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**BIOASSAY OF
TRIMETHYLTHIOUREA
FOR POSSIBLE CARCINOGENICITY**

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health



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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF TRIMETHYLTHIOUREA
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CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of trimethylthiourea conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of trimethylthiourea was conducted by Litton Bionetics, Inc., Bethesda, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4,5) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed by Dr. F. M. Garner (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. F. M. Garner (4), and the diagnoses included in this report represent the interpretation of this pathologist. 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 (8,9) and Mr. R. M. Helfand (8), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (10).

This report was prepared at METREK, a Division of The MITRE Corporation (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), task leader Ms. P. Walker (8), senior biologist Mr. M. Morse (8), biochemist Mr. S. C. Drill (8), chemist Dr. N. Zimmerman (8), and technical editor Ms. P. A. Miller (8). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,11), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,12), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. G. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of trimethylthiourea was conducted using Fischer 344 rats and B6C3F1 mice. A mixture containing 80 percent trimethylthiourea and 15 percent dimethylthiourea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of trimethylthiourea were, respectively, 500 and 250 ppm for rats and 1000 and 500 ppm for mice. The compound was administered in the diet for 77 weeks, followed by an observation period of 29 weeks for rats and 14 weeks for mice.

There were no significant positive associations between the dosage of trimethylthiourea administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. For high dose female rats and for dosed mice of both sexes, compound-related mean body weight depression was observed, indicating that the dosages of trimethylthiourea administered to these animals may have approximated the maximum tolerated dosages. Since no mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of trimethylthiourea to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

The incidences of follicular-cell carcinomas of the thyroid in female rats were dose-related, and there was a significant difference between the incidences in the high dose and control. This same relationship was established for the combination of follicular-cell carcinomas and follicular-cell adenomas in female rats.

Under the conditions of this bioassay, dietary administration of trimethylthiourea was carcinogenic in female Fischer 344 rats, inducing follicular-cell carcinomas of the thyroid. There was not sufficient evidence for the carcinogenicity of the compound in male Fischer 344 rats or in B6C3F1 mice of either sex.

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I. INTRODUCTION

Trimethylthiourea (Figure 1) (NCI No. C02186), useful in a wide variety of industrial applications, was selected for bioassay by the National Cancer Institute because it is a derivative of thiourea, a liver carcinogen in Osborne-Mendel rats (Radomski et al., 1965).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is trimethylthiourea.* It is also called N,N,N'-trimethylthiourea and 1,1,3-trimethyl-2-thiourea.

Trimethylthiourea can be used to accelerate the vulcanization of various types of rubber, including polychloropropene and diene-nitrile rubber (Langlade, 1976; Starmer, 1976a,b). Trimethylthiourea has also been used as a component of adhesives (Hauser and Malofsky, 1974; Ohashi et al., 1971) and degradable polyethylene films (Kirkpatrick, 1973); as an intermediate in the synthesis of substituted acyl derivatives of α -aminoacylpenicillins (Rosati, 1973) and phthalocyanine pigments (Inuzuka et al., 1976); and as an inhibitor of ozone fading of dyed polyamides (Lofquist and Saunders, 1974); in a mixture with an aqueous acid to emboss nylon carpet by shrinking certain areas (Palmer and Conger, 1974); and to prepare an antishrinkage treatment for keratinous fibers (e.g., wool) (Vandenberg and Willis, 1974).

Specific production data for trimethylthiourea are not available; however, this compound is produced in commercial quantities (in excess

*The CAS registry number is 2489-77-2.

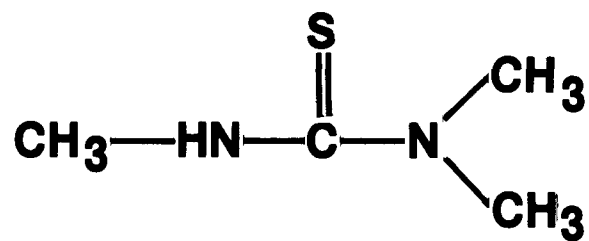


FIGURE 1
CHEMICAL STRUCTURE OF TRIMETHYLTHIOUREA

of 1000 pounds or \$1000 in value annually) by one U.S. company (Stanford Research Institute, 1977).

A potential for exposure to trimethylthiourea may exist among workers in a variety of industries, including the chemical, elastomer, textile and dye, and pharmaceutical manufacturing industries.

II. MATERIALS AND METHODS

A. Chemicals

Trimethylthiourea was purchased from R. T. Vanderbilt Co., Inc., Norwalk, Connecticut. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. According to the manufacturer's specifications, the product was 80 percent trimethylthiourea, 15 percent 1,3-dimethyl-2-thiourea and 5 percent Zeolex 80. Thin-layer chromatography was performed utilizing two solvent systems (i.e., benzene:acetone and chloroform:acetone). Each plate was visualized with ultraviolet and visible light, iodine vapor, and Grote's reagent spray. Two spots were apparent on each plate, one major spot and one minor spot that was positive to Grote's reagent and identified as 1,3-dimethyl-2-thiourea. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected on the basis of the structure of the compound.

Throughout this report the term trimethylthiourea is used to represent this material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox[®] (Allied Mills, Inc., Chicago, Illinois). Trimethylthiourea was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the ground feed using a mortar and

pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 1250 and 625 ppm of trimethylthiourea were analyzed spectrophotometrically. The mean result immediately after preparation was 106.7 percent of theoretical (ranging from 97.6 to 124.4 percent), including correction for the analytical method of recovery used. After 10 days, at ambient room temperature, the mean result was 102.9 percent of theoretical (ranging from 100.3 to 108.0 percent), including correction for the analytical method of recovery used.

C. Animals

Two animal species, Fischer 344 rats and B6C3F1 mice, were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined for visible signs of disease or parasites. Obviously ill or runted animals were culled. The remaining animals were quarantined for 2 weeks prior to initiation of test.

Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 22° to 26°C and the relative humidity was maintained between 45 and 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

All rats were housed four per cage by sex and all mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri[®] hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device that was checked daily

for diluting accuracy. Water bottles were changed and washed twice weekly and sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox[®] meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* Michler's ketone (90-94-8); p-chloroaniline (106-47-8); and p-nitrosodiphenylamine (156-10-5).

All dosed and control mice were housed in a room with other mice receiving diets containing Michler's ketone (90-94-8); 4,4'-methylene-bis(N,N-dimethyl)-benzenamine (101-61-1); 1-phenyl-2-thiourea (103-85-5); 3-chloro-p-toluidine (95-74-9); dibutyltin diacetate (1067-33-0); 2-nitro-p-phenylenediamine (5307-14-2); p-chloroaniline (106-47-8); 5-chloro-o-toluidine (95-79-4); and p-aminodiphenylamine (2198-59-6).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of trimethylthiourea for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among six groups, each consisting of five males and five females. Trimethylthiourea was incorporated into

* CAS registry numbers are given in parentheses.

the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 315, 680, 1465, 3155, and 6800 ppm. The remaining rat group served as a control group, receiving only the basal laboratory diet.

Mice were distributed among nine groups, each consisting of five males and five females. Trimethylthiourea was incorporated into the basal laboratory diet and supplied ad libitum to seven of the nine mouse groups in concentrations of 370, 550, 810, 1180, 1740, 2550 and 5500 ppm. The remaining two mouse groups served as control groups, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the observation period, all survivors were sacrificed and necropsied.

At the end of the subchronic test, mean body weight gain among male rats dosed with 315 ppm was 2 percent greater than the mean body weight gain of their controls, while female rats receiving the same concentration displayed a mean body weight gain 4 percent greater than that of their controls. Mean body weight gain among male rats dosed with 680 ppm was 15 percent less than that of their controls, while female rats receiving the same concentrations displayed a mean

body weight gain 11 percent less than that of their controls. No deaths were reported among male or female rats. The high concentration selected for administration to dosed rats in the chronic bioassay was 500 ppm.

At the end of the subchronic test, mean body weight gain among male mice dosed with 810 ppm was 3 percent less than the mean body weight gain of their controls, while female mice receiving the same concentration displayed a mean body weight gain 6 percent less than that of their controls. Mean body weight gain among male mice dosed with 1180 ppm was 14 percent less than that of their controls, while female mice receiving the same concentration displayed a mean body weight gain 10 percent less than that of their controls. No deaths were reported among male or female mice. The high concentration selected for administration to dosed mice in the chronic bioassay was 1000 ppm.

F. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated, and were placed on test simultaneously. The dietary concentrations of trimethylthiourea utilized for rats were 500 and

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
TRIMETHYLTHIOUREA FEEDING EXPERIMENT

	<u>INITIAL GROUP SIZE</u>	<u>TRIMETHYLTHIOUREA CONCENTRATION^a</u>	<u>OBSERVATION PERIOD</u>	
			<u>TREATED (WEEKS)</u>	<u>UNTREATED (WEEKS)</u>
<u>MALE</u>				
CONTROL	20	0	0	106
LOW DOSE	50	250 0	77	29
HIGH DOSE	50	500 0	77	29
<u>FEMALE</u>				
CONTROL	20	0	0	106
LOW DOSE	50	250 0	77	29
HIGH DOSE	50	500 0	77	29

^aConcentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
 TRIMETHYLTHIOUREA FEEDING EXPERIMENT

	<u>INITIAL GROUP SIZE</u>	<u>TRIMETHYLTHIOUREA CONCENTRATION^a</u>	<u>OBSERVATION PERIOD</u>	
			<u>TREATED (WEEKS)</u>	<u>UNTREATED (WEEKS)</u>
<u>MALE</u>				
CONTROL	20	0	0	91
LOW DOSE	50	500 0	77	14
HIGH DOSE	50	1000 0	77	14
<u>FEMALE</u>				
CONTROL	20	0	0	91
LOW DOSE	50	500 0	77	14
HIGH DOSE	50	1000 0	77	14

^aConcentrations given in parts per million.

250 ppm. Throughout this report, those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed rats were supplied with feed containing trimethylthiourea for 77 weeks followed by a 29-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated, and were placed on test simultaneously. The concentrations of trimethylthiourea utilized for mice were 1000 and 500 ppm. Throughout this report, those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed mice were supplied with feed containing trimethylthiourea for 77 weeks followed by a 14 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. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group. Body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and once a month for the remainder of the bioassay.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed

when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide asphyxiation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of all major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

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 (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, tunica vaginalis, and ovary.

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 recorded in each group at the time that the test was initiated.

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) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when 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.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

No evidence of mean body weight depression, in relation to controls, was apparent in dosed male rats. The mean body weight of high dose female rats was depressed relative to the control group (Figure 2).

No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and trimethylthiourea-dosed groups are shown in Figure 3. For both males and females, the Tarone test did not indicate a significant positive association between dosage and mortality.

There were adequate numbers of male rats at risk from late-developing tumors, as 46/50 (92 percent) of the high dose, 45/50 (90 percent) of the low dose and 18/20 (90 percent) of the control group survived on test for at least 100 weeks.

For females, with 32/50 (64 percent) of the high dose, 38/50 (76 percent) of the low dose, and 17/20 (85 percent) of the control group alive on test until the termination of the study, there were adequate numbers at risk from late-developing tumors.

C. Pathology

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

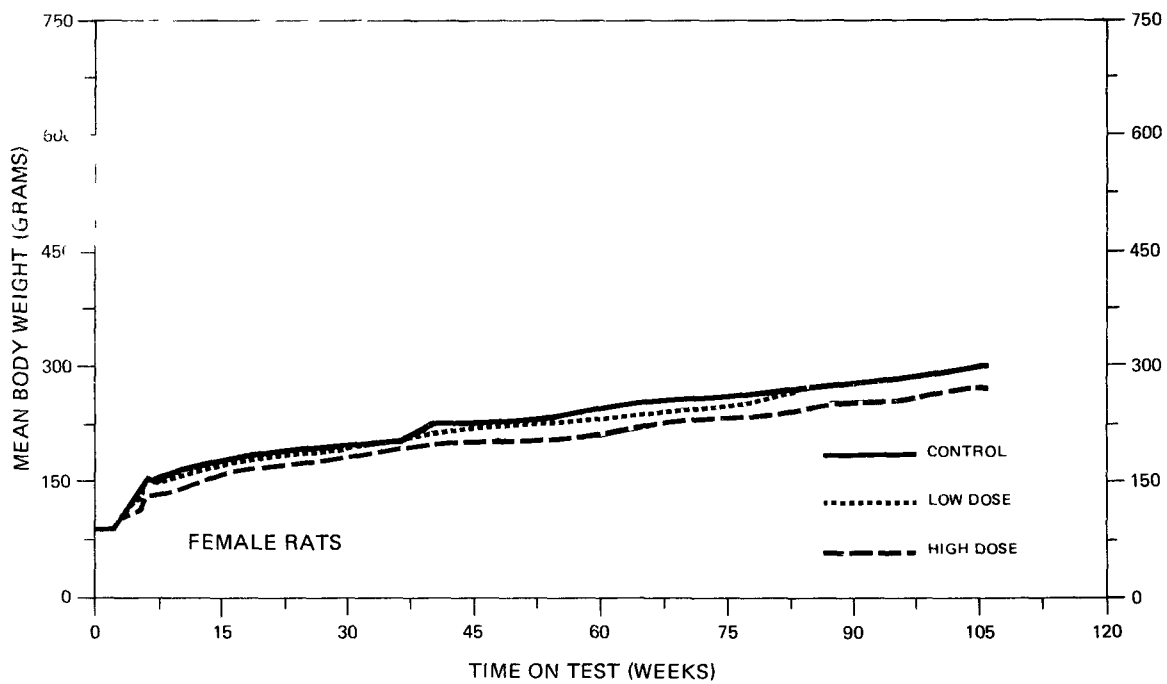
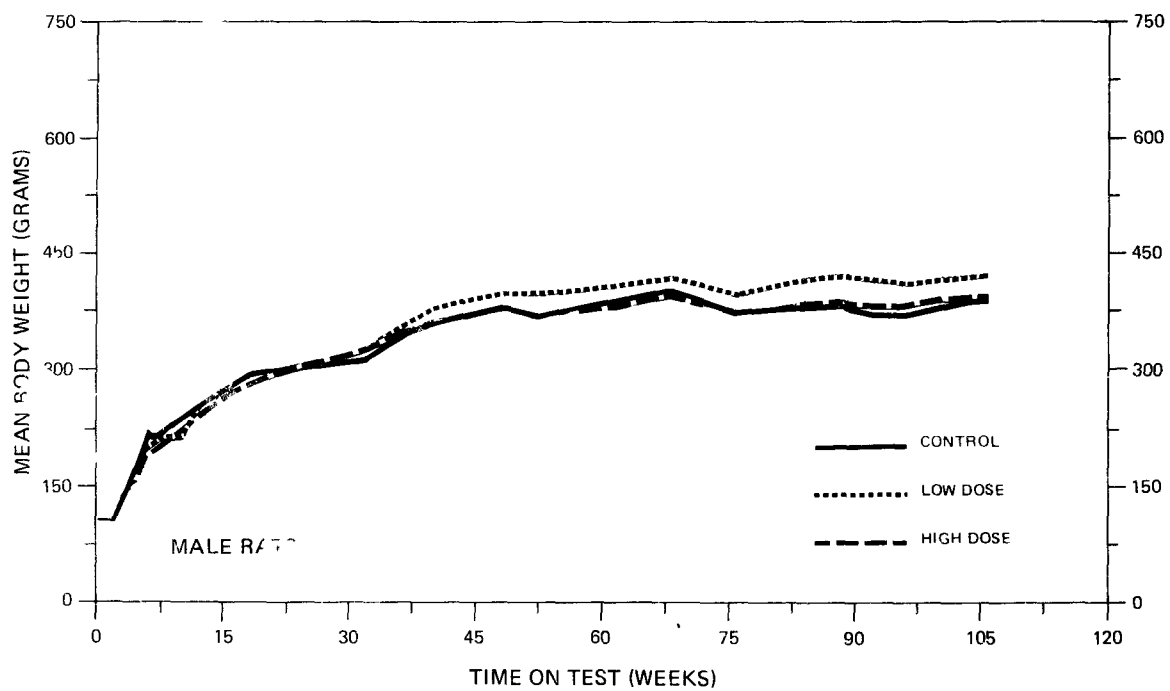


FIGURE 2
GROWTH CURVES FOR TRIMETHYLTHIOUREA CHRONIC STUDY RATS

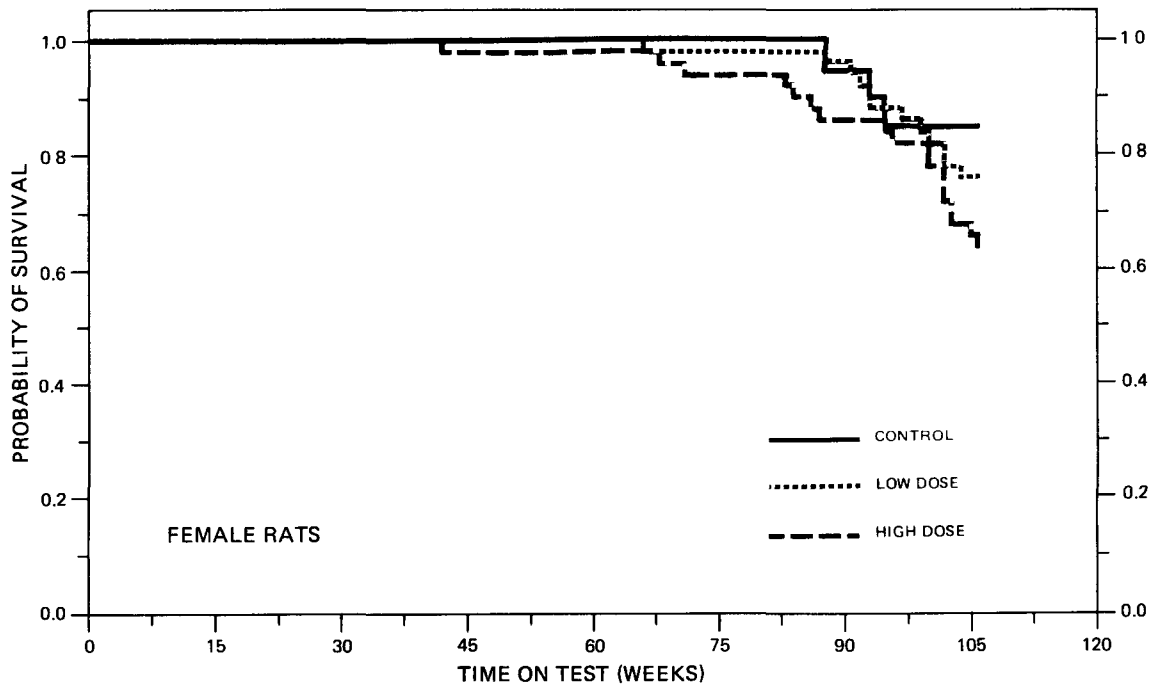
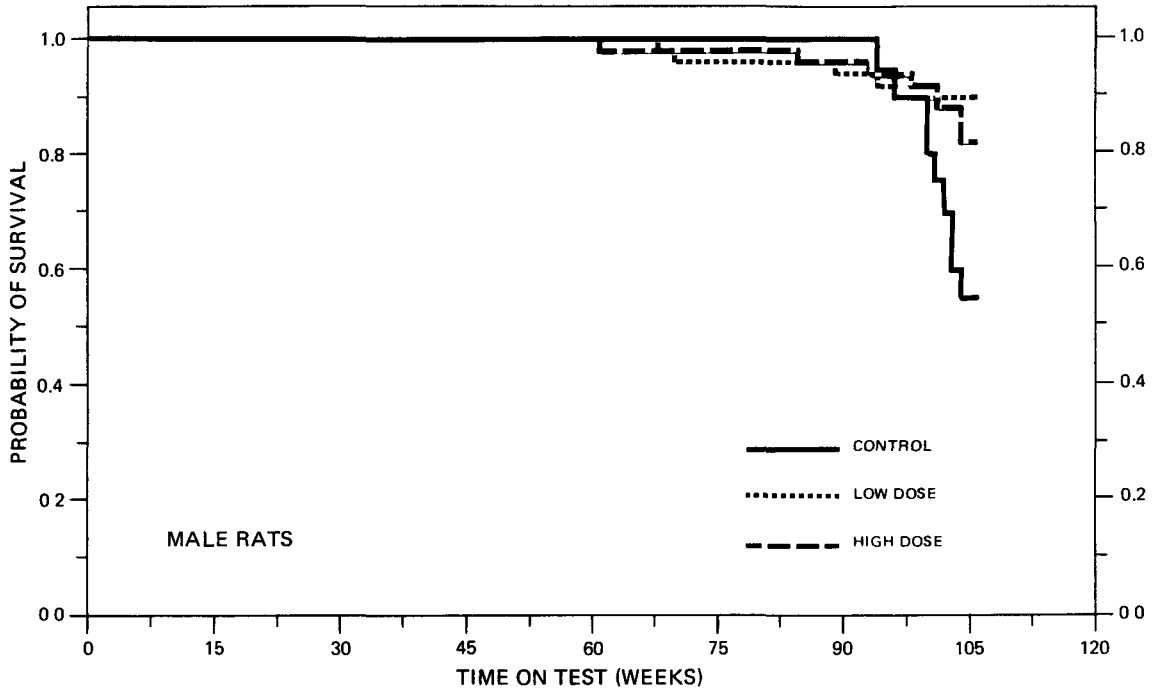


FIGURE 3
SURVIVAL COMPARISONS OF TRIMETHYLTHIOUREA CHRONIC STUDY RATS

A variety of neoplasms was observed in both the dosed and control groups. Each of these types has been encountered previously as a spontaneous lesion in this strain of rat.

The lesions of interest observed at necropsy were those associated with the thyroid, particularly in the dosed females. The incidences of follicular and C-cell adenomas and carcinomas of the thyroid gland were observed to be higher in the dosed animals than in the controls. The tumors were most common in the high dose females as shown in the following table:

	<u>Control</u>	<u>Low Dose</u>	<u>High Dose</u>
<u>Males</u>			
<u>Number of Animals with Thyroid Examined Histopathologically</u>	(17)	(37)	(39)
Follicular-Cell Adenoma	0	0	0
Follicular-Cell Carcinoma	0	1(3%)	3(8%)
C-Cell Adenoma	2(12%)	1(3%)	0
C-Cell Carcinoma	0	0	2(5%)
<u>Females</u>			
<u>Number of Animals with Thyroid Examined Histopathologically</u>	(17)	(38)	(47)
Follicular-Cell Adenoma	0	0	9(19%)
Follicular-Cell Carcinoma	0	1(3%)	14(30%)
C-Cell Adenoma	1(6%)	2(5%)	2(4%)
C-Cell Carcinoma	0	1(3%)	2(4%)

Adenomas were recognized as well-defined nodules of either para-follicular or follicular cells. Many of the latter had papillary or

cystic features as well. Features distinguishing carcinomas from adenomas include capsular invasion, increased mitotic activity, hyperchromatism, loss of cell polarity to basement membranes, piling up of cells, and, in the follicular-cell carcinomas, a tendency toward medullary rather than acinar proliferation. Metastases were observed in only one rat; however, local invasion from thyroid neoplasms did occur.

The usual variety of nonneoplastic lesions was present in both dosed and control animals. Such lesions have been encountered previously in the Fischer 344 rat and are considered to be spontaneous. Mortality toward the end of the study exceeded 10 percent in all groups. Most deaths were due to neoplasia complicated by chronic murine pneumonia.

The results of this pathologic examination indicated that trimethylthiourea was carcinogenic in female Fischer 344 rats at the doses given, inducing neoplasms of the thyroid.

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 is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or trimethylthiourea-dosed groups and where such tumors were observed in at least 5 percent of the group.

TABLE 3
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RAT TREATED WITH TRIMETHYLTHIOUREA^a

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	2/20(0.10)	4/50(0.08)	5/50(0.10)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.800	1.000
Lower Limit	---	0.128	0.184
Upper Limit	---	8.436	10.007
Weeks to First Observed Tumor	94	68	101
Thyroid: Follicular-Cell Carcinoma ^b	0/17(0.00)	1/37(0.03)	3/39(0.08)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.026	0.276
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	93
Thyroid: C-Cell Carcinoma ^b	0/17(0.00)	0/37(0.00)	2/39(0.05)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	---	Infinite
Lower Limit	---	---	0.135
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	106

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma or C-Cell Adenoma ^b	2/17(0.12)	1/37(0.03)	2/39(0.05)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.230	0.436
Lower Limit	---	0.004	0.035
Upper Limit	---	4.175	5.686
Weeks to First Observed Tumor	106	106	106
Testis: Interstitial-Cell Tumor ^b	18/20(0.90)	42/50(0.84)	45/49(0.92)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.933	1.020
Lower Limit	---	0.816	0.895
Upper Limit	---	1.226	1.256
Weeks to First Observed Tumor	100	94	93

^aTreated groups received doses of 250 or 500 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

^dThe 95% confidence interval on 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 TRIMETHYLTHIOUREA^a

TOPOGRAPHY;MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	0/20(0.00)	1/50(0.02)	3/50(0.06)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.022	0.250
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	100
<hr/>			
Liver: Hepatocellular Carcinoma or Neoplastic Nodule ^b	2/20(0.10)	2/49(0.04)	0/49(0.00)
P Values ^c	P = 0.041(N)	N.S.	N.S.
Relative Risk (Control) ^d	---	0.408	0.000
Lower Limit	---	0.032	0.000
Upper Limit	---	5.381	1.372
Weeks to First Observed Tumor	106	93	---
<hr/>			
Pituitary: Chromophobe Adenoma ^b	2/18(0.11)	8/44(0.18)	11/47(0.23)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.636	2.106
Lower Limit	---	0.378	0.535
Upper Limit	---	14.919	18.439
Weeks to First Observed Tumor	95	93	84

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma ^b	0/17(0.00)	1/38(0.03)	14/47(0.30)
P Values ^c	P < 0.001	N.S.	P = 0.007
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.025	1.667
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	68
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma ^b	0/17(0.00)	1/38(0.03)	23/47(0.49)
P Values ^c	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend ^e	P = 0.015	---	---
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.025	2.873
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	106	68
Thyroid: C-Cell Carcinoma or C-Cell Adenoma ^b	1/17(0.06)	3/38(0.08)	4/47(0.09)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.342	1.447
Lower Limit	---	0.120	0.161
Upper Limit	---	68.559	69.681
Weeks to First Observed Tumor	95	99	84

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma ^b	1/20(0.05)	3/50(0.06)	6/50(0.12)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.200	2.400
Lower Limit	---	0.106	0.325
Upper Limit	---	61.724	108.021
Weeks to First Observed Tumor	88	106	95
Mammary Gland: Adenocarcinoma NOS or Papillary Adenocarcinoma ^b	2/20(0.10)	1/50(0.02)	0/50(0.00)
P Values ^c	P = 0.035(N)	N.S.	N.S.
Relative Risk (Control) ^d	---	0.200	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	3.681	1.345
Weeks to First Observed Tumor	95	88	---
Mammary Gland: Fibroadenoma, Adenocar- cinoma NOS, or Papillary Adenocarcinoma ^b	3/20(0.15)	4/50(0.08)	6/50(0.12)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.533	0.800
Lower Limit	---	0.102	0.195
Upper Limit	---	3.410	4.615
Weeks to First Observed Tumor	88	88	95

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp ^b	2/20(0.10)	0/50(0.00)	0/49(0.00)
P Values ^c	P = 0.025(N)	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.043	---	---
Relative Risk (Control) ^d	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.345	1.372
Weeks to First Observed Tumor	106	---	---

^aTreated groups received doses of 250 or 500 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

For females, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of follicular-cell carcinomas of the thyroid. The Fisher exact test comparing high dose to control was also significant ($P = 0.007$). Furthermore, the combined incidences of follicular-cell carcinomas or follicular-cell adenomas of the thyroid also were significant for both the Cochran-Armitage test ($P < 0.001$) and the high dose Fisher exact test ($P < 0.001$). Based upon these statistical results, the administration of trimethylthiourea was associated with the increased incidence of follicular-cell carcinomas of the thyroid in female Fischer 344 rats.

None of the statistical tests for any site in male rats indicated a significant positive association between chemical administration and tumor incidence.

In female rats, the Cochran-Armitage test indicated significant negative associations between dosage and the combined incidence of hepatocellular carcinomas or neoplastic nodules, between dosage and mammary gland adenocarcinomas, and between dosage and the incidence of endometrial stromal polyps. In all cases, however, the Fisher exact tests comparing high dose to control and low dose to control were not significant.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Both high and low dose male and female mice had severe mean body weight depression relative to the control group. Distinct dose-related mean body weight depression was observed in female mice after week 25 (Figure 4).

No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and trimethylthiourea-dosed groups are shown in Figure 5. For both male and female mice the Tarone test did not indicate a significant positive association between dosage and mortality.

There were adequate numbers of male mice at risk from late-developing tumors as 50/50 (100 percent) of the high dose, 44/50 (88 percent) of the low dose, and 18/20 (90 percent) of the control group survived on test until the termination of the study. Only one natural death, in the control group at week 62, was reported among all dosed and control male mice.

For females, with 43/50 (86 percent) of the high dose, 43/50 (86 percent) of the low dose, and 15/20 (75 percent) of the control group surviving on test until termination of the study, there were adequate numbers at risk from late-developing tumors. Two high dose females were missing starting in week 87, 4 low dose females were missing starting in weeks 23, 58, and 68, and one control female was missing starting in week 30.

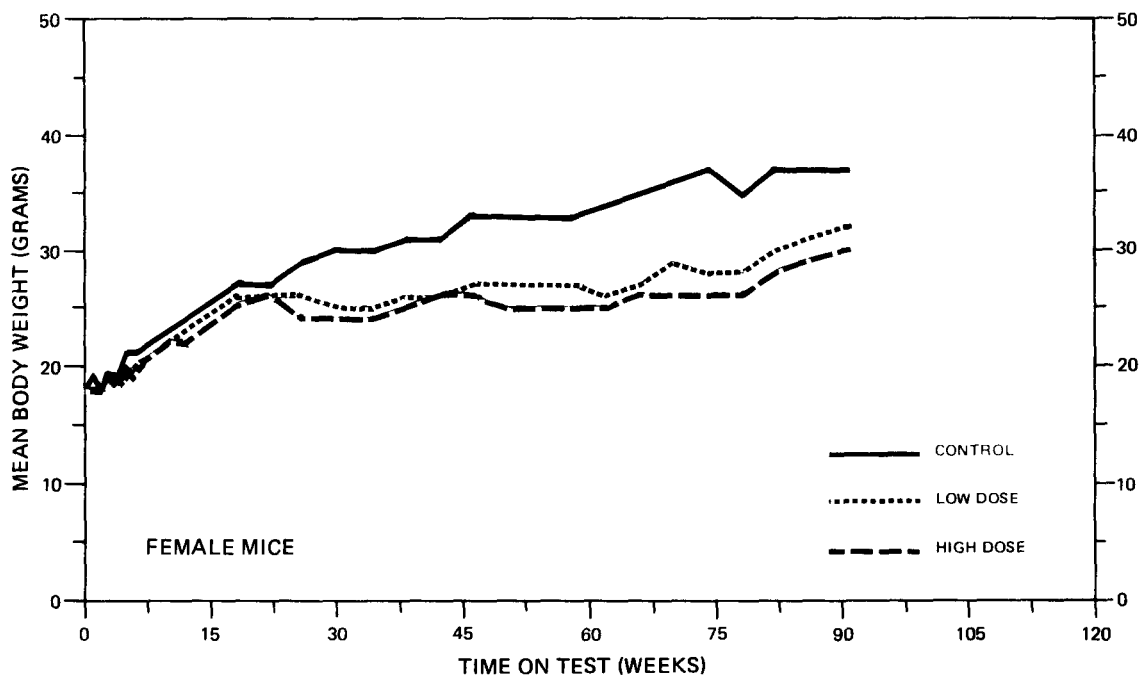
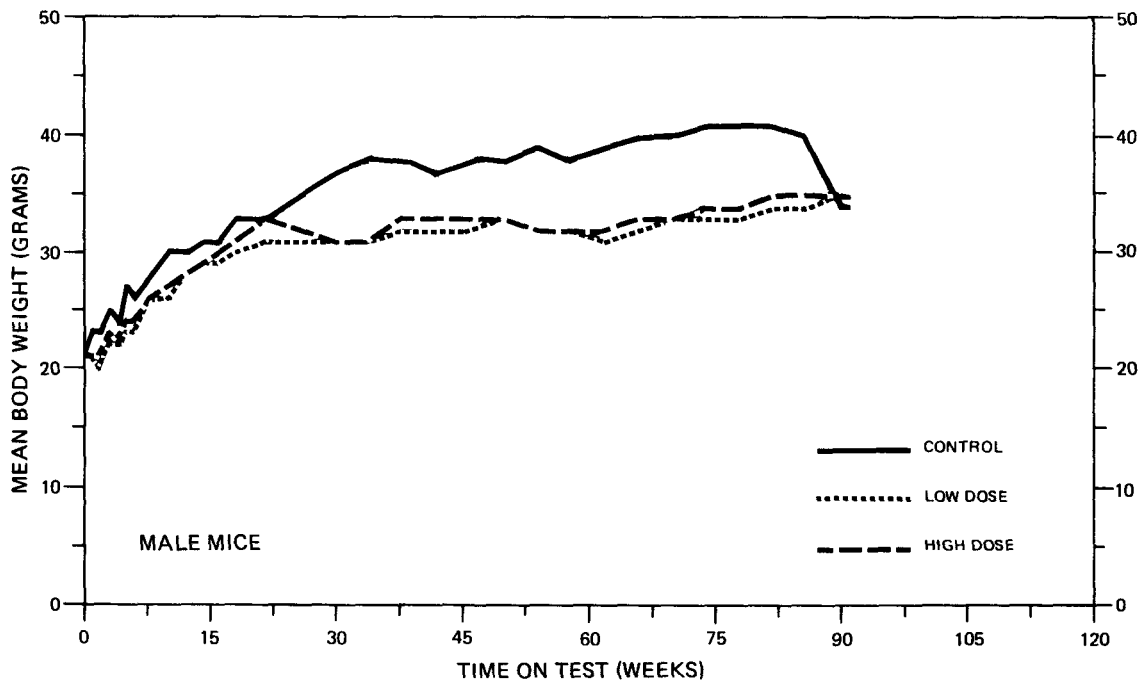


FIGURE 4
GROWTH CURVES FOR TRIMETHYLTHIOUREA CHRONIC STUDY MICE

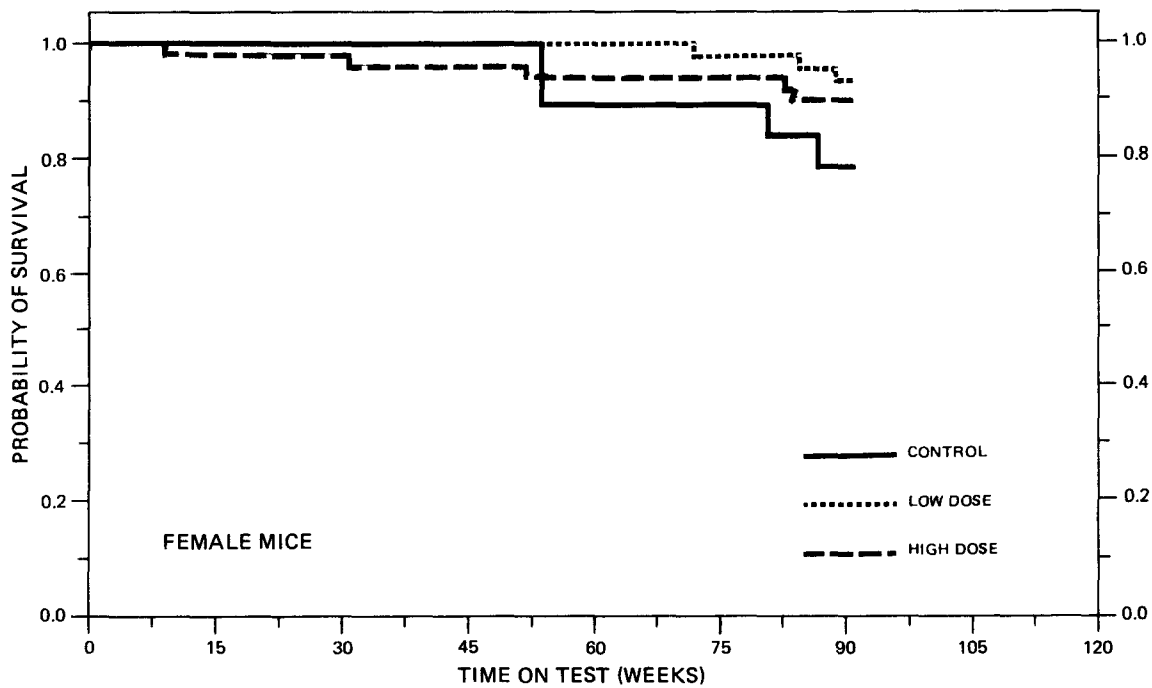
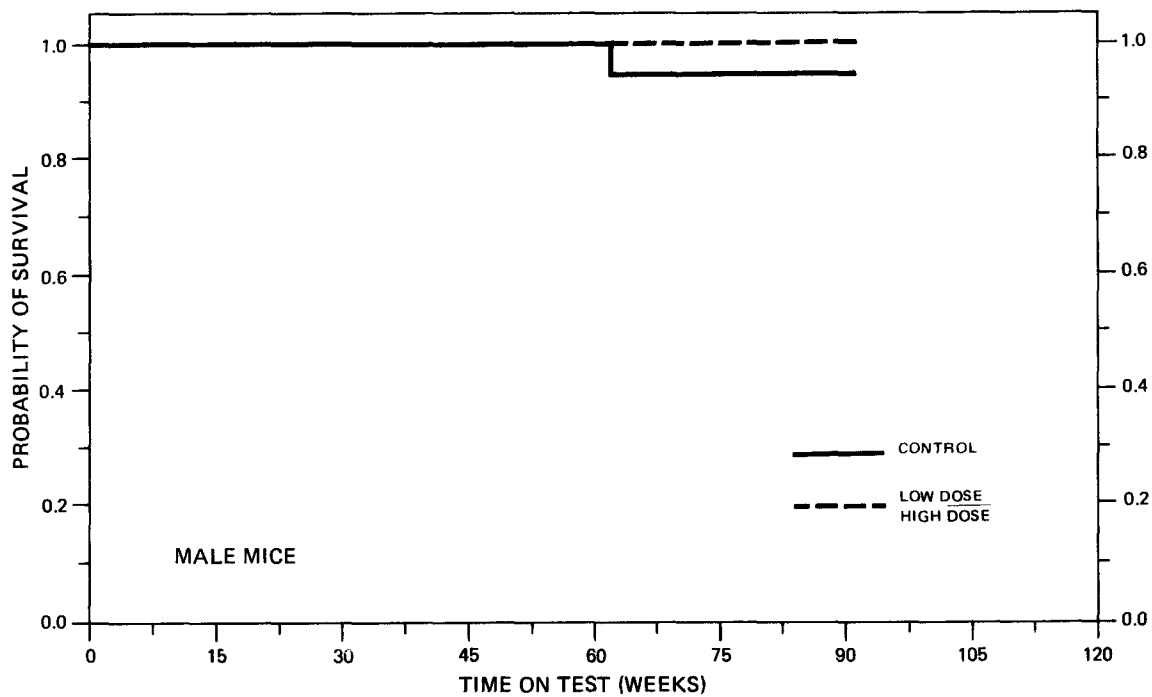


FIGURE 5
SURVIVAL COMPARISONS OF TRIMETHYLTHIOUREA CHRONIC STUDY MICE

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).

A variety of neoplastic and nonneoplastic lesions occurred in both dosed and control animals. The type, incidence, and distribution of these lesions were similar to those expected in aged B6C3F1 mice; therefore, these lesions were considered to be spontaneous and not related to compound administration.

The results of this pathologic examination indicated that trimethylthiourea was not carcinogenic in B6C3F1 mice under the conditions of this study.

D. Statistical Analyses of Results

For male mice the results of the statistical analyses are included in Table 5 for every type of malignant tumor where at least two such tumors were observed in at least one of the control or trimethylthiourea-dosed groups and where such tumors were observed in at least 5 percent of the group. There were no sites in female mice where the above criteria were met.

None of the statistical tests for any site in mice of either sex indicated a significant positive association between chemical administration and tumor incidence. Based upon these statistical results there was no evidence that trimethylthiourea was a carcinogen in B6C3F1 mice under the conditions of this bioassay.

TABLE 5
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH TRIMETHYLTHIOUREA^a

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma ^b	1/18(0.06)	5/44(0.11)	8/50(0.16)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.045	2.880
Lower Limit	---	0.258	0.439
Upper Limit	---	94.395	124.908
Weeks to First Observed Tumor	91	91	91
Liver: Hepatocellular Adenoma or Neoplastic Nodule ^b	6/19(0.32)	2/44(0.05)	2/50(0.04)
P Values ^c	P = 0.003(N)	P = 0.007(N)	P = 0.004(N)
Departure from Linear Trend ^e	P = 0.020	---	---
Relative Risk (Control) ^d	---	0.144	0.127
Lower Limit	---	0.016	0.014
Upper Limit	---	0.729	0.645
Weeks to First Observed Tumor	91	91	91

TABLE 5 (CONCLUDED)

^aTreated groups received doses of 500 or 1000 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. 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. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

In male mice the Cochran-Armitage test and the Fisher exact tests comparing high dose to control and low dose to control indicated a significant negative association between dose and the combined incidence of hepatocellular adenomas or neoplastic nodules of the liver. However, historical control data from the same laboratory indicate an incidence of 32/272 (12 percent) of these tumors in untreated male B6C3F1 mice as compared with the higher 6/19 (32 percent) incidence in control males in this bioassay.

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 Table 5 based upon the observed tumor incidence rates. In many of the intervals shown in Table 5, 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 trimethylthiourea that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the dosage of trimethylthiourea administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. For high dose female rats and for dosed mice of both sexes compound-related mean body weight depression was observed, indicating that the dosages of trimethylthiourea administered to these animals may have approximated the maximum tolerated dosages. Since no mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of trimethylthiourea to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

For female rats, there was a significant positive association between dosage and the incidences of follicular-cell carcinomas (i.e., 0/17, 1/38 [3 percent], and 14/47 [30 percent] in the control, low dose, and high dose groups, respectively). The high dose to control Fisher exact comparison was also significant for this neoplasm in female rats. In addition, when female rats having follicular-cell carcinomas and follicular-cell adenomas were combined and the resulting tumor incidences analyzed statistically, the Cochran-Armitage test and the high dose to control Fisher exact comparison were, once again, significant.

There were no significant positive associations between compound administration and tumor incidence in male rats or in mice of either sex.

Under the conditions of this bioassay, dietary administration of trimethylthiourea was carcinogenic to female Fischer 344 rats, inducing follicular-cell carcinomas of the thyroid. There was not sufficient evidence for the carcinogenicity of the compound in male Fischer 344 rats or in B6C3F1 mice of either sex.

VI. BIBLIOGRAPHY

- Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.
- Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.
- Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.
- Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.
- Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.
- Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.
- Hauser, M. and B.M. Malofsky, "Surface Activator for Anaerobic Adhesives." Ger. Offen. 2,401,744 (Loctite Corp.), August 1, 1974; Chemical Abstracts 82, 17949g.
- Inuzuka, Y., N. Kameyama, and K. Watanabe, "Phthalocyanine Pigments." Japan. Kokai 76 26,933 (Toyo Ink Mfg. Co., Ltd.), March 5, 1976; Chemical Abstracts 85, 7367p.
- Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.
- Kirkpatrick, G., "Degradable Polyethylene Films." Ger. Offen. 2,261,778 (Union Carbide Corp.), July 19, 1973; Chemical Abstracts 79, 147239z.
- Langlade, A., "Extrusion and Continuous Vulcanization of Polychloropropene Rubbers." Hule Mex. Plast. 32(364):18-20, 22-24, 1976; Chemical Abstracts 85, 161530t.
- Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

- Lofquist, R.A. and P.R. Saunders, "Water-Soluble Thioureas to Inhibit Ozone Fading of Dyed Polyamides." U.S. Patent 3,822,996 (Allied Chemical Corp.), July 9, 1974; Chemical Abstracts 83, 61478x.
- Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.
- Ohashi, K., S. Kusayama, and W. Ota, "Adhesives." Ger. Offen. 2,106,155 (Sumitomo Chemical Co., Ltd.), August 19, 1971; Chemical Abstracts 76, 4623x.
- Palmer, L.B. and R.P. Conger, "Carpet Embossing in Register With Print." U.S. Patent 3,849,159 (Congoleum Industries, Inc.), November 19, 1974; Chemical Abstracts 82, 99931g.
- Radomski, J.L., W.B. Deichmann, W.E. MacDonald, and E.M. Glass, "Synergism Among Oral Carcinogens. I. Results of the Simultaneous Feeding of Four Tumorigens to Rats." Toxicology and Applied Pharmacology 7:652-656, 1965.
- Rosati, R.L., "Substituted Acyl Derivatives of α -Aminopenicillins." Ger. Offen. 2,316,170 (Pfizer, Inc.), December 6, 1973; Chemical Abstracts 80, 82954c.
- Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.
- Stanford Research Institute, 1977 Directory of Chemical Producers, U.S.A. Menlo Park, California, 1977.
- Starmer, P.H., "Vulcanizable Compositions Containing Diene/Nitrile Polymers Having Active Halogen Sites." U.S. Patent 3,976,625 (B. F. Goodrich Co.), August 24, 1976; Chemical Abstracts 85, 161608z.
- Starmer, P.H., "Vulcanizable Mixtures." Ger. Offen. 2,557,559 (B. F. Goodrich Co.), July 16, 1976; Chemical Abstracts 85, 125521g.
- Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.
- Vandenberg, E.J. and W.D. Willis, "Treatment of Keratinous Fibers." British Patent 1,349,306 (Hercules, Inc.), April 3, 1974; Chemical Abstracts 81, 51035v.

Review of the Bioassay of Trimethylthiourea* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to 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 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 reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Trimethylthiourea for carcinogenicity.

The primary reviewer noted that Trimethylthiourea induced an increased incidence of thyroid follicular-cell neoplasms in only treated female rats. Significant increases in tumor incidences were not found in other treatment groups. After a brief description of the experimental design, the primary reviewer commented on the fact that not all of the thyroid glands were examined. She suggested that this may have caused a bias in the results. Despite the experimental shortcomings, the primary reviewer concurred with the conclusion in the report that Trimethylthiourea was carcinogenic in female rats. She added that the data were insufficient, however, to base an estimate of the human risk posed by Trimethylthiourea.

A motion was approved unanimously that the report on the bioassay of Trimethylthiourea be accepted as written.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin School of Medicine

Joseph Highland, Environmental Defense Fund

Michael Shimkin, University of California at San Diego

Louise Strong, University of Texas Health Sciences Center

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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.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN RATS TREATED WITH TRIMETHYLTHIOUREA

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH TRIMETHYLTHIOUREA

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS	1 (5%)	1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (5%)		
FIBROMA			1 (2%)
NEURILEMMA	1 (5%)		
RESPIRATORY SYSTEM			
*TRACHEA	(19)	(50)	(49)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
*LUNG	(20)	(45)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	1 (2%)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
SARCOMA, NOS, METASTATIC		1 (2%)	
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	3 (6%)	2 (4%)
LEUKEMIA, NOS		1 (2%)	2 (4%)
*SPLEEN	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS.			

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(49)
NEOPLASTIC NODULE	1 (5%)		1 (2%)
*ESOPHAGUS	(19)	(50)	(49)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
*CECUM	(20)	(49)	(49)
PAPILLARY ADENOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*PITUITARY	(17)	(45)	(36)
CHROMOPHOBE ADENOMA	1 (6%)	1 (2%)	1 (3%)
*ADRENAL	(20)	(50)	(49)
PHEOCHROMOCYTOMA	1 (5%)	2 (4%)	2 (4%)
*THYROID	(17)	(37)	(39)
FOLLICULAR-CELL CARCINOMA		1 (3%)	3 (8%)
C-CELL ADENOMA	2 (12%)	1 (3%)	
C-CELL CARCINOMA			2 (5%)
*PANCREATIC ISLETS	(20)	(49)	(49)
ISLET-CELL ADENOMA		2 (4%)	
REPRODUCTIVE SYSTEM			
*TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	18 (90%)	42 (84%)	45 (92%)
NERVOUS SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
SPECIAL SENSE ORGANS			
*EXTERNAL EAR FIBROSARCOMA	(20)	(50)	(50) 1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*EPICARDIUM SARCOMA, NOS, METASTATIC	(20)	(50) 1 (2%)	(50)
*TUNICA VAGINALIS MESOTHELIOMA, NOS	(20)	(50)	(50) 1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS MESOTHELIOMA, NOS	(20)	(50) 1 (2%)	(50)
THORAX OSTEOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	8	4	7
MORIBUND SACRIFICE	1	1	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	45	41
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	45	47
TOTAL PRIMARY TUMORS	30	57	63
TOTAL ANIMALS WITH BENIGN TUMORS	19	43	47
TOTAL BENIGN TUMORS	25	51	50
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	5	10
TOTAL MALIGNANT TUMORS	4	5	11
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	2
TOTAL SECONDARY TUMORS		2	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	1	2
TOTAL UNCERTAIN TUMORS	1	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCEPTAIN 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 TRIMETHYLTHIOUREA

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROMA			2 (4%)
RESPIRATORY SYSTEM			
*LUNG	(20)	(46)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
HEMATOPOIETIC SYSTEM			
*BRAIN	(20)	(50)	(49)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			3 (6%)
LEUKEMIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(49)
HEPATOCELLULAR ADENOMA	1 (5%)		
NEOPLASTIC NODULE	1 (5%)	2 (4%)	
*DUODENUM	(20)	(50)	(49)
LEIOMYOMA	1 (5%)		
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*PITUITARY CHROMOPHOBE ADENOMA	(18) 2 (11%)	(44) 8 (18%)	(47) 11 (23%)
*ADRENAL CORTICAL ADENOMA PHEOCHROMOCYTOMA	(20) 1 (5%)	(50)	(49) 1 (2%)
*THYROID FOLLICULAR-CELL ADENOMA FOLLICULAR-CELL CARCINOMA C-CELL ADENOMA C-CELL CARCINOMA	(17) 1 (6%)	(38) 1 (3%) 2 (5%) 1 (3%)	(47) 9 (19%) 14 (30%) 2 (4%) 2 (4%)
*PANCREATIC ISLETS ISLET-CELL ADENOMA	(20)	(50) 1 (2%)	(47)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND ADENOCARCINOMA, NOS PAPILLARY ADENOCARCINOMA FIBROADENOMA	(20) 1 (5%) 1 (5%) 1 (5%)	(50) 1 (2%) 3 (6%)	(50) 6 (12%)
*UTERUS ENDOMETRIAL STROMAL POLYP	(20) 2 (10%)	(50)	(49)
*OVARY GRANULOSA-CELL TUMOR	(20)	(49) 1 (2%)	(49)
NERVOUS SYSTEM			
*BRAIN GLIOMA, NOS	(20)	(50)	(49) 1 (2%)
SPECIAL SENSE ORGANS			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY ADENOCARCINOMA, NOS, METASTATIC	(20)	(50)	(50) 1 (2%)
*ABDOMINAL VISCERA SARCOMA, NCS	(20)	(50)	(50) 1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS ADENOCARCINOMA, NOS, METASTATIC	(20)	(50)	(50) 1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH ^a	2	8	11
MORBUND SACRIFICE	1	4	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	38	32
ANIMAL MISSING			
^a INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	20	35
TOTAL PRIMARY TUMORS	12	23	53
TOTAL ANIMALS WITH BENIGN TUMORS	6	13	23
TOTAL BENIGN TUMORS	9	15	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	21
TOTAL MALIGNANT TUMORS	2	5	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			3
TOTAL SECONDARY TUMORS			3
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	3	
TOTAL UNCERTAIN TUMORS	1	3	
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 TRIMETHYLTHIOUREA

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH TRIMETHYLTHIOUREA

	CONTROL (UNTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	6	
ANIMALS NECROPSIED	19	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	44	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(18)	(44)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (7%)	6 (12%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (6%)	2 (5%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(44)	(50)
LEUKEMIA, NOS	1 (5%)		
*SPLEEN	(17)	(41)	(49)
HEMANGIOMA		1 (2%)	
LYMPHOCYTIC LEUKEMIA		1 (2%)	
*MESENTERY	(19)	(44)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(19)	(44)	(50)
HEPATOCELLULAR ADENOMA	5 (26%)	2 (5%)	2 (4%)
NEOPLASTIC NODULE	1 (5%)		
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
HEMANGIOSARCOMA			1 (2%)
*SMALL INTESTINE SARCOMA, NOS	(19)	(44)	(50) 1 (2%)
*DUODENUM ADENOCARCINOMA, NOS	(19)	(44)	(50) 1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*THYROID FOLLICULAR-CELL ADENOMA FOLLICULAR-CELL CARCINOMA	(12) 1 (8%)	(32)	(40) 1 (3%)
*PANCREATIC ISLETS ISLET-CELL ADENOMA	(19)	(41) 1 (2%)	(47)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND PAPILLARY CYSTADENOCARCINOMA, NOS	(19)	(44) 1 (2%)	(50)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH	1		
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	44	50
ANIMAL MISSING	1	6	
a INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	11	15
TOTAL PRIMARY TUMORS	9	11	15
TOTAL ANIMALS WITH BENIGN TUMORS	6	7	8
TOTAL BENIGN TUMORS	6	7	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	4	7
TOTAL MALIGNANT TUMORS	2	4	7
TOTAL ANIMALS WITH SECONDARY TUMORS*			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
* SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH TRIMETHYLTHIOUREA

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	4	2
ANIMALS NECROPSIED	19	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	46	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(19)	(46)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(46)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA, NOS	1 (5%)	1 (2%)	
*PULMONARY LYMPH NODE	(13)	(37)	(39)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
*KIDNEY	(19)	(46)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*OVARY PAPILLOMA, NOS	(19)	(45) 1 (2%)	(42)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS FIBROSARCOMA	(19)	(46) 1 (2%)	(48)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH ²	3	1	5
MORIBUND SACRIFICE	1	2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	43	43
ANIMAL MISSING	1	4	2
² INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	3	4
TOTAL PRIMARY TUMORS	1	5	4
TOTAL ANIMALS WITH BENIGN TUMORS		1	
TOTAL BENIGN TUMORS		2	
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	2	4
TOTAL MALIGNANT TUMORS	1	3	4
TOTAL ANIMALS WITH SECONDARY TUMORS*			
TOTAL SECONDARY TUMORS			
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 TRIMETHYLTHIOUREA

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH TRIMETHYLTHIOUREA

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
MULTILOCLAR CYST		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(45)	(49)
BRONCHOPNEUMONIA, FOCAL			1 (2%)
PNEUMONIA, CHRONIC MURINE	8 (40%)	6 (13%)	25 (51%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
*MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS	1 (5%)	3 (6%)	2 (4%)
DEGENERATION, NOS		1 (2%)	4 (8%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(49)
GRANULOMA, NOS			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	1 (2%)	1 (2%)
*LIVER/CENTRILOBULAR	(20)	(49)	(49)
NECROSIS, NOS	1 (5%)		
*LIVER/PERIPORTAL	(20)	(49)	(49)
FIBROSIS	2 (10%)		

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTF) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
*LIVER/HEPATOCTES NECROSIS, DIFFUSE	(20)	(49)	(49) 1 (2%)
*PANCREATIC ACINUS ATROPHY, NOS	(20) 1 (5%)	(49)	(49) 3 (6%)
*SMALL INTESTINE HYPERPLASIA, LYMPHOID	(20)	(49) 1 (2%)	(49)
*LARGE INTESTINE NEMATODIASIS	(20) 1 (5%)	(49) 1 (2%)	(49) 4 (8%)
URINARY SYSTEM			
*KIDNEY CYST, NOS	(20)	(50) 1 (2%)	(49)
INFLAMMATION, CHRONIC	12 (60%)	31 (62%)	35 (71%)
*URINARY BLADDER CALCULUS, NOS	(19)	(46) 2 (4%)	(45)
ENDOCRINE SYSTEM			
*PITUITARY HEMORRHAGIC CYST	(17)	(45) 1 (2%)	(36)
*ADRENAL HEMORRHAGIC CYST	(20)	(50) 1 (2%)	(49) 1 (2%)
LIPOIDOSIS		1 (2%)	
*ADRENAL MEDULLA CYST, NOS	(20) 1 (5%)	(50)	(49)
HYPERPLASIA, NOS			1 (2%)
*THYROID HYPERPLASIA, C-CELL	(17)	(37)	(39) 1 (3%)
*PANCREATIC ISLETS HYPERPLASIA, NOS	(20)	(49) 1 (2%)	(49)
REPRODUCTIVE SYSTEM			
*TESTIS GRANULOMA, SPERMATIC	(20)	(50) 1 (2%)	(49)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1045	LOW DOSE 11-1043	HIGH DOSE 11-1041
CYTOMEGALY HYPERPLASIA, INTERSTITIAL CELL		1 (2%) 1 (2%)	
*TESTIS/TUBULE MINERALIZATION	(20) 1 (5%)	(50)	(49)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA HYDPOTHORAX	(20) 1 (5%)	(50)	(50)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED AUTO/NECROPSY/NO HISTO		2	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 TRIMETHYLTHIOUREA

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(46)	(49)
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
PNEUMONIA, CHRONIC MURINE	3 (15%)	8 (17%)	9 (18%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
REACTION, FOREIGN BODY		1 (2%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
*MYOCARDIUM	(19)	(47)	(39)
INFLAMMATION, NOS		1 (2%)	
FIBROSIS		1 (2%)	
DEGENERATION, NOS		1 (2%)	2 (5%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(49)
CYST, NOS	1 (5%)		

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
NECROSIS, FOCAL METAMORPHOSIS FATTY		1 (2%) 4 (8%)	4 (8%)
*LIVER/PERIportal METAMORPHOSIS FATTY	(20)	(49)	(49) 1 (2%)
*PANCREATIC ACINUS ATROPHY, NOS	(20) 1 (5%)	(50) 1 (2%)	(47)
*STOMACH EPIDERMAL INCLUSION CYST	(20)	(50)	(49) 1 (2%)
*SMALL INTESTINE HYPERPLASIA, LYMPHOID	(20)	(50)	(49) 1 (2%)
*LARGE INTESTINE NEMATODIASIS	(20) 1 (5%)	(50) 1 (2%)	(49) 2 (4%)
UPINARY SYSTEM			
*KIDNEY HYDRONEPHROSIS INFLAMMATION, NOS INFLAMMATION, CHRONIC CALCIFICATION, FOCAL	(20) 2 (10%) 1 (5%)	(50) 16 (32%)	(50) 1 (2%) 1 (2%) 20 (40%)
ENDOCRINE SYSTEM			
*PITUITARY CYST, NOS HEMORRHAGIC CYST	(18) 1 (6%)	(44) 2 (5%) 3 (7%)	(47) 3 (6%)
*ADRENAL HEMORRHAGIC CYST METAMORPHOSIS FATTY LIPOIDOSIS	(20) 1 (5%)	(50) 3 (6%) 1 (2%) 2 (4%)	(49) 1 (2%) 1 (2%)
*ADRENAL CORTEX HYPERPLASIA, NOS HYPERPLASIA, FOCAL	(20)	(50) 1 (2%)	(49) 1 (2%)
*THYROID FOLLICULAR CYST, NOS	(17)	(38) 2 (5%)	(47)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, C-CELL		1 (3%)	
HYPERPLASIA, FOLLICULAR-CELL			3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
INFLAMMATION, VESICULAR			1 (2%)
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
*UTERUS	(20)	(50)	(49)
THROMBUS, ORGANIZED		1 (2%)	
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
PYOMETRA			1 (2%)
INFLAMMATION, ACUTE	1 (5%)		
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
*UTERUS/ENDOMETRIUM	(20)	(50)	(49)
CYST, NOS	1 (5%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, VESICULAR		1 (2%)	1 (2%)
*OVARY/OVIDUCT	(20)	(50)	(49)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
*OVARY	(20)	(49)	(49)
CYST, NOS		1 (2%)	2 (4%)
PAROVARIAN CYST	3 (15%)	2 (4%)	6 (12%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1046	LOW DOSE 11-1044	HIGH DOSE 11-1042
BODY CAVITIES			
*EPICARDIUM INFLAMMATION, NOS	(20)	(50)	(50) 1 (2%)
*MESENTERY NECROSIS, FAT	(20)	(50)	(50) 1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED AUTO/NECROPSY/HISTO PERF	6	10 1	1 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 TRIMETHYLTHIOUREA

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH TRIMETHYLTHIOUREA

	CONTROL (UMTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	6	
ANIMALS NECROPSIED	19	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY ** 19		44	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(18)	(44)	(50)
PNEUMONIA, CHRONIC MURINE		1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
*SPLEEN	(17)	(41)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	2 (4%)
*MESENTERIC L. NODE	(14)	(43)	(47)
INFLAMMATION, HEMORRHAGIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS	1 (7%)		
HYPERPLASIA, LYMPHOID		2 (5%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(19)	(44)	(50)
INFLAMMATION, NOS			1 (2%)
GRANULOMA, NOS			2 (4%)
NECROSIS, NOS			1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
HYPERPLASIA, NOS			1 (2%)
*PANCREAS	(19)	(41)	(47)
ECTOPIA		1 (2%)	
CYSTIC DUCTS		1 (2%)	
*SMALL INTESTINE	(19)	(44)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
*LARGE INTESTINE	(19)	(44)	(50)
NEMATODIASIS	4 (21%)	8 (18%)	9 (18%)
URINARY SYSTEM			
*KIDNEY	(19)	(44)	(50)
HYDRONEPHROSIS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	3 (16%)	1 (2%)	
NEPHROPATHY		1 (2%)	
ENDOCRINE SYSTEM			
*THYROID	(12)	(32)	(40)
GOITER COLLOID			1 (3%)
HYPERPLASIA, C-CELL			2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(44)	(50)
DILATATION/DUCTS			1 (2%)
NERVOUS SYSTEM			
*BRAIN	(19)	(44)	(49)
CORPORA AMYLACEA	6 (32%)	11 (25%)	17 (35%)
SPECIAL SENSE ORGANS			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2045	LOW DOSE 22-2043	HIGH DOSE 22-2041
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE INFLAMMATION, NOS	(19)	(44)	(50)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	19	15
ANIMAL MISSING/NO NECROPSY	1	6	
* 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 TRIMETHYLTHIOUREA

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	4	2
ANIMALS NECROPSIED	19	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY ** 19	19	46	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(19)	(46)	(46)
LYMPHOCYTOIC INFLAMMATORY INFILTR		1 (2%)	
PNEUMONIA, CHRONIC MURINE	4 (21%)	3 (7%)	
GRANULOMATOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
*SPLEEN	(19)	(42)	(47)
HEMORRHAGIC CYST			1 (2%)
*LYMPH NODE	(13)	(37)	(39)
HYPERPLASIA, NOS			1 (3%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
*MESENTERIC L. NODE	(13)	(37)	(39)
HYPERPLASIA, LYMPHOID	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(19)	(45)	(48)
INFLAMMATION, ACUTE FOCAL	1 (5%)	1 (2%)	
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
GRANULOMA, NOS			1 (2%)
HYPERPLASIA, NODULAR	1 (5%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS		1 (2%)	
*PANCREAS	(18)	(44)	(45)
CYSTIC DUCTS			1 (2%)
*PANCREATIC ACINUS	(18)	(44)	(45)
ATROPHY, NOS			1 (2%)
*PEYERS PATCH	(18)	(46)	(47)
HYPERPLASIA, LYMPHOID	1 (6%)		
*LARGE INTESTINE	(17)	(45)	(45)
NEMATODIASIS	1 (6%)		
URINARY SYSTEM			
*KIDNEY	(19)	(46)	(47)
INFLAMMATION, CHRONIC	2 (11%)	2 (4%)	
PERIVASCULAR CUFFING		1 (2%)	
ENDOCRINE SYSTEM			
*THYROID	(16)	(37)	(38)
GOITER COLLOID	1 (6%)		
*PANCREATIC ISLETS	(18)	(44)	(45)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*UTERUS	(19)	(46)	(45)
HYDROMETRA	2 (11%)		1 (2%)
HEMORRHAGE			1 (2%)
PYOMETRA		1 (2%)	
ABSCESS, NOS			1 (2%)
*CERVIX UTERI	(19)	(46)	(45)
INFLAMMATION, NOS			1 (2%)
*UTERUS/ENDOMETRIUM	(19)	(46)	(45)
CYST, NOS	1 (5%)		1 (2%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
INFLAMMATION, NOS	1 (5%)	2 (4%)	
INFLAMMATION, SUPPURATIVE	1 (5%)		
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
HYPERTROPHIA, NOS		2 (4%)	1 (2%)
HYPERTROPHIA, CYSTIC	6 (32%)	12 (26%)	14 (31%)
*OVARY	(19)	(45)	(42)
CYST, NOS	5 (26%)	5 (11%)	8 (19%)
FOLLICULAR CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
*BRAIN/MENINGES	(19)	(45)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)	1 (2%)	1 (2%)
*BRAIN	(19)	(45)	(48)
CORPORA AMYLACEA	4 (21%)	13 (29%)	17 (35%)
INCLUSION, CYTOPLASMIC		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(46)	(48)
PERIVASCULAR CUFFING		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	10	6
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2046	LOW DOSE 22-2044	HIGH DOSE 22-2042
ANIMAL MISSING/NO NECROPSY	1	4	2
AUTO/NECROPSY/HISTO PERF	1		3
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

