED SACRIFICE	5	5		5
ACCIDENTALLY KILLED	3			
TERMINAL SACRIFICE	41	37	45	39
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	21	13	13
TOTAL PRIMARY TUMORS	26	25	15	14
TOTAL ANIMALS WITH BENIGN TUMORS	6	8	3	1
TOTAL BENIGN TUMORS	7	8	3	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	15	10	13
TOTAL MALIGNANT TUMORS	19	17	12	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1		
TOTAL SECONDARY TUMORS	1	1		
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 B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS MISSING			3	
ANIMALS NECROPSIED	47	46	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	46	46	50
INTEGUMENTARY SYSTEM				
*SKIN	(47)	(46)	(46)	(50)
FIBROSARCOMA		2 (4%)		
RESPIRATORY SYSTEM				
#LUNG	(46)	(45)	(46)	(48)
CARCINOMA, NOS, METASTATIC	1 (2%)			
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	3 (7%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(47)	(46)	(46)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)	3 (7%)	2 (4%)	4 (8%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)		2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)	6 (13%)	1 (2%)	
LYMPHOCYTIC LEUKEMIA		1 (2%)		
#SPLEEN	(45)	(43)	(44)	(48)
HEMANGIOSARCOMA	1 (2%)			
MALIGNANT LYMPHOMA, NOS				1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#LIVER	(46)	(45)	(46)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)			
KUPFFER-CELL SARCOMA			1 (2%)	
#PEYERS PATCH	(45)	(43)	(42)	(44)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
DIGESTIVE SYSTEM				
#LIVER	(46)	(45)	(46)	(50)
CARCINOMA, NOS, METASTATIC	1 (2%)			
HEPATOCELLULAR CARCINOMA	4 (9%)	4 (9%)	1 (2%)	1 (2%)
#STOMACH	(45)	(42)	(44)	(41)
SQUAMOUS CELL PAPILLOMA		3 (7%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(37)	(37)	(38)	(34)
ADENOMA, NOS	3 (8%)	6 (16%)	3 (8%)	
CHROMOPHOBE ADENOMA	1 (3%)			
#ADRENAL	(44)	(43)	(42)	(45)
CORTICAL ADENOMA		1 (2%)		
#THYROID	(44)	(30)	(40)	(42)
PAPILLARY CARCINOMA				1 (2%)
#PANCREATIC ISLETS	(39)	(41)	(44)	(41)
ISLET-CELL ADENOMA		1 (2%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(47)	(46)	(46)	(50)
ADENOCARCINOMA, NOS		1 (2%)		
FIBROADENOMA	1 (2%)			
#UTERUS	(43)	(43)	(44)	(43)
ADENOCARCINOMA, NOS				1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (2%)			
ENDOMETRIAL STROMAL SARCOMA				1 (2%)
#UTERUS/ENDOMETRIUM	(43)	(43)	(44)	(43)
CARCINOMA,NOS	1 (2%)			
#OVARY/OVIDUCT	(43)	(43)	(44)	(43)
PAPILLARY ADENOMA	1 (2%)			

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

TABLE B2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
INTRADUCTAL PAPILLOMA	1 (2%)			
#OVARY	(44)	(41)	(44)	(37)
LUTEOMA		1 (2%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(47)	(46)	(46)	(50)
PAPILLARY CYSTADENOMA, NOS			2 (4%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(47)	(46)	(46)	(50)
HEMANGIOSARCOMA	1 (2%)			
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	5	8	5	8
MORIBUND SACRIFICE	1	2		1
SCHEDULED SACRIFICE	5	5		5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	39	35	42	36
ANIMAL MISSING			3	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	22	14	11
TOTAL PRIMARY TUMORS	21	32	15	11
TOTAL ANIMALS WITH BENIGN TUMORS	7	12	8	
TOTAL BENIGN TUMORS	8	13	8	
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	18	7	11
TOTAL MALIGNANT TUMORS	13	19	7	11
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			
TOTAL SECONDARY TUMORS	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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2,4-DINITROTOLUENE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
ANIMALS INITIALLY IN STUDY	50	25	50	50
ANIMALS NECROPSIED	46	25	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	45	25	49	49
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(25)	(49)	(49)
EPIDERMAL INCLUSION CYST			1 (2%)	
COLLAGEN DISEASE				1 (2%)
FIBROSIS			1 (2%)	
NECROSIS, NOS		1 (4%)		
*SUBCUT TISSUE	(46)	(25)	(49)	(49)
EPIDERMAL INCLUSION CYST				1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
NECROSIS, NOS				1 (2%)
KELOID	1 (2%)			
FIBROUS DYSPLASIA	1 (2%)			
RESPIRATORY SYSTEM				
*LARYNX	(46)	(25)	(49)	(49)
INFLAMMATION ACUTE AND CHRONIC		1 (4%)		
INFLAMMATION, CHRONIC		7 (28%)		
#TRACHEA	(45)	(11)	(44)	(49)
INFLAMMATION, NOS		1 (9%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (4%)		3 (7%)	
INFLAMMATION, ACUTE/CHRONIC	24 (53%)		29 (66%)	3 (6%)
INFLAMMATION, CHRONIC				26 (53%)
#LUNG/BRONCHUS	(45)	(25)	(48)	(49)
BRONCHIECTASIS		2 (8%)	1 (2%)	4 (8%)
INFLAMMATION, FOCAL		1 (4%)		
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
HYPERPLASIA, LYMPHOID	2 (4%)			3 (6%)
#LUNG/BRONCHIOLE	(45)	(25)	(48)	(49)
BRONCHIOLIECTASIS	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
LYMPHOCYTIC INFLAMMATORY INFILTR	5 (11%)		2 (4%)	
INFLAMMATION, ACUTE/CHRONIC	3 (7%)			
HYPERPLASIA, LYMPHOID	6 (13%)		1 (2%)	
#LUNG	(45)	(25)	(48)	(49)
EMPHYSEMA, NOS	1 (2%)			
ATELECTASIS	1 (2%)			
EDEMA, NOS			1 (2%)	
HEMORRHAGE			1 (2%)	
INFLAMMATION, INTERSTITIAL	4 (9%)	2 (8%)	1 (2%)	3 (6%)
BRONCHOPNEUMONIA, ACUTE		1 (4%)		
ABSCESS, NOS	2 (4%)	1 (4%)		
PNEUMONIA, CHRONIC MURINE		11 (44%)		1 (2%)
GRANULOMA, NOS		1 (4%)		
FIBROSIS, DIFFUSE				2 (4%)
PERIVASCULITIS	5 (11%)		3 (6%)	
HYPERPLASIA, NOS				2 (4%)
HYPERPLASIA, ADENOMATOUS	1 (2%)			
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)			
#LUNG/ALVEOLI	(45)	(25)	(48)	(49)
CALCIFICATION, NOS				1 (2%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(45)	(25)	(44)	(46)
HEMORRHAGE	1 (2%)			
KARYORRHEXIS	1 (2%)			
ATROPHY, NOS			1 (2%)	
MYELOFIBROSIS			1 (2%)	
HYPERPLASIA, HEMATOPOIETIC	4 (9%)	2 (8%)		
ERYTHROPOIESIS	1 (2%)		1 (2%)	
MYELOPOIESIS	1 (2%)			
#SPLEEN	(45)	(25)	(48)	(49)
CONGESTION, NOS	1 (2%)			
HEMORRHAGE				1 (2%)
HEMATOMA, NOS				1 (2%)
FIBROSIS				2 (4%)
HEMOSIDEROSIS		1 (4%)		1 (2%)
ATROPHY, NOS				2 (4%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)	1 (4%)		
HYPERPLASIA, ERYTHROID		1 (4%)		
HYPERPLASIA, RETICULUM CELL				1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)			

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
HEMATOPOIESIS	1 (2%)		1 (2%)	1 (2%)
ERYTHROPOIESIS				1 (2%)
MYELOPOIESIS	1 (2%)			
#SPLENIC CAPSULE	(45)	(25)	(48)	(49)
HEMORRHAGE	1 (2%)			
#LYMPH NODE	(41)	(24)	(37)	(44)
PLASMACYTOSIS		1 (4%)		
#PAROTID LYMPH NODE	(41)	(24)	(37)	(44)
HYPERPLASIA, NOS			1 (3%)	
#MANDIBULAR L. NODE	(41)	(24)	(37)	(44)
LYMPHANGIECTASIS			2 (5%)	
#MEDIASTINAL L.NODE	(41)	(24)	(37)	(44)
LYMPHANGIECTASIS			1 (3%)	
HEMORRHAGE			1 (3%)	
#THYMUS	(35)	(22)	(26)	(34)
HYPERPLASIA, EPITHELIAL	1 (3%)			
CIRCULATORY SYSTEM				
#HEART	(45)	(25)	(48)	(49)
PERIARTERITIS		1 (4%)		
PERIVASCULITIS	2 (4%)			
#MYOCARDIUM	(45)	(25)	(48)	(49)
INFLAMMATION, FOCAL	1 (2%)		2 (4%)	
INFLAMMATION, INTERSTITIAL			2 (4%)	2 (4%)
INFLAMMATION, ACUTE/CHRONIC	3 (7%)		2 (4%)	
FIBROSIS	1 (2%)		1 (2%)	2 (4%)
FIBROSIS, FOCAL		1 (4%)	2 (4%)	
FIBROSIS, DIFFUSE			15 (31%)	1 (2%)
DEGENERATION, NOS	13 (29%)	10 (40%)	3 (6%)	
CALCIFICATION, NOS				1 (2%)
#ENDOCARDIUM	(45)	(25)	(48)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
*AORTA	(46)	(25)	(49)	(49)
MINERALIZATION			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
MEDIAL CALCIFICATION				2 (4%)
CALCIFICATION, FOCAL		1 (4%)		
*CORONARY ARTERY	(46)	(25)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
*PULMONARY ARTERY	(46)	(25)	(49)	(49)
MEDIAL CALCIFICATION				1 (2%)
*ANTERIOR MEDIASTINAL	(46)	(25)	(49)	(49)
MEDIAL CALCIFICATION				1 (2%)
*SPLENIC ARTERY	(46)	(25)	(49)	(49)
MEDIAL CALCIFICATION				1 (2%)
*MESENTERIC ARTERY	(46)	(25)	(49)	(49)
MEDIAL CALCIFICATION				1 (2%)
*RENAL ARTERY	(46)	(25)	(49)	(49)
HYPERPLASIA, FOCAL			1 (2%)	
DIGESTIVE SYSTEM				
#PAROTID GLAND	(43)	(24)	(43)	(48)
INFLAMMATION, INTERSTITIAL	1 (2%)			
#SUBMAXILLARY GLAND	(43)	(24)	(43)	(48)
HYPERPLASIA, FOCAL	1 (2%)			
#LIVER	(45)	(25)	(49)	(48)
CONGESTION, NOS	1 (2%)			
CONGESTION, CHRONIC PASSIVE		1 (4%)		
HEMORRHAGE			1 (2%)	
INFLAMMATION, FOCAL			2 (4%)	
INFLAMMATION, ACUTE FOCAL	2 (4%)		1 (2%)	
CHOLANGIOFIBROSIS		1 (4%)		
PERIVASCULITIS			1 (2%)	
DEGENERATION, NOS	2 (4%)			
NECROSIS, FOCAL	1 (2%)	1 (4%)	1 (2%)	
NECROSIS, DIFFUSE				1 (2%)
METAMORPHOSIS FATTY	3 (7%)	4 (16%)	21 (43%)	12 (25%)
HYPERPLASIA, NODULAR			4 (8%)	
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	8 (18%)		25 (51%)	2 (4%)
#LIVER/CENTRILOBULAR	(45)	(25)	(49)	(48)
NECROSIS, NOS				1 (2%)

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
METAMORPHOSIS FATTY	1 (2%)			
#LIVER/HEPATOCYTES	(45)	(25)	(49)	(48)
HYPERPLASIA, FOCAL	6 (13%)			
*BILE DUCT	(46)	(25)	(49)	(49)
INFLAMMATION, NOS	5 (11%)		3 (6%)	
HYPERPLASIA, NOS	11 (24%)	6 (24%)	7 (14%)	
HYPERPLASIA, FOCAL			7 (14%)	
#PANCREAS	(45)	(25)	(45)	(48)
INFLAMMATION, NOS	1 (2%)	1 (4%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	2 (4%)		5 (11%)	
SCAR				1 (2%)
PERIARTERITIS			2 (4%)	1 (2%)
METAMORPHOSIS FATTY			1 (2%)	
HYPERPLASTIC NODULE				1 (2%)
#PANCREATIC DUCT	(45)	(25)	(45)	(48)
HYPERPLASIA, NOS		1 (4%)		
#PANCREATIC ACINUS	(45)	(25)	(45)	(48)
ATROPHY, NOS	13 (29%)			
ATROPHY, FOCAL	2 (4%)			
#ESOPHAGUS	(44)	(25)	(42)	(49)
INFLAMMATION, ACUTE FOCAL	1 (2%)			
#STOMACH	(45)	(24)	(45)	(49)
EPIDERMAL INCLUSION CYST		1 (4%)		
HYPERKERATOSIS	1 (2%)			
#GASTRIC MUCOSA	(45)	(24)	(45)	(49)
CALCIFICATION, NOS				2 (4%)
#GASTRIC MUSCULARIS	(45)	(24)	(45)	(49)
CALCIFICATION, NOS				2 (4%)
#SMALL INTESTINE	(45)	(24)	(45)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)	
#PEYERS PATCH	(45)	(24)	(45)	(49)
HYPERPLASIA, NOS		2 (8%)		
HYPERPLASIA, LYMPHOID	1 (2%)			
#ILEUM	(45)	(24)	(45)	(49)
METAPLASIA, OSSEOUS			1 (2%)	

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
#COLON	(44)	(24)	(43)	(46)
PARASITISM			4 (9%)	1 (2%)
URINARY SYSTEM				
#KIDNEY	(45)	(24)	(48)	(49)
HYDRONEPHROSIS				1 (2%)
CONGESTION, NOS	1 (2%)			
GLOMERULONEPHRITIS, NOS	34 (76%)	5 (21%)	37 (77%)	5 (10%)
GLOMERULONEPHRITIS, FOCAL			2 (4%)	
INFLAMMATION, INTERSTITIAL	5 (11%)			
GLOMERULONEPHRITIS, CHRONIC			1 (2%)	
NEPHROPATHY		1 (4%)		
NEPHROSIS, NOS		16 (67%)		43 (88%)
GLOMERULOSCLEROSIS, NOS			1 (2%)	
CALCIFICATION, NOS				1 (2%)
#KIDNEY/MEDULLA	(45)	(24)	(48)	(49)
MULTIPLE CYSTS	1 (2%)			
#KIDNEY/GLOMERULUS	(45)	(24)	(48)	(49)
INFLAMMATION, MEMBRANOUS	9 (20%)		4 (8%)	
#KIDNEY/TUBULE	(45)	(24)	(48)	(49)
CALCIFICATION, NOS				1 (2%)
#URINARY BLADDER	(44)	(23)	(44)	(49)
CALCULUS, NOS		3 (13%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
HYPERPLASIA, EPITHELIAL			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(21)	(44)	(35)
CYST, NOS			1 (2%)	
CONGESTION, NOS	1 (2%)			
HEMORRHAGE			1 (2%)	
HYPERPLASIA, FOCAL	6 (14%)		5 (11%)	2 (6%)
#PITUITARY/BASOPHIL	(44)	(21)	(44)	(35)
NODULE		1 (5%)		
#ADRENAL	(45)	(25)	(46)	(45)
METAMORPHOSIS FATTY			1 (2%)	

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
HYPERPLASIA, FOCAL				1 (2%)
#ADRENAL CORTEX	(45)	(25)	(46)	(45)
NODULE	1 (2%)			
METAMORPHOSIS FATTY			2 (4%)	
HYPERTROPHY, FOCAL		1 (4%)		
HYPERPLASIA, NODULAR	1 (2%)			
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, FOCAL	7 (16%)		3 (7%)	
#ADRENAL MEDULLA	(45)	(25)	(46)	(45)
HYPERPLASIA, NOS	2 (4%)			
HYPERPLASIA, FOCAL	4 (9%)			
ANGIECTASIS			2 (4%)	
#THYROID	(42)	(23)	(41)	(47)
THYROGLOSSAL DUCT CYST				1 (2%)
HYPERPLASIA, FOCAL	2 (5%)		1 (2%)	
HYPERPLASIA, C-CELL	1 (2%)		1 (2%)	
HYPERPLASIA, FOLLICULAR-CELL				1 (2%)
#PANCREATIC ISLETS	(45)	(25)	(45)	(48)
HYPERPLASIA, NOS	2 (4%)			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(46)	(25)	(49)	(49)
CYST, NOS			1 (2%)	
HYPERPLASIA, NOS	3 (7%)	3 (12%)	1 (2%)	
LACTATION		7 (28%)		
*PREPUTIAL GLAND	(46)	(25)	(49)	(49)
ABSCESS, NOS	2 (4%)			
#PROSTATE	(45)	(23)	(45)	(48)
INFLAMMATION, NOS		1 (4%)		
INFLAMMATION, FOCAL	1 (2%)			
INFLAMMATION, ACUTE	10 (22%)		5 (11%)	1 (2%)
INFLAMMATION, ACUTE FOCAL	4 (9%)		14 (31%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
DEGENERATION, NOS	13 (29%)			
ATROPHY, NOS	2 (4%)	4 (17%)		
HYPERPLASIA, FOCAL			1 (2%)	
HYPERPLASIA, PAPILLARY	2 (4%)			

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
HYPERPLASIA, ADENOMATOUS	1 (2%)			
METAPLASIA, SQUAMOUS			1 (2%)	
*SEMINAL VESICLE	(46)	(25)	(49)	(49)
ATROPHY, NOS	26 (57%)	1 (4%)	1 (2%)	
HYPERPLASIA, PAPILLARY	1 (2%)			
*COAGULATING GLAND	(46)	(25)	(49)	(49)
ATROPHY, NOS	3 (7%)			
#TESTIS	(45)	(24)	(46)	(49)
DEGENERATION, NOS			32 (70%)	
CALCIFICATION, FOCAL		4 (17%)		
ATROPHY, NOS	1 (2%)	12 (50%)		
HYPERPLASIA, INTERSTITIAL CELL		2 (8%)	2 (4%)	5 (10%)
#TESTIS/TUBULE	(45)	(24)	(46)	(49)
DEGENERATION, NOS	10 (22%)		3 (7%)	2 (4%)
NERVOUS SYSTEM				
#BRAIN	(45)	(25)	(45)	(49)
HEMORRHAGE		2 (8%)		
CALCIFICATION, FOCAL		1 (4%)		
#CEREBRAL CORTEX	(45)	(25)	(45)	(49)
HEMORRHAGE	1 (2%)			
MALACIA	1 (2%)			
SPECIAL SENSE ORGANS				
*EYE	(46)	(25)	(49)	(49)
SYNECHIA, POSTERIOR	1 (2%)			
CATARACT	1 (2%)			
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(46)	(25)	(49)	(49)
CALCIFICATION, FOCAL		1 (4%)		
BODY CAVITIES				
NONE				

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

TABLE C1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 01-0030	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE 01-0025	HIGH DOSE 01-0087
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, ACUTE/CHRONIC			1	
SPECIAL MORPHOLOGY SUMMARY				
AUTC/NECROPSY/HISTO PERF			1	
AUTC/NECROPSY/NC HISTO	1			
AUTOLYSIS/NO NECROPSY	4		1	1

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
ANIMALS INITIALLY IN STUDY	50	25	50	50
ANIMALS NECROPSIED	48	23	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	23	49	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(48)	(23)	(49)	(50)
COLLAGEN DISEASE				1 (2%)
RESPIRATORY SYSTEM				
*NASAL SEPTUM	(48)	(23)	(49)	(50)
INFLAMMATION, CHRONIC				1 (2%)
*LARYNX	(48)	(23)	(49)	(50)
INFLAMMATION ACUTE AND CHRONIC		1 (4%)		
INFLAMMATION, CHRONIC		3 (13%)		
#TRACHEA	(47)	(5)	(48)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (9%)		4 (8%)	
INFLAMMATION, ACUTE/CHRONIC	18 (38%)		25 (52%)	1 (2%)
INFLAMMATION, CHRONIC				20 (40%)
POLYP, INFLAMMATORY	1 (2%)			
#LUNG/BRONCHUS	(47)	(23)	(49)	(50)
BRONCHIECTASIS	2 (4%)			
INFLAMMATION, ACUTE FOCAL			1 (2%)	
FIBROSIS	1 (2%)			
#LUNG/BRONCHIOLE	(47)	(23)	(49)	(50)
INFLAMMATION, NOS	4 (9%)			
LYMPHOCYTIC INFLAMMATORY INFILTR	7 (15%)		4 (8%)	
INFLAMMATION, ACUTE/CHRONIC	5 (11%)			
HYPERPLASIA, LYMPHOID	2 (4%)			
#LUNG	(47)	(23)	(49)	(50)
CONGESTION, NOS			2 (4%)	
INFLAMMATION, FOCAL			1 (2%)	
INFLAMMATION, INTERSTITIAL	4 (9%)	3 (13%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
BRONCHOPNEUMONIA, ACUTE				1 (2%)
PNEUMONIA, CHRONIC MURINE		8 (35%)		
GRANULOMA, FOREIGN BODY		1 (4%)		
PERIVASCULITIS	15 (32%)		4 (8%)	
CALCIFICATION, FOCAL		1 (4%)		
HYPERPLASIA, EPITHELIAL		1 (4%)		
HYPERPLASIA, ADENOMATOUS				1 (2%)
#LUNG/ALVEOLI	(47)	(23)	(49)	(50)
EPITHELIALIZATION	1 (2%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(47)	(22)	(48)	(45)
HYPOPLASIA, NOS	1 (2%)			
OSTEOSCLEROSIS				1 (2%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)	1 (5%)		
#SPLEEN	(47)	(23)	(49)	(50)
CONGESTION, NOS	1 (2%)			
HEMATOMA, NOS		1 (4%)		
HEMOSIDEROSIS		2 (9%)		1 (2%)
ATROPHY, NOS				1 (2%)
HYPERPLASIA, HEMATOPOIETIC		3 (13%)		
HYPERPLASIA, ERYTHROID		4 (17%)		
HEMATOPOIESIS	2 (4%)	3 (13%)		1 (2%)
ERYTHROPOIESIS				2 (4%)
#MANDIBULAR L. NODE	(42)	(21)	(44)	(47)
HYPERPLASIA, NOS				1 (2%)
#PANCREATIC L.NODE	(42)	(21)	(44)	(47)
HYPERPLASIA, NOS			1 (2%)	
#MESENTERIC L. NODE	(42)	(21)	(44)	(47)
HYPERPLASIA, NOS				1 (2%)
#THYMUS	(36)	(20)	(27)	(32)
HYPERPLASIA, RETICULUM CELL			1 (4%)	
CIRCULATORY SYSTEM				
#HEART	(47)	(23)	(49)	(49)
PERIARTERITIS	2 (4%)		1 (2%)	

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
PERIVASCULITIS	1 (2%)			
HYPERTROPHY, NOS	1 (2%)			
#MYOCARDIUM	(47)	(23)	(49)	(49)
INFLAMMATION, NOS			1 (2%)	
INFLAMMATION, FOCAL	1 (2%)			
INFLAMMATION, INTERSTITIAL		1 (4%)	1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	2 (4%)		5 (10%)	2 (4%)
FIBROSIS, DIFFUSE			4 (8%)	
DEGENERATION, NOS	2 (4%)	4 (17%)		3 (6%)
#ENDOCARDIUM	(47)	(23)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC	3 (6%)			
#CARDIAC VALVE	(47)	(23)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
*RENAL ARTERY	(48)	(23)	(49)	(50)
THROMBOSIS, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(47)	(23)	(49)	(50)
CONGESTION, CHRONIC PASSIVE		1 (4%)		
HEMORRHAGE	1 (2%)			
INFLAMMATION, FOCAL	1 (2%)		1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE				1 (2%)
SCLEROSIS	1 (2%)			
CHOLANGIOFIBROSIS		1 (4%)		
PERIVASCULITIS	1 (2%)			
NECROSIS, FOCAL	1 (2%)		1 (2%)	
NECROSIS, COAGULATIVE	1 (2%)			
METAMORPHOSIS FATTY	4 (9%)	2 (9%)	8 (16%)	4 (8%)
BASOPHILIC CYTO CHANGE		4 (17%)		
HYPERTROPHY, NOS	1 (2%)			
HYPERPLASIA, NODULAR			4 (8%)	
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	21 (45%)	3 (13%)	33 (67%)	6 (12%)
HYPERPLASIA, DIFFUSE	1 (2%)			
HYPERPLASIA, RETICULUM CELL			1 (2%)	
#LIVER/HEPATOCYTES	(47)	(23)	(49)	(50)
HYPERPLASIA, FOCAL	2 (4%)			
*BILE DUCT	(48)	(23)	(49)	(50)
INFLAMMATION, NOS			1 (2%)	1 (2%)

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
INFLAMMATION WITH FIBROSIS	1 (2%)			
HYPERPLASIA, NOS	4 (8%)	2 (9%)	7 (14%)	1 (2%)
HYPERPLASIA, FOCAL			7 (14%)	
#PANCREAS	(46)	(22)	(47)	(48)
INFLAMMATION, INTERSTITIAL	2 (4%)		2 (4%)	
INFLAMMATION, ACUTE/CHRONIC			5 (11%)	
ATROPHY, NOS	1 (2%)			
#PANCREATIC ACINUS	(46)	(22)	(47)	(48)
DEGENERATION, GRANULAR	1 (2%)			
ATROPHY, NOS	4 (9%)			
ATROPHY, FOCAL	1 (2%)			
#STOMACH	(46)	(23)	(49)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
#SMALL INTESTINE	(47)	(23)	(47)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		2 (4%)	
#PEYERS PATCH	(47)	(23)	(47)	(50)
HYPERPLASIA, NOS		4 (17%)		
#COLON	(46)	(22)	(46)	(49)
ULCER, FOCAL	1 (2%)			
PARASITISM		2 (9%)	4 (9%)	2 (4%)
URINARY SYSTEM				
#KIDNEY	(47)	(23)	(49)	(49)
GLOMERULONEPHRITIS, NOS	32 (68%)	4 (17%)	19 (39%)	
GLOMERULONEPHRITIS, FOCAL			3 (6%)	
INFLAMMATION, INTERSTITIAL	2 (4%)		2 (4%)	
PYELONEPHRITIS, ACUTE		1 (4%)		
PYELONEPHRITIS, CHRONIC		1 (4%)		
NEPHROSIS, NOS		10 (43%)		40 (82%)
POSTMORTEM CHANGE			1 (2%)	
CALCIFICATION, FOCAL		1 (4%)		
#KIDNEY/CORTEX	(47)	(23)	(49)	(49)
CYST, NOS	1 (2%)			
#KIDNEY/GLOMERULUS	(47)	(23)	(49)	(49)
INFLAMMATION, MEMBRANOUS	7 (15%)		4 (8%)	

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
#KIDNEY/TUBULE	(47)	(23)	(49)	(49)
NECROSIS, NOS		1 (4%)		
ENDOCRINE SYSTEM				
#PITUITARY	(46)	(21)	(45)	(40)
CYST, NOS			2 (4%)	
HEMORRHAGIC CYST		1 (5%)		
NECROSIS, FOCAL	1 (2%)			
HYPERTROPHY, FOCAL	1 (2%)			
HYPERPLASIA, FOCAL	6 (13%)	1 (5%)	1 (2%)	
#ADRENAL	(47)	(23)	(49)	(50)
METAMORPHOSIS FATTY	1 (2%)		2 (4%)	
HYPERPLASIA, FOCAL	1 (2%)			
#ADRENAL CORTEX	(47)	(23)	(49)	(50)
HEMORRHAGE	2 (4%)			
NODULE	4 (9%)			
DEGENERATION, NOS	2 (4%)			
NECROSIS, FOCAL	1 (2%)			
METAMORPHOSIS FATTY	1 (2%)		2 (4%)	
PIGMENTATION, NOS	1 (2%)			
HYPERPLASIA, NODULAR	1 (2%)			
HYPERPLASIA, FOCAL	9 (19%)		1 (2%)	
#ADRENAL MEDULLA	(47)	(23)	(49)	(50)
HYPERPLASIA, NODULAR	1 (2%)			
HYPERPLASIA, FOCAL	2 (4%)			
#THYROID	(45)	(21)	(45)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
HYPERPLASIA, FOCAL	3 (7%)			
HYPERPLASIA, C-CELL	1 (2%)	3 (14%)		1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(23)	(49)	(50)
GALACTOCELE	6 (13%)	1 (4%)	9 (18%)	
MULTIPLE CYSTS				1 (2%)
INFLAMMATION, ACUTE			1 (2%)	
HYPERPLASIA, NOS	1 (2%)	1 (4%)	12 (24%)	
HYPERPLASIA, CYSTIC	1 (2%)			2 (4%)

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
LACTATION		9 (39%)		
*CLITORAL GLAND	(48)	(23)	(49)	(50)
ABSCESS, NOS	1 (2%)			
HYPERPLASIA, PAPILLARY			1 (2%)	
*VAGINA	(48)	(23)	(49)	(50)
POLYP	1 (2%)			
#UTERUS	(46)	(23)	(47)	(49)
HYDROMETRA	2 (4%)		2 (4%)	3 (6%)
INFLAMMATION, NOS				1 (2%)
PYOMETRA		3 (13%)		1 (2%)
INFLAMMATION, ACUTE				1 (2%)
FIBROSIS				1 (2%)
HYPERPLASIA, ADENOMATOUS				2 (4%)
#UTERUS/ENDOMETRIUM	(46)	(23)	(47)	(49)
CYST, NOS			3 (6%)	
INFLAMMATION, NOS		1 (4%)		
INFLAMMATION, SUPPURATIVE				7 (14%)
INFLAMMATION, ACUTE	6 (13%)		20 (43%)	4 (8%)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
INFLAMMATION, CHRONIC		1 (4%)		
HYPERTROPHY, NOS	1 (2%)			
HYPERPLASIA, NOS	1 (2%)	1 (4%)		
HYPERPLASIA, FOCAL			1 (2%)	
HYPERPLASIA, CYSTIC	7 (15%)	1 (4%)	7 (15%)	2 (4%)
#OVARY/OVIDUCT	(46)	(23)	(47)	(49)
RETENTION FLUID	1 (2%)			
INFLAMMATION, SUPPURATIVE	1 (2%)			
INFLAMMATION, ACUTE	1 (2%)	1 (4%)		
ABSCESS, NOS		1 (4%)		
#OVARY	(47)	(22)	(47)	(49)
CYST, NOS	4 (9%)	3 (14%)	1 (2%)	1 (2%)
PAROVARIAN CYST			1 (2%)	
HYPERPLASIA, NOS	1 (2%)			
NERVOUS SYSTEM				
#BRAIN	(47)	(23)	(48)	(48)
HYDROCEPHALUS, NOS		1 (4%)		

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

TABLE C2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 02-0030	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE 02-0025	HIGH DOSE 02-0087
HEMORRHAGE		1 (4%)		
CALCIFICATION, FOCAL		1 (4%)		
SPECIAL SENSE ORGANS				
*EYE	(48)	(23)	(49)	(50)
CATARACT	1 (2%)		1 (2%)	
*EYE/CORNEA	(48)	(23)	(49)	(50)
INFLAMMATION, INTERSTITIAL			1 (2%)	
*EYE/RETINA	(48)	(23)	(49)	(50)
DEGENERATION, NOS	1 (2%)		1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PLEURA	(48)	(23)	(49)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
HYPERPLASIA, ADENOMATOUS				1 (2%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(48)	(23)	(49)	(50)
ECSTMOPTEM CHANGE			1 (2%)	
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/NO HISTO	1			
AUTOLYSIS/NO NECROPSY	2	2	1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2,4-DINITROTOLUENE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	46	46	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	46	45	48	49
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(46)	(48)	(49)
GRANULOMA, PYOGENIC	1 (2%)			
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(46)	(45)	(48)	(48)
INFLAMMATION, FOCAL	1 (2%)			
#LUNG	(46)	(45)	(48)	(48)
EMPHYSEMA, NOS	1 (2%)			
EDEMA, NOS			1 (2%)	
HEMORRHAGE	1 (2%)			
INFLAMMATION, INTERSTITIAL	7 (15%)			
ARTERIOSCLEROSIS, NOS		1 (2%)		
#LUNG/ALVEOLI	(46)	(45)	(48)	(48)
INFLAMMATION, NOS	1 (2%)			
HEMATOPOIETIC SYSTEM				
#SPLEEN	(46)	(45)	(47)	(44)
FIBROSIS		1 (2%)		
HYPERPLASIA, HEMATOPOIETIC			1 (2%)	
HYPERPLASIA, ERYTHROID	1 (2%)			
HYPERPLASIA, RETICULUM CELL		3 (7%)		
HEMATOPOIESIS	1 (2%)	1 (2%)		1 (2%)
#LYMPH NODE	(34)	(35)	(40)	(34)
HEMORRHAGE	1 (3%)			
HYPERPLASIA, NOS	1 (3%)			
HYPERPLASIA, PLASMA CELL				1 (3%)
#MANDIBULAR L. NODE	(34)	(35)	(40)	(34)
HYPERPLASIA, RETICULUM CELL	1 (3%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
#MESENTERIC L. NODE	(34)	(35)	(40)	(34)
THROMBOSIS, NOS	1 (3%)			
CONGESTION, NOS				1 (3%)
HEMORRHAGE	1 (3%)		1 (3%)	
HYPERPLASIA, NOS				2 (6%)
HYPERPLASIA, PLASMA CELL				1 (3%)
HYPERPLASIA, RETICULUM CELL			1 (3%)	
HYPERPLASIA, LYMPHOID				1 (3%)
CIRCULATORY SYSTEM				
#ENDOCARDIUM	(46)	(44)	(48)	(47)
INFLAMMATION PROLIFERATIVE	1 (2%)			
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(37)	(43)	(44)	(47)
PERIVASCULITIS	5 (14%)			
#LIVER	(46)	(45)	(47)	(48)
INFLAMMATION, NECROTIZING	1 (2%)			
DEGENERATION, NOS		1 (2%)		
NECROSIS, FOCAL	2 (4%)			
NECROSIS, HEMORRHAGIC	1 (2%)			
METAMORPHOSIS FATTY	8 (17%)	3 (7%)	1 (2%)	
HYPERPLASIA, NODULAR			1 (2%)	
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	1 (2%)			2 (4%)
#LIVER/PERIPORTAL	(46)	(45)	(47)	(48)
INFLAMMATION, NOS		1 (2%)		
#LIVER/KUPFFER CELL	(46)	(45)	(47)	(48)
HYPERPLASIA, NOS		2 (4%)		
*BILE DUCT	(46)	(46)	(48)	(49)
INFLAMMATION, NOS		1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)			
#PANCREAS	(44)	(44)	(46)	(42)
DILATATION/DUCTS			1 (2%)	
INFLAMMATION, FOCAL	2 (5%)			
INFLAMMATION, INTERSTITIAL	1 (2%)			

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

TABLE D1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
INFLAMMATION, ACUTE/CHRONIC			2 (4%)	
HYPERPLASIA, FOCAL	2 (5%)			
#PANCREATIC DUCT	(44)	(44)	(46)	(42)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
#PANCREATIC ACINUS	(44)	(44)	(46)	(42)
ATROPHY, FOCAL	1 (2%)			
HYPERPLASIA, FOCAL			1 (2%)	
#STOMACH	(45)	(42)	(45)	(45)
INFLAMMATION, NOS	2 (4%)			
INFLAMMATION, ACUTE	1 (2%)			1 (2%)
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, EPITHELIAL	1 (2%)			
HYPERPLASIA, FOCAL		1 (2%)		
HYPERPLASIA, ADENOMATOUS	2 (4%)			
#GASTRIC SEROSA	(45)	(42)	(45)	(45)
PERIARTERITIS				1 (2%)
#ILEUM	(46)	(43)	(45)	(45)
ABSCESS, NOS			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(46)	(45)	(48)	(48)
CALCULUS, NOS		20 (44%)		
GLOMERULONEPHRITIS, NOS	3 (7%)			
INFLAMMATION, INTERSTITIAL	7 (15%)	5 (11%)	16 (33%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)		
PERIVASCULITIS		2 (4%)		
ARTERIOSCLEROSIS, NOS		1 (2%)		
NEPHROSIS, NOS		1 (2%)		
HYPERPLASIA, TUBULAR CELL		2 (4%)		
METAPLASIA, OSSEOUS	1 (2%)			
#KIDNEY/TUBULE	(46)	(45)	(48)	(48)
DEGENERATION, NOS		1 (2%)		
METAMORPHOSIS FATTY		9 (20%)		
#KIDNEY/PELVIS	(46)	(45)	(48)	(48)
INFLAMMATION, ACUTE/CHRONIC	3 (7%)		14 (29%)	
#URINARY BLADDER	(46)	(44)	(46)	(45)
PERIVASCULAR CUFFING				1 (2%)

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

TABLE D1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
HYPERPLASIA, EPITHELIAL	2 (4%)			
ENDOCRINE SYSTEM				
#PITUITARY	(39)	(36)	(33)	(42)
CYST, NOS			1 (3%)	
HYPERPLASIA, FOCAL	1 (3%)		1 (3%)	
#ADRENAL	(44)	(43)	(46)	(45)
NECROSIS, FOCAL	1 (2%)			
HYPERPLASIA, FOCAL			2 (4%)	
#ADRENAL CORTEX	(44)	(43)	(46)	(45)
HYPERPLASIA, NOS	2 (5%)			
HYPERPLASIA, FOCAL	14 (32%)		25 (54%)	
#PARATHYROID	(17)	(18)	(21)	(20)
CYST, NOS	1 (6%)			
REPRODUCTIVE SYSTEM				
*PREPUTIAL GLAND	(46)	(46)	(48)	(49)
INFLAMMATION, ACUTE				1 (2%)
#PROSTATE	(46)	(44)	(45)	(47)
HYPERPLASIA, EPITHELIAL	1 (2%)			
#TESTIS	(46)	(45)	(47)	(47)
MINERALIZATION			1 (2%)	
DEGENERATION, NOS			4 (9%)	
#TESTIS/TUBULE	(46)	(45)	(47)	(47)
DEGENERATION, NOS	3 (7%)	2 (4%)		
*EPIDIDYMIS	(46)	(46)	(48)	(49)
NECROSIS, NOS			1 (2%)	
NERVOUS SYSTEM				
*BRAIN	(46)	(45)	(46)	(45)
MINERALIZATION			18 (39%)	
SPECIAL SENSE ORGANS				
NONE				

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

TABLE D1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 05-0030	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE 05-0025	HIGH DOSE 05-0088
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(46)	(46)	(48)	(49)
PERIARTERITIS				1 (2%)
BODY CAVITIES				
*ABDOMINAL CAVITY	(46)	(46)	(48)	(49)
STEATITIS		1 (2%)		
NECROSIS, FAT	1 (2%)			
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(46)	(46)	(48)	(49)
RETICULUM CELL SARCOMA			1 (2%)	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2	8	1	28
ACCIDENTAL DEATH	3			
AUTO/NECROPSY/HISTO PERF			1	2
AUTO/NECROPSY/NO HISTO		1		
AUTOLYSIS/NO NECROPSY	1	4	2	1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 2,4-DINITROTOLUENE

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS MISSING			3	
ANIMALS NECROPSIED	47	46	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	46	46	50
INTEGUMENTARY SYSTEM				
*SKIN	(47)	(46)	(46)	(50)
FIBROSIS		1 (2%)		
FIBROSIS, FOCAL		1 (2%)		
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(46)	(45)	(46)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)	
METAPLASIA, NOS			1 (2%)	
#LUNG	(46)	(45)	(46)	(48)
CONGESTION, NOS			1 (2%)	
EDEMA, NOS			1 (2%)	
INFLAMMATION, FOCAL			1 (2%)	
INFLAMMATION, INTERSTITIAL	2 (4%)	2 (4%)		
PERIARTERITIS		1 (2%)		
#LUNG/ALVEOLI	(46)	(45)	(46)	(48)
EMPHYSEMA, NOS	1 (2%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(45)	(44)	(45)	(45)
HYPOPLASIA, NOS	1 (2%)			
MYELOFIBROSIS	1 (2%)		1 (2%)	
HYPERPLASIA, HEMATOPOIETIC			1 (2%)	
#SPLEEN	(45)	(43)	(44)	(48)
LYMPHOCYTOSIS	2 (4%)			
HYPERPLASIA, HEMATOPOIETIC			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
HYPERPLASIA, RETICULUM CELL		2 (5%)		
HYPERPLASIA, LYMPHOID	3 (7%)	4 (9%)	3 (7%)	
HEMATOPOIESIS	2 (4%)	1 (2%)	1 (2%)	1 (2%)
ERYTHROPOIESIS				3 (6%)
#MANDIBULAR L. NODE	(27)	(41)	(42)	(38)
INFLAMMATION, ACUTE			1 (2%)	
#MEDIASTINAL L.NODE	(27)	(41)	(42)	(38)
INFLAMMATION, ACUTE			1 (2%)	
#PANCREATIC L.NODE	(27)	(41)	(42)	(38)
HYPERPLASIA, RETICULUM CELL	1 (4%)			
#MESENTERIC L. NODE	(27)	(41)	(42)	(38)
INFLAMMATION, ACUTE FOCAL			1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)	
HYPERPLASIA, PLASMA CELL				1 (3%)
HYPERPLASIA, LYMPHOID				1 (3%)
CIRCULATORY SYSTEM				
#HEART/ATRIUM	(46)	(45)	(46)	(47)
CALCIFICATION, FOCAL	1 (2%)			
#MYOCARDIUM	(46)	(45)	(46)	(47)
CALCIFICATION, FOCAL		1 (2%)		
*PULMONARY ARTERY	(47)	(46)	(46)	(50)
HYPERPLASIA, NOS		1 (2%)		
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(29)	(43)	(44)	(48)
PERIVASCULITIS	1 (3%)			
#LIVER	(46)	(45)	(46)	(50)
MINERALIZATION			1 (2%)	
INFLAMMATION, NOS			1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)	1 (2%)
NECROSIS, FOCAL	1 (2%)		1 (2%)	
NECROSIS, COAGULATIVE	1 (2%)			
METAMORPHOSIS FATTY			1 (2%)	
HYPERPLASIA, NODULAR	2 (4%)			

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

TABLE D2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)	
HYPERPLASIA, DIFFUSE		1 (2%)		
ANGIECTASIS	1 (2%)			
#LIVER/PERIPORTAL	(46)	(45)	(46)	(50)
INFLAMMATION, NOS		1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)			
#LIVER/KUPFFER CELL	(46)	(45)	(46)	(50)
HYPERPLASIA, NOS		1 (2%)		
*BILE DUCT	(47)	(46)	(46)	(50)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, ACUTE/CHRONIC			3 (7%)	
INFLAMMATION, CHRONIC DIFFUSE				1 (2%)
#PANCREAS	(39)	(41)	(44)	(41)
INFLAMMATION, INTERSTITIAL				1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
FIBROSIS				1 (2%)
ATROPHY, FOCAL			2 (5%)	
#PANCREATIC DUCT	(39)	(41)	(44)	(41)
INFLAMMATION, NOS	1 (3%)			
#STOMACH	(45)	(42)	(44)	(41)
INFLAMMATION, NOS	3 (7%)			
ULCER, FOCAL			1 (2%)	
INFLAMMATION, ACUTE FOCAL			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)	
#SMALL INTESTINE	(45)	(43)	(42)	(44)
AMYLOIDOSIS			1 (2%)	
#PEYERS PATCH	(45)	(43)	(42)	(44)
HYPERPLASIA, NOS	1 (2%)			1 (2%)
#DUODENUM	(45)	(43)	(42)	(44)
ECTOPIA	1 (2%)			
#ILEUM	(45)	(43)	(42)	(44)
ABSCESS, NOS			1 (2%)	
*RECTUM	(47)	(46)	(46)	(50)
PROLAPSE				1 (2%)

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

TABLE D2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
URINARY SYSTEM				
#KIDNEY	(45)	(43)	(46)	(46)
GLOMERULONEPHRITIS, NOS	3 (7%)			
INFLAMMATION, INTERSTITIAL	9 (20%)	3 (7%)	20 (43%)	1 (2%)
GLOMERULONEPHRITIS, CHRONIC				1 (2%)
PERIVASCULITIS		4 (9%)		
#KIDNEY/GLOMERULUS	(45)	(43)	(46)	(46)
AMYLOIDOSIS		1 (2%)		
#KIDNEY/PELVIS	(45)	(43)	(46)	(46)
INFLAMMATION, NOS			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	1 (2%)	8 (17%)	
#URINARY BLADDER	(42)	(41)	(42)	(44)
INFLAMMATION, ACUTE/CHRONIC			9 (21%)	
HYPERPLASIA, EPITHELIAL			3 (7%)	
ENDOCRINE SYSTEM				
#PITUITARY	(37)	(37)	(38)	(34)
HYPERPLASIA, FOCAL			7 (18%)	
#ADRENAL	(44)	(43)	(42)	(45)
CONGESTION, NOS			1 (2%)	
#ADRENAL CORTEX	(44)	(43)	(42)	(45)
NODULE	1 (2%)			
HYPERPLASIA, NOS	2 (5%)		3 (7%)	
HYPERPLASIA, FOCAL			36 (86%)	
#THYROID	(44)	(30)	(40)	(42)
COLLOID CYST			1 (3%)	
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
HYPERPLASIA, FOCAL			1 (3%)	
REPRODUCTIVE SYSTEM				
#UTERUS	(43)	(43)	(44)	(43)
HYDROMETRA	4 (9%)	4 (9%)		1 (2%)
INFLAMMATION, SUPPURATIVE				2 (5%)
INFLAMMATION, ACUTE				2 (5%)

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

TABLE D2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
ABSCESS, NOS			2 (5%)	
NECROSIS, FOCAL			1 (2%)	
METAPLASIA, SQUAMOUS	1 (2%)			
#UTERUS/ENDOMETRIUM	(43)	(43)	(44)	(43)
CYST, NOS		2 (5%)		
INFLAMMATION, NOS	1 (2%)			1 (2%)
INFLAMMATION, SUPPURATIVE				3 (7%)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
HYPERPLASIA, NOS		1 (2%)	5 (11%)	1 (2%)
HYPERPLASIA, FOCAL			1 (2%)	
HYPERPLASIA, CYSTIC	33 (77%)	35 (81%)	34 (77%)	23 (53%)
#OVARY	(44)	(41)	(44)	(37)
CYST, NOS	5 (11%)	1 (2%)	5 (11%)	2 (5%)
HEMORRHAGIC CYST			1 (2%)	
INFLAMMATION, SUPPURATIVE				1 (3%)
INFLAMMATION, ACUTE				2 (5%)
INFLAMMATION, CHRONIC				4 (11%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(47)	(46)	(46)	(50)
SYNECHIA, NOS			1 (2%)	
CATARACT			1 (2%)	
*EYE/RETINA	(47)	(46)	(46)	(50)
DEGENERATION, NOS			1 (2%)	
*EYE/CRYSTALLINE LENS	(47)	(46)	(46)	(50)
SYNECHIA, ANTERIOR			1 (2%)	
*HARDERIAN GLAND	(47)	(46)	(46)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*VERTEBRA	(47)	(46)	(46)	(50)
OSTEOSCLEROSIS		1 (2%)		

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

TABLE D2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 06-0030	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE 06-0025	HIGH DOSE 06-0088
BODY CAVITIES				
*PLEURA	(47)	(46)	(46)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)			
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(47)	(46)	(46)	(50)
ECSINOPHIL CHANGE			1 (2%)	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	1		6
ANIMAL MISSING/NO NECROPSY			3	
AUTO/NECROPSY/HISTO PERF	1	2	1	6
AUTOLYSIS/NO NECROPSY	3	4	1	

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

Review of the Bioassay of 2,4-Dinitrotoluene* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

January 18, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which 2,4-Dinitrotoluene was reviewed.

The primary reviewer said that he agreed with the staff's conclusion that, under the conditions of test, 2,4-Dinitrotoluene induced benign tumors in rats but showed no evidence of carcinogenicity in either sex of mice. He noted that a different strain of mouse was used for the prechronic study than for the chronic phase. After briefly describing the experimental design, the primary reviewer said that the study was deficient in that the maximum tolerated dose was not adequately defined during the subchronic studies. Therefore, it had to be approximated for the chronic phase. He opined that the hemangiosarcomas in the treated rats may be biologically significant. In summary, the primary reviewer said that the tumors in the treated rats must be viewed with concern, especially since the maximum tolderated dose may not have been attained. He felt that the data did not allow an assessment of human risk.

The secondary reviewer noted that 2,4-Dinitrotoluene is an intermediate in the production of dyes. He added

that there may be considerable human exposure from residues of 2,4-Dinitrotoluene in dye products. He continued that there may be a potential for human risk because of the increased tumor incidence seen in the treated rats. He suggested that another study be done, possibly using another species and route of exposure.

A Program staff member commented that there was a treatment-related biological effect produced by 2,4-Dinitrotoluene in the rats. He added that the dose levels in the mice and male rats approached the maximum tolerated dose, as suggested by the growth curves.

A Subgroup member suggested that the biological activity of 2,4-Dinitrotoluene may be due to its conversion to the diamine compound. The rate of its enzymatic conversion may limit its activity. He added that skin exposure would be a more appropriate route if 2,4-Dinitrotoluene is to be retested. Another Subgroup member suggested that the staff consult with Dr. Harris at North Carolina and Dr. Peters at Harvard, both of whom are doing epidemiologic studies among workers exposed in the TDI process. The consultation would be helpful in considering the need to retest 2,4-Dinitrotoluene.

It was moved that, in view of the significant number of benign tumors in the treated rats and widespread human exposure, 2,4-Dinitrotoluene be considered for retest. The motion was seconded and approved unanimously.

Members Present Were:

Arnold Brown (Acting Chairman), Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph Highland, Environmental Defense Fund
Charles Kensler, Arthur D. Little Company
Verald K. Rowe, Dow Chemical, U.S.A.
Sheldon Samuels, Industrial Union Department, AFL-CIO
Louise Strong, University of Texas Health Sciences Center
Sidney Wolfe, Health Research Group

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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