National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 47
1978

BIOASSAY OF 4,4'-THIODIANILINE FOR POSSIBLE CARCINOGENICITY

CAS No. 139-65-1

NCI-CG-TR-47

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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Bethesda, Maryland 20014

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DHEW Publication No. (NIH) 78-847

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FOREWORD: This report presents the results of the bioassay of 4,4′-thiodianiline 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 environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as 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 that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 4,4′-thiodianiline was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and feeding of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in the bioassay were analyzed under the direction of Dr. E. Murrill[7], and analytical results were reviewed by Dr. S. S. Olin[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

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SUMMARY

A bioassay of 4,4′-thiodianiline for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were administered 4,4′-thiodianiline 5 days per week at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 2,500 or 5,000 ppm for the mice. The period of administration of the chemical was 68–72 weeks for the rats and 77 or 79 weeks for the mice, depending on the length of survival time of the animals. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex. All surviving matched-control rats were killed at 104 weeks; all surviving matched-control mice were killed at 91 weeks.

The administration of 4,4′-thiodianiline resulted in marked reduction in mean body weights of the rats and mice of each sex, and all dosed animals died prior to the scheduled end of the study.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls 0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males were both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high-dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth. Only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of the chemical. Adenocarcinoma of the colon occurred in six low-dose male rats and in one high-dose male rat, but not in any of the controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of 4,4′-thiodianiline.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the females administered the test chemical at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33). However, no such tumors occurred among the 235 historical-control female rats at this laboratory; thus, the tumors of the ear canal are considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at statistically significant incidences ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant ($P = 0.002$) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of

females were significantly higher (low-dose P = 0.025, high-dose P < 0.001) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

It is concluded that under the conditions of this bioassay, 4,4′-thiodianiline was carcinogenic for Fischer 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4′-Thiodianiline was carcinogenic for B6C3F1 mice, inducing tumors in the liver and thyroid of both males and females.

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I. INTRODUCTION

4,4′-Thiodianiline (CAS 139-65-1; NCI C01707) is an intermediate in the manufacture of several diazo dyes (Am. Assoc. Text. Chem. Color., 1958). It is an analog of 4,4′-sulfonyldianiline, which is the antileprosy drug dapsone; 4,4′-sulfonyldianiline differs from 4,4′-thiodianiline by the oxidation of the sulfide linkage to the sulfone. 4,4′-Thiodianiline has been considered to be weakly active in inducing mammary tumors in female Sprague-Dawley rats (Griswold et al., 1968).

4,4′-Thiodianiline was selected for the Carcinogenesis Testing Program because of this activity and for comparison with the analog 4,4′-sulfonyldianiline, which was tested at the same time.

II. MATERIALS AND METHODS

A. Chemical

The 4,4′-thiodianiline used in the chronic study was manufactured by Carroll Products, Wood River Junction, Rhode Island. Its stated purity was 97% (nitrite absorption). Purity as determined by nonaqueous titration of the amine groups was 99.11 ± 0.01%. Thin-layer chromatography (tlc) revealed one impurity at the origin and three trace impurities. The trace impurities were shown by two-dimensional chromatography to result from decomposition of 4,4′-thiodianiline on the tlc plate. One impurity, estimated as 0.05%, was detected by vapor-phase chromatography. The melting point was 107-109°C (literature: 108-109°C). Elemental analyses (C, H, N, S) were correct for $C_{12}H_{12}N_2S$, the molecular formula of the chemical. Infrared and nuclear magnetic resonance spectra were consistent with spectra for 4,4′-thiodianiline in the literature.

The single batch of the chemical used in the chronic studies was stored at 5°C in amber bottles.

B. Dietary Preparation

Test diets were prepared every 2 weeks by mixing a known amount of sifted 4,4′-thiodianiline with a small amount of Wayne® Lab

Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal meal and mixing in a twin-shell blender for 10 minutes. No analyses of concentration or determinations of stability of the chemical in feed were performed. The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

For the subchronic studies, male Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic studies, male and female Fischer 344 rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories. On arrival at the laboratory, the animals, which were 30 or 31 days of age, were quarantined (rats for 17 days, mice for 16 days). Animals with no clinical signs of disease were then assigned to control or dosed groups and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. Room air was changed 15 times

per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and the cage tops were covered with disposable filter bonnets; the mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective dosed animals. Animals administered 4,4′-thiodianiline were maintained in the same rooms as animals of the same species administered the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)

5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine
hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide
(acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide
(chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine
hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)

acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of 4,4′-thiodianiline, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. Male Sprague-Dawley rats were administered the chemical in feed at concentrations of 1,200, 3,000, 6,000, 15,000, or 30,000 ppm. Male Swiss mice were administered the chemical at concentrations of 2,000, 5,000 10,000, 25,000, or 50,000 ppm. The animals were fed the test diet 7 days per week for 45 days and were then observed for an additional 45 days. Five male animals of each species were dosed at each concentra-

tion, and 20 male animals of each species were maintained as untreated controls.

At 45 days, the mean body weight gain in rats administered 1,200 ppm was 56% of that of the controls; at 3,000 ppm, 27%; and at 6,000 ppm, 10%. The mean body weights of animals administered 15,000 ppm were below the initial mean. At 90 days, mean body weights of the dosed groups were still less than those of the controls. All rats fed at 30,000 ppm died during week 3 on study, and one animal fed at 3,000 ppm died during week 2. No gross abnormalities were found at necropsy. For rats, the low and high doses for the chronic studies were set at 1,500 and 3,000 ppm.

At 45 days, the mean body weight gain in mice administered 2,000 ppm was 77% of that of the controls; at 5,000 ppm, 46%; and at 10,000 ppm, 46%. At 90 days, mice administered 2,000 or 10,000 ppm had mean body weight gains which were comparable to those of controls. In the group administered 5,000 ppm, however, mean body weight gain was only 65% of that of the controls. All mice fed diets containing 25,000 or 50,000 ppm had died by week 3, and two animals fed a diet containing 10,000 ppm died during week 3. No gross abnormalities were found at necropsy. For mice, the low and high doses for the chronic studies were set at 2,500 and 5,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. The time on study varied slightly for the dosed groups, because of differences in the times of death.

G. Clinical and Pathologic Examinations

All animals were observed twice per day for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed once every 2 weeks for the entire study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and 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, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Whenever possible, peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in

Table 1. Design of 4,4′-Thiodianiline Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	4,4′-Thiodianiline in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		104
Low-Dose	35	1,500	68[c]	
High-Dose	35	3,000	68[c]	
Female				
Matched-Control	15	0		104
Low-Dose	35	1,500	72[c]	
High-Dose	35	3,000	69[c]	

[a]All rats were 47 or 48 days of age when placed on study.

[b]Dosed animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Administration of the chemical to rats terminated at the times indicated due to death of all animals.

Table 2. Design of 4,4′-Thiodianiline Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	4,4′-Thiodianiline in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	14	0		91
Low-Dose	35	2,500	79[c]	
High-Dose	35	5,000	77[c]	
Female				
Matched-Control	14	0		91
Low-Dose	35	2,500	79[c]	
High-Dose	35	5,000	77[c]	

[a]All mice were 46 or 47 days of age when placed on study.

[b]Dosed animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Administration of the chemical to mice terminated at the times indicated due to death of all animals.

paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

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 Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is 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 is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions 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) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed 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) 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), was also used. Under the assumption of a linear trend, this test determines 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 is a positive dose relation-

ship. 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 an animal died naturally or was 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 with 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 dosed 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 dosed 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 dosed 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% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically signifi-

cant result ($P < 0.025$ 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. RESULTS - RATS

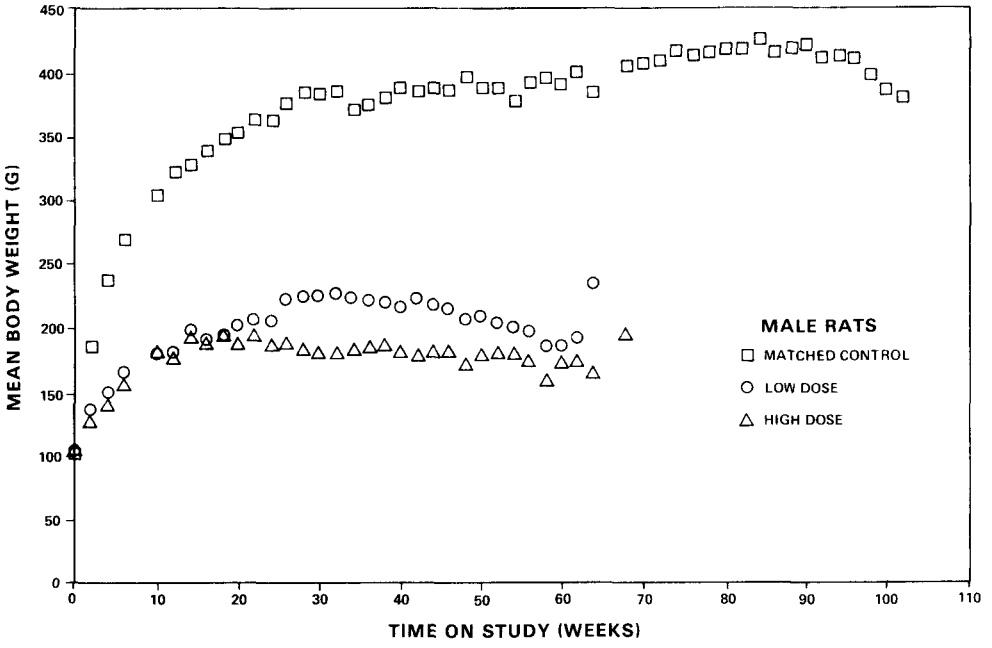
A. Body Weights and Clinical Signs (Rats)

Mean body weights of low- and high-dose groups of rats of each sex were markedly depressed in comparison with those of the controls during the entire period of administration of the chemical (figure 1). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. Several dosed rats had palpable masses in the external ear canal. No other clinical signs associated with administration of 4,4′-thiodianiline were recorded.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed 4,4′-thiodianiline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2.

In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant ($P < 0.001$), and a significant departure from linear trend ($P < 0.001$) is observed in male rats. In male rats, 18/35 (51%) of the high-dose group, 23/35 (66%) of the low-dose group, and all of the 15 matched



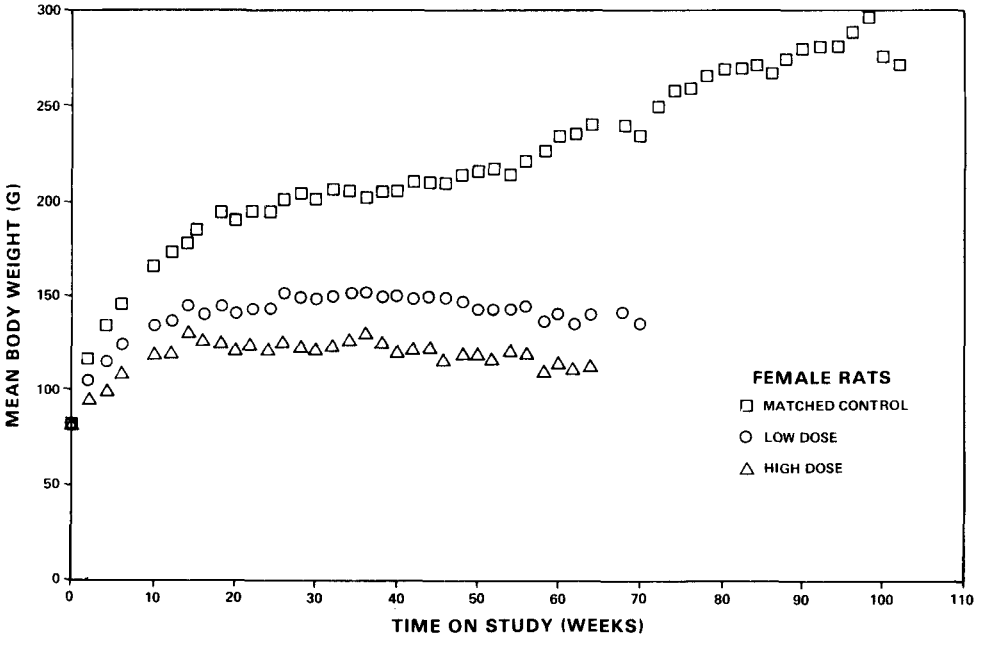


Figure 1. Growth Curves for Rats Fed 4, 4'-Thiodianiline in the Diet

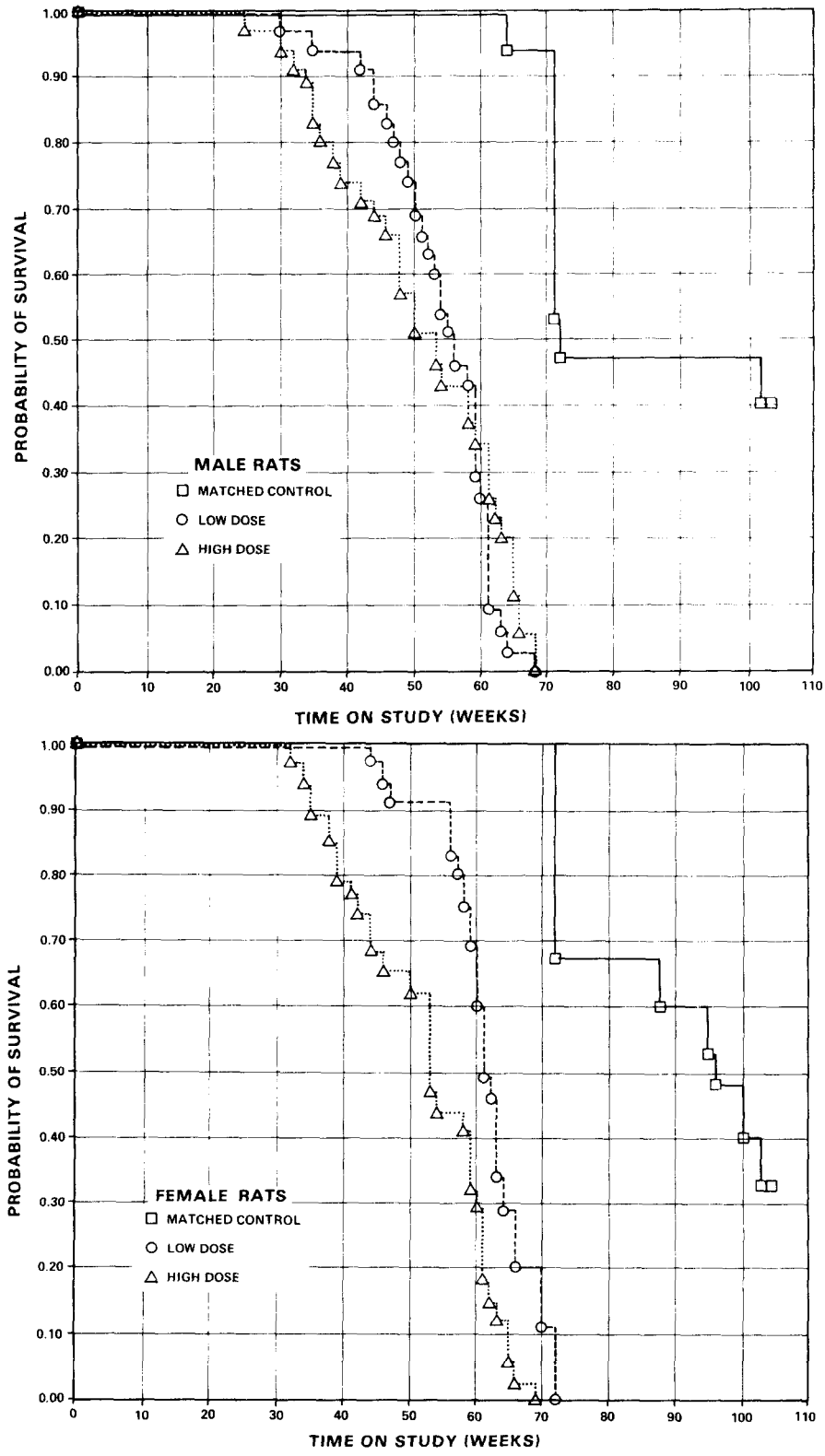


Figure 2. Survival Curves for Rats Fed 4, 4'-Thiodianiline in the Diet

control animals lived at least as long as week 52 on study. In females, 21/35 (60%) of the high-dose group, 32/35 (91%) of the low-dose group, and all of the 15 matched control animals lived beyond 1 year on study.

C. Pathology (Rats)

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.

A variety of neoplasms occurred in both the control and dosed groups. Some types of neoplasms occurred only, or with a greater frequency, in rats of dosed groups when compared with controls. These lesions are not uncommon in this strain of rat independent of the administration of any chemical. However, many of the tumors observed in the rats appeared to be chemically induced. These tumors occurred at a high incidence in the dosed groups when compared with the controls; most were malignant, and many had metastasized to one or more locations. The incidences of these tumors were as follows:

	RATS					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Skin*	(15)	(33)	(33)	(15)	(33)	(33)
Squamous-cell papilloma	0	4	0	0	0	0
Squamous-cell carcinoma	0	1	0	0	0	0
Ear Canal*	(15)	(33)	(33)	(15)	(33)	(33)
Squamous-cell papilloma	0	10	2	0	5	3
Squamous-cell carcinoma	0	5	6	0	1	0
Lungs**	(15)	(33)	(33)	(15)	(32)	(32)
Squamous-cell carcinoma	0	4	0	0	0	0
Alveolar/bronchiolar adenoma	0	1	0	0	0	0
Alveolar/bronchiolar carcinoma	0	3	0	0	2	0
Adenosquamous carcinoma	0	0	0	0	1	0
Liver**	(15)	(33)	(33)	(15)	(32)	(33)
Hepatocellular adenoma	0	2	2	0	1	2
Hepatocellular carcinoma	0	21	10	0	5	1
Colon**	(15)	(32)	(33)	(14)	(33)	(32)
Adenocarcinoma, NOS (not otherwise specified)	0	6	1	0	0	0
Thyroid**	(15)	(33)	(33)	(14)	(33)	(32)
Follicular-cell adenoma	0	2	0	0	0	0
Follicular-cell carcinoma	0	28	32	0	24	32
Uterus**				(15)	(33)	(32)
Adenocarcinoma				0	31	23

*Number of rats necropsied
**Number of rats with tissue examined microscopically

The majority of squamous-cell tumors arising from the skin were located near the commissure of the mouth. The squamous-cell carcinomas were characterized by large, polyhedral cells that

extended deep into the underlying dermis and subcutis. These well-differentiated neoplastic cells had a large, vesicular nucleus and prominent nucleolus, and many exhibited intercellular bridges. Numerous keratin pearls and some individual cell keratinization were also observed within the neoplasm.

Squamous-cell tumors involving the external ear canal and the subcutaneous tissues adjacent to the ear canal were observed as early as 25 weeks in male rats. They probably originated from the sebaceous glands of the ear canals (Zymbal's glands). In 1/33 (3%) high-dose males and 1/33 (3%) low-dose males, the ear canal papillomas were observed bilaterally. Squamous-cell carcinomas were metastatic to the lungs in 1/33 (3%) high-dose and 1/33 (3%) low-dose males. The morphology of the squamous-cell carcinomas and papillomas involving the ear canal is well documented (Pliss, 1973).

Malignant tumors arising from the lungs consisted of squamous-cell carcinomas, alveolar-cell adenocarcinomas, and an adenosquamous carcinoma. The morphology of the pulmonary squamous-cell carcinomas resembled that described for the skin, with marked keratinization and pearl formation being present. Neoplastic squamous cells had invaded through the pleura and transplanted to the parietal pleura in one case. The alveolar-cell carcinomas were comprised of cuboidal to columnar cells

aligned along the alveolar septa. Often the cells projected into the alveolar spaces, resulting in the formation of numerous papillary structures. The adenosquamous carcinoma present in one low-dose female was comprised of poorly differentiated cells arranged in a lobular pattern. The lobules were separated by a fine fibrovascular stroma. The neoplastic cells were characterized by a large vesicular nucleus and an eosinophilic cytoplasm. Few nucleoli or mitotic figures were observed. A bronchiolar adenoma was seen in one low-dose male.

In the hepatocellular carcinomas, there was considerable hepatocytomegaly, with many large hepatocytes having a large vesicular nucleus and a finely vacuolated cytoplasm. The disorganized hepatic cords were often surrounded by distended sinusoidal spaces, resulting in a trabecular pattern. In 1/33 (3%) low-dose males the neoplastic hepatocytes had invaded through the capsule, and numerous tumor transplantations were present throughout the mesentery. These tumors were frequently multiple in individual animals. Hepatocellular carcinomas had metastasized to the lungs in 7/33 (21%) low-dose and 1/33 (3%) high-dose males.

The colonic adenocarcinomas, present in low-dose and high-dose male rats, were polypoid-shaped masses comprised of large columnar epithelial cells, having a large, basophilic, elongated or flattened nucleus, and were attached to a fibrous connective

tissue stalk. The neoplastic glandular epithelium was devoid of mucous cells. The majority of the colonic masses were well differentiated and probably represented carcinoma in situ; however, in a few of the tumors the lymphatics of the lamina propria had been infiltrated by neoplastic epithelial cells.

In the majority of rats involved, the thyroid follicular-cell adenocarcinomas were bilateral in location. The neoplastic cells were arranged primarily into acini with the high cuboidal to columnar cells being aligned along a basement membrane. Many of the acini had papillary infoldings of epithelial cells projecting into the lumen, and the acini were separated by a fine fibrovascular stroma. The fibrous stroma was so abundant in some tumors that a sclerotic adenocarcinoma resulted. Neoplastic cell infiltration of blood vessels, lymphatics, and underlying tracheal and esophageal tissues was a frequent finding. Metastasis to the regional (mediastinal) lymph nodes was observed in three high-dose males and in one high-dose female. Pulmonary metastases were present in 17/33 (52%) high-dose and 15/33 (45%) low-dose males and in 11/32 (34%) high-dose females. Also, hepatic and splenic metastases were each observed in 1/33 (3%) high-dose males. Thyroid follicular-cell adenomas were present in 2/33 (6%) low-dose males.

Cells of the uterine adenocarcinomas had large, vesicular nuclei,

and prominent eosinophilic nucleoli, and were arranged into glands. The glands were usually separated by a fine fibrovascular stroma; however, the stroma became so abundant in some tumors that the result was a sclerotic adenocarcinoma. The neoplastic endometrial glandular tissues both projected into the uterine lumen and infiltrated the underlying muscle layers. In 12/33 (36%) low-dose and 2/33 (6%) high-dose rats the cells had infiltrated through the uterine wall and transplanted to the mesentery. Pulmonary metastasis was observed in 14/32 (44%) low-dose females, and metastasis to the mesenteric (abdominal) lymph nodes was observed in three low-dose females.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. Many of these nonneoplastic lesions are commonly seen in aged rats; however, some were considered to be chemically induced. The incidences of the chemical-related lesions were as follows:

	RATS					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Lungs*	(15)	(33)	(33)	(15)	(32)	(32)
Epidermal inclusion cyst	0	5	0	0	2	0
Alveolar-cell hyperplasia	0	15	0	0	13	2
Squamous metaplasia (alveolar and bronchiolar)	0	12	0	0	4	0
Liver*	(15)	(33)	(33)	(15)	(32)	(33)
Hyperplasia, nodular or NOS	0	4	10	0	1	9
Bile Duct**	(15)	(33)	(33)	(15)	(33)	(33)
Hyperplasia, NOS, or cystic	0	8	25	0	6	12
Thyroid*	(15)	(33)	(33)	(14)	(33)	(32)
Follicular-cell hyperplasia	0	1	0	0	7	0

*Number of rats with tissue examined microscopically.
**Number of rats necropsied.

Squamous metaplasia of alveolar and bronchiolar epithelium was often observed with inclusion cysts. The metaplastic as well as the hyperplastic foci were often multiple or locally extensive, and their incidence was highest in those dosed groups in which the incidence of pulmonary squamous-cell carcinomas and alveolar-cell adenocarcinomas were the highest.

The incidence of nodular hyperplasias of the liver was greatest in the high-dose animals, whereas the incidence of liver tumors

was greatest in the low-dose rats. The incidence of bile duct hyperplasia paralleled that of nodular hepatocellular hyperplasia.

Feeding Fischer 344 rats 4,4′-thiodianiline for 18 months resulted in an increased incidence of tumors in all dosed groups. These tumors were all epithelial in origin, and included squamous-cell papillomas and a carcinoma of the skin; squamous-cell papillomas and carcinomas of the external ear canal; squamous-cell carcinomas, alveolar-cell carcinomas, and a bronchiolar adenoma of the lungs; hepatocellular adenomas and carcinomas; adenocarcinomas of the colon; follicular-cell adenomas and carcinomas of the thyroid; and adenocarcinomas of the uterus. Also, the high incidence of alveolar-cell hyperplasias and alveolar and bronchiolar squamous metaplasias with inclusion cyst formation in the lungs, hepatocellular nodular hyperplasias and bile duct hyperplasias and thyroid follicular-cell hyperplasias appeared to be chemically induced.

In the judgment of the pathologists, 4,4′-thiodianiline was carcinogenic for Fischer 344 rats under the conditions of this study.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses

of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In male rats, the Fisher exact comparisons of the incidences of hepatocellular carcinoma in dosed groups with that in the control group show that the incidences in the low- and high-dose groups are significantly higher than that in the control group ($P < 0.001$ and $P = 0.014$, respectively). These statistical tests indicate that the incidence of hepatocellular carcinoma in male rats is associated with 4,4′-thiodianiline at the doses of this experiment. Although the results of the Cochran-Armitage test for positive dose-related trend on these incidences are not significant, a significant departure from linearity is observed ($P < 0.001$), because the incidence in the low-dose group is higher than that in the high-dose group. This may reflect differences in early mortality in the low- and high- dose groups.

In each sex, follicular-cell carcinomas of the thyroid are found in high incidences in all the dosed groups (male rats: low-dose 28/33 [85%], high-dose 32/33 [97%]; female rats: low-dose 24/33 [73%], high-dose 32/32 [100%]). The results of the Cochran-Armitage test and of the Fisher exact test are all significant ($P < 0.001$). Because there is a sharp increase of incidences in the dosed groups, an indicated departure from linear trend is

observed (males: $P < 0.001$; females: $P = 0.025$). The statistical conclusion is that the incidence of follicular-cell carcinoma of the thyroid in rats is associated with administration of the chemical.

In male rats, the results of the Fisher exact test show that the incidence of squamous-cell papilloma or carcinoma of the ear canal in the low-dose group (15/33 [45%]) is significantly higher ($P = 0.001$) than that in the controls. The results of the Fisher exact comparison of incidences in the high-dose and control groups indicates a probability level of 0.037, which is above the 0.025 significance level required by the Bonferroni inequality criterion when multiple comparison is considered. No such tumors are seen in the controls. Although the results of the Cochran-Armitage test on the combined incidences of squamous-cell papilloma and carcinoma of the ear canal are not significant, an indicated departure from linear trend is observed ($P = 0.002$), because the incidence in the low-dose group is greater than that in the high-dose group. This difference may reflect the early mortality of the high-dose group when compared with that of the low-dose group. The first such tumor in the high-dose group was observed at 25 weeks.

In female rats, tumors of the ear canal appear in incidences of 0/15 in the controls, 6/33 (18%) in the low-dose, and 3/33 (9%)

in the high-dose groups. While these incidences are not statistically significant, each of the dosed groups has animals with tumors, and none appear in the controls.

Adenocarcinomas of the uterus are observed in the dosed females, but not in the controls. The results of the Cochran-Armitage test and of the Fisher exact test are all significant ($P < 0.001$), with a similarly high significant departure from linear trend ($P < 0.001$), due to the steep increase in incidence in the low-dose group. The statistical conclusion is that the incidence of these tumors of the uterus in female rats is dose related.

In summary, the administration of 4,4′-thiodianiline in Fischer 344 rats at the doses of this experiment is statistically associated with tumors of the liver and of the ear canal in males, of the thyroid in males and females, and of the uterus in females.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were markedly reduced (figure 3). High and low doses caused about the same reduction in weight. Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. No other clinical signs of toxicity were recorded.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed 4,4′-thiodianiline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4.

In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant ($P \leq 0.001$). In each sex, 29/35 (83%) of the high-dose animals and 34/35 (97%) of the low-dose animals lived at least as long as week 52 on study. In the control animals, all (14/14) of the males and 12/14 (86%) of the females lived beyond week 52 on study.

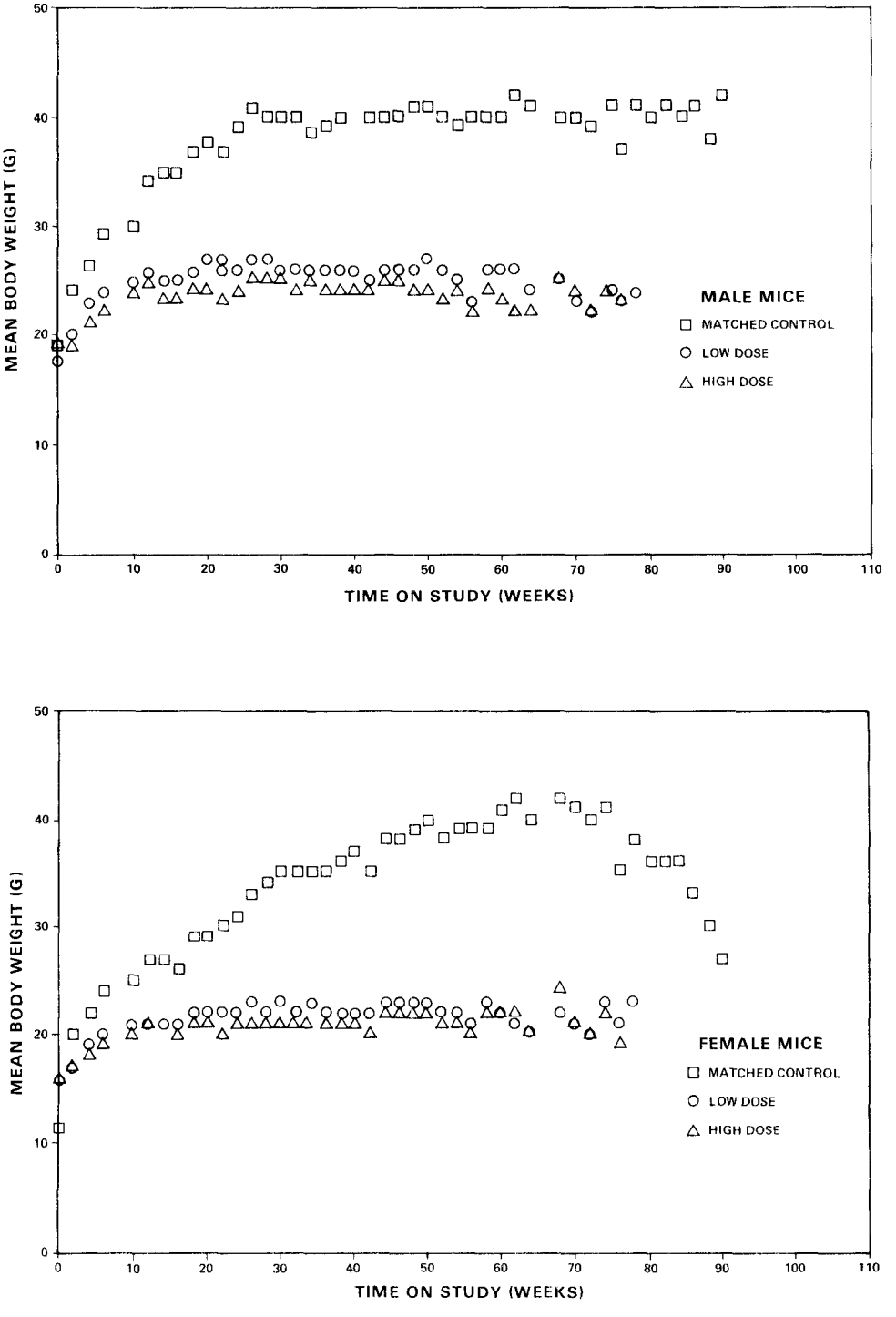


Figure 3. Growth Curves for Mice Fed 4, 4'-Thiodianiline in the Diet

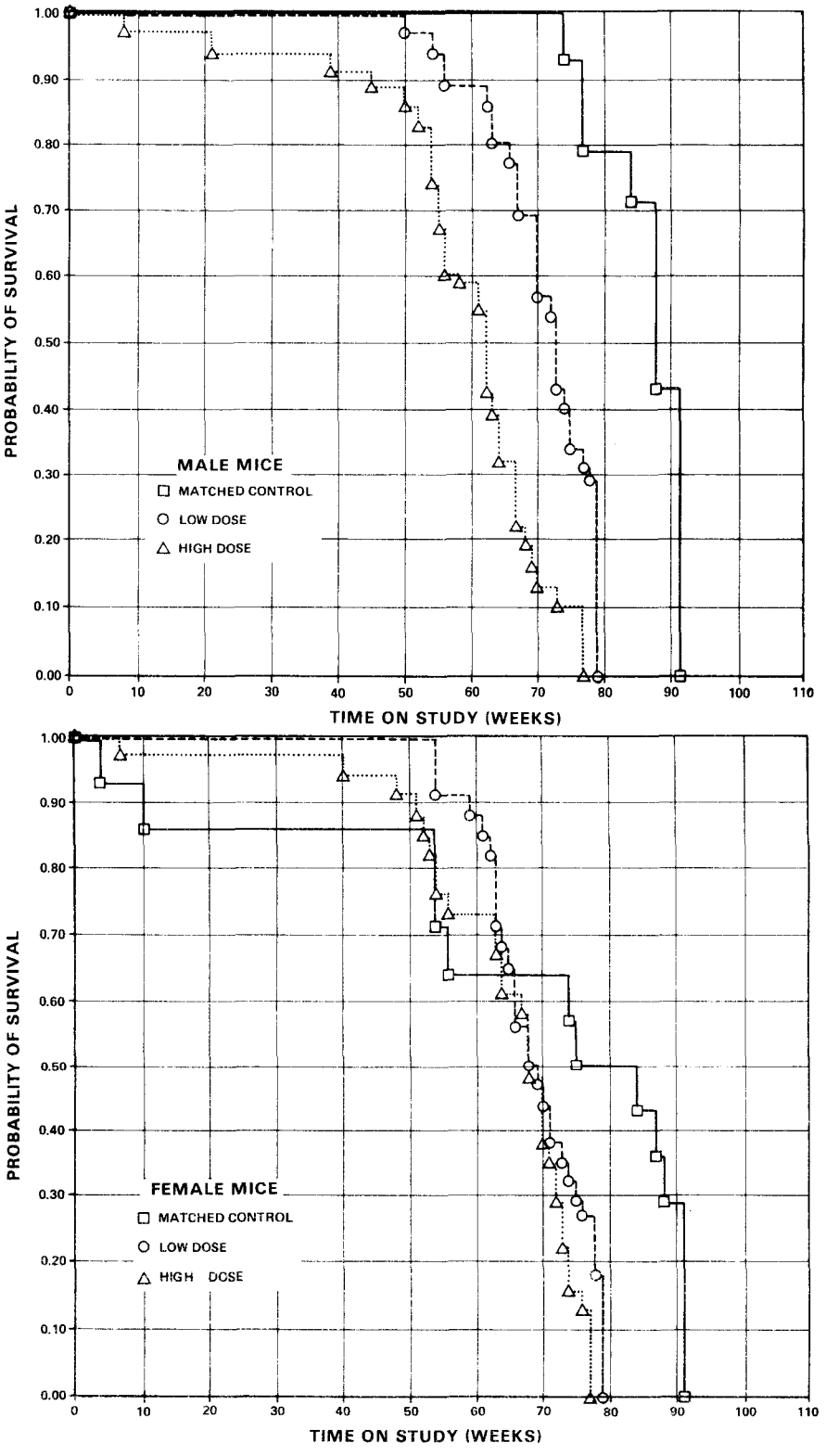


Figure 4. Survival Curves for Mice Fed 4, 4'-Thiodianiline in the Diet

C. Pathology (Mice)

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 neoplasms occurred in both the control and dosed groups. Some types of neoplasms occurred only, or with a greater frequency, in mice of dosed groups when compared with controls. These lesions are not uncommon in this strain of mouse independent of the administration of any chemical. However, many of the tumors observed in the mice appeared to be chemically induced. These tumors occurred at a high incidence in the dosed groups when compared with the controls; most were malignant, and some had metastasized to one or more locations. The incidences of these tumors were as follows:

	MICE					
	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Liver*	(13)	(34)	(24)	(12)	(34)	(31)
Hepatocellular carcinoma	1	32	22	0	32	30
Thyroid*	(14)	(33)	(23)	(11)	(33)	(30)
Follicular-cell adenoma	0	8	0	0	9	5
Follicular-cell carcinoma	0	15	20	0	3	15
Adenoma, NOS (small-cell)	0	0	0	0	0	2

*Number of mice with tissue examined microscopically.

The morphology of the hepatocellular carcinomas was similar to that described in the rats, with most carcinomas being trabecular in appearance. In 3/26 (12%) high-dose males, 3/32 (9%) high-dose females, and 2/34 (6%) low-dose females, the neoplastic cells had invaded through the hepatic capsule and had transplanted to the mesentery. Carcinomas were metastatic to the lungs in 4/28 (14%) high-dose and 3/34 (9%) low-dose females and to the kidneys in 1/34 (3%) low-dose males and 1/31 (3%) high-dose females.

The morphology of the thyroid follicular-cell adenocarcinomas was similar to that described in the rats. Invasion of blood vessels, lymphatics, and adjacent tracheal and esophageal tissues was also observed, but not as frequently as in the rats. Pulmonary metastases were observed in 2/23 (9%) high-dose and 1/34 (3%) low-dose males.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. Most of these nonneoplastic lesions are commonly seen in aged mice; however, the follicular-cell hyperplasias involving the thyroid were often bilateral and observed in only the dosed mice.

Feeding B6C3F1 mice 4,4′-thiodianiline for 18 months resulted in

an increased incidence of tumors in all dosed groups. The tumors were all of epithelial origin, and included hepatocellular carcinomas and thyroid follicular-cell adenomas, carcinomas, and adenomas (small-cell type). Also, the high incidence of thyroid follicular-cell hyperplasia appeared to be chemically related.

In the judgment of the pathologists, 4,4′-thiodianiline was carcinogenic for B6C3F1 mice under the conditions of this study.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of hepatocellular carcinoma and of the Fisher exact comparisons of incidences in the dosed and control groups are all significant ($P < 0.001$). An indicated departure from linear trend ($P < 0.001$) is also observed in each sex, due to the sharp increase of incidences in the dosed group (males: matched controls 1/13 [8%], low-dose 32/34 [94%], high-dose 22/24 [92%]; females: matched controls 0/12, low-dose 32/34 [94%], high-dose 30/31 [97%]). The statistical conclusion is that the incidence of hepatocellular

carcinoma in mice is associated with 4,4′-thiodianiline at the doses of this experiment.

In both male and female mice, follicular-cell carcinomas of the thyroid were observed exclusively in the dosed animals, and the results of the Cochran-Armitage test are significant ($P < 0.001$). In the male mice, the results of the Fisher exact test show that the incidences in both the high- and low-dose groups are significantly higher than those in the controls ($P \leq 0.001$); in the females, the incidence in the high-dose group, but not the low-dose group, is significantly higher than that in the controls ($P = 0.002$). The statistical conclusion is that the incidence of follicular-cell carcinoma of the thyroid in mice is dose associated. When the incidences of follicular-cell adenoma and carcinoma are grouped for analyses, increased significance is observed in each sex over those of adenoma or carcinoma, taken separately.

V. DISCUSSION

In this bioassay, 4,4′-thiodianiline was toxic to both rats and mice, since there was a marked depression in mean weights in each of the dosed groups compared with corresponding control groups over the entire study, and all animals died prior to the end of the scheduled period of administration of the chemical. The low- and high-dose male rats and the high-dose female rats died by week 68 or 69, and the low-dose female rats by week 72. The low-dose male and female mice died by week 79, and the high-dose male and female mice by week 77.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals. Metastases were observed from many of these tumors. In addition, hyperplasias of the liver, of the follicular cells of the thyroid, and of the alveolar cells of the lungs were found in greater numbers in the dosed than in the control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls

0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males are both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high-dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth, and only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of 4,4′-thiodianiline. Adenocarcinoma of the colon occurred in six low-dose rats and in one high-dose rat, but in no controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of the chemical.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the dosed females at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33); however, no such tumors occurred among the 235 historical-control female rats at this laboratory. The tumors of the ear canal are therefore considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at a statistically significant incidence ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant

(P = 0.002) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of females were significantly higher (low-dose P = 0.025, high-dose P < 0.001) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

No long-term studies with continuous administration of 4,4′-thiodianiline have been previously reported. The chemical was considered to be weakly active in a mammary tumor test system using young female Sprague-Dawley rats. In this system, the test chemicals were administered by gavage on 10 consecutive days, and the animals were necropsied 9 months later (Griswold et al., 1968). No evidence of mammary neoplasms was seen in the present bioassay with Fischer 344 rats.

It is concluded that under the conditions of this bioassay, 4,4′-thiodianiline was carcinogenic for Fisher 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4′-Thiodianiline was carcinogenic for B6C3F1 mice, inducing tumors in the liver and thyroid of both males and females.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED 4,4′-THIODIANILINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		4 (12%)	
SQUAMOUS CELL CARCINOMA		1 (3%)	
BASAL-CELL CARCINOMA		1 (3%)	1 (3%)
*SUBCUT TISSUE	(15)	(33)	(33)
SEBACEOUS ADENOMA		1 (3%)	
SARCOMA, NOS	1 (7%)	1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA		4 (12%)	
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	1 (3%)
HEPATOCELLULAR CARCINOMA, METAST		7 (21%)	1 (3%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (9%)	
FOLLICULAR-CELL CARCINOMA, METAS		15 (45%)	17 (52%)
SARCOMA, NOS, METASTATIC	1 (7%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(15)	(32)	(33)
FOLLICULAR-CELL CARCINOMA, METAS			1 (3%)
#MEDIASTINAL L.NODE			(3)
FOLLICULAR-CELL CARCINOMA, METAS			3 (100%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(33)
HEPATOCELLULAR ADENOMA		2 (6%)	2 (6%)
HEPATOCELLULAR CARCINOMA		21 (64%)	10 (30%)
FOLLICULAR-CELL CARCINOMA, METAS			1 (3%)
SARCOMA, NOS, METASTATIC	1 (7%)		
#SMALL INTESTINE	(15)	(32)	(33)
MUCINOUS ADENOCARCINOMA			1 (3%)
#COLON	(15)	(32)	(33)
ADENOCARCINOMA, NOS		6 (19%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
HAMARTOMA	1 (7%)		
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(24)	(27)
CHROMOPHOBE ADENOMA			1 (4%)
#THYROID	(15)	(33)	(33)
FOLLICULAR-CELL ADENOMA		2 (6%)	
FOLLICULAR-CELL CARCINOMA		28 (85%)	32 (97%)
C-CELL CARCINOMA	1 (7%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(15)	(33)	(33)
ADENOMA, NOS			1 (3%)
#TESTIS	(15)	(30)	(33)
INTERSTITIAL-CELL TUMOR	10 (67%)		
NERVOUS SYSTEM			
#BRAIN	(15)	(33)	(33)
ASTROCYTOMA	1 (7%)		1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		10 (30%)	2 (6%)
SQUAMOUS CELL CARCINOMA		5 (15%)	6 (18%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA, METASTA		1 (3%)	
*MESENTERY	(15)	(33)	(33)
HEPATOCELLULAR CARCINOMA, METAST		1 (3%)	
MUCINOUS ADENOCARCINOMA, METASTA			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@		7	3
MORIBUND SACRIFICE	9	28	32
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	6		
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	32	33
TOTAL PRIMARY TUMORS	14	90	58
TOTAL ANIMALS WITH BENIGN TUMORS	10	15	5
TOTAL BENIGN TUMORS	11	20	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	30	33
TOTAL MALIGNANT TUMORS	3	70	52
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	20	18
TOTAL SECONDARY TUMORS	2	25	25
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(33)	(33)
SARCOMA, NOS			1 (3%)
FIBROMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(32)	(32)
ADENOCARCINOMA, NOS, METASTATIC		14 (44%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (6%)	
FOLLICULAR-CELL CARCINOMA, METAS			11 (34%)
ADENOSQUAMOUS CARCINOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(33)	(33)
UNDIFFERENTIATED LEUKEMIA	1 (7%)		
#MEDIASTINAL L.NODE		(3)	(1)
FOLLICULAR-CELL CARCINOMA, METAS			1 (100%)
#ABDOMINAL LYMPH NODE		(3)	(1)
ADENOCARCINOMA, NOS, METASTATIC		1 (33%)	
#MESENTERIC L. NODE		(3)	(1)
ADENOCARCINOMA, NOS, METASTATIC		2 (67%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(32)	(33)
HEPATOCELLULAR ADENOMA		1 (3%)	2 (6%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA		5 (16%)	1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(22)	(27)
CHROMOPHOBE ADENOMA	3 (23%)		
#THYROID	(14)	(33)	(32)
FOLLICULAR-CELL CARCINOMA		24 (73%)	32 (100%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(33)
ADENOCARCINOMA, NOS		1 (3%)	
FIBROADENOMA	2 (13%)		
*PREPUTIAL GLAND	(15)	(33)	(33)
ADENOMA, NOS		2 (6%)	
*VAGINA	(15)	(33)	(33)
SQUAMOUS CELL CARCINOMA		1 (3%)	
#UTERUS	(15)	(33)	(32)
ADENOCARCINOMA, NOS		31 (94%)	23 (72%)
SARCOMA, NOS	1 (7%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(33)	(33)
SQUAMOUS CELL PAPILLOMA		5 (15%)	3 (9%)
SQUAMOUS CELL CARCINOMA		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(15)	(33)	(33)
ADENOCARCINOMA, NOS, METASTATIC		12 (36%)	2 (6%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	16	7
MORIBUND SACRIFICE	8	19	27
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	5		
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	32	33
TOTAL PRIMARY TUMORS	7	75	62
TOTAL ANIMALS WITH BENIGN TUMORS	4	7	4
TOTAL BENIGN TUMORS	5	9	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	32	33
TOTAL MALIGNANT TUMORS	2	66	57
TOTAL ANIMALS WITH SECONDARY TUMORS#		18	12
TOTAL SECONDARY TUMORS		29	14
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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED 4,4′-THIODIANILINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	14	34	26
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	26
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(34)	(23)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	
FOLLICULAR-CELL CARCINOMA, METAS		1 (3%)	2 (9%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(24)
HEPATOCELLULAR ADENOMA	3 (23%)	1 (3%)	1 (4%)
HEPATOCELLULAR CARCINOMA	1 (8%)	32 (94%)	22 (92%)
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(25)
HEPATOCELLULAR CARCINOMA, METAST		1 (3%)	
TUBULAR-CELL ADENOMA		1 (3%)	
TUBULAR-CELL ADENOCARCINOMA		1 (3%)	
#URINARY BLADDER	(14)	(33)	(23)
TRANSITIONAL-CELL CARCINOMA		1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(14)	(33)	(23)
FOLLICULAR-CELL ADENOMA		8 (24%)	
FOLLICULAR-CELL CARCINOMA		15 (45%)	20 (87%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(14)	(34)	(26)
HEPATOCELLULAR CARCINOMA, METAST			3 (12%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	14	35	35
NATURAL DEATH@	3	13	14
MORIBUND SACRIFICE	11	22	18
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE			
ANIMAL MISSING			3

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	34	24
TOTAL PRIMARY TUMORS	4	60	43
TOTAL ANIMALS WITH BENIGN TUMORS	3	10	1
TOTAL BENIGN TUMORS	3	10	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	34	23
TOTAL MALIGNANT TUMORS	1	50	42
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	5
TOTAL SECONDARY TUMORS		2	5
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 FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	12	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(12)	(34)	(32)
BASAL-CELL CARCINOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(12)	(34)	(28)
HEPATOCELLULAR CARCINOMA, METAST		3 (9%)	4 (14%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(12)	(34)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(12)	(34)	(31)
HEPATOCELLULAR ADENOMA			2 (6%)
HEPATOCELLULAR CARCINOMA		32 (94%)	30 (97%)
URINARY SYSTEM			
#KIDNEY	(12)	(34)	(31)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
TUBULAR-CELL ADENOCARCINOMA		2 (6%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(12)	(34)	(30)
TRANSITIONAL-CELL PAPILLOMA		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(11)	(33)	(30)
ADENOMA, NOS			2 (7%)
FOLLICULAR-CELL ADENOMA		9 (27%)	5 (17%)
FOLLICULAR-CELL CARCINOMA		3 (9%)	15 (50%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(34)	(32)
FIBROADENOMA	1 (8%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(12)	(34)	(32)
HEPATOCELLULAR CARCINOMA, METAST		2 (6%)	3 (9%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	14	35	35
NATURAL DEATH@	2	20	11
MORIBUND SACRIFICE	12	14	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE			
ANIMAL MISSING			3
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	33	30
TOTAL PRIMARY TUMORS	2	49	54
TOTAL ANIMALS WITH BENIGN TUMORS	1	10	8
TOTAL BENIGN TUMORS	1	10	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	32	30
TOTAL MALIGNANT TUMORS	1	39	45
TOTAL ANIMALS WITH SECONDARY TUMORS#		5	8
TOTAL SECONDARY TUMORS		5	8
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 FED 4,4′-THIODIANILINE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	1 (3%)
RESPIRATCRY SYSTEM			
#LUNG	(15)	(33)	(33)
EMPHYSEMA, NOS			4 (12%)
EPIDERMAL INCLUSION CYST		5 (15%)	
ERONCHOPNEUMONIA SUPPURATIVE		5 (15%)	2 (6%)
ERONCHOPNEUMONIA CHRONIC SUPPURA		6 (18%)	3 (9%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		15 (45%)	
METAPLASIA, SQUAMOUS		7 (21%)	
#LUNG/ALVEOLI	(15)	(33)	(33)
METAPLASIA, SQUAMOUS		5 (15%)	
HEMATOPCIETIC SYSTEM			
#BONE MARROW	(12)	(33)	(32)
ATRCPHY, NOS	6 (50%)		
CIRCULATORY SYSTEM			
NCNE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(33)
INFLAMMATION, GRANULOMATOUS			1 (3%)
CYTOPLASMIC CHANGE, NOS		1 (3%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR		4 (12%)	10 (30%)
*BILE DUCT	(15)	(33)	(33)
HYPERPLASIA, NOS		8 (24%)	25 (76%)
#PANCREAS	(15)	(32)	(33)
INFLAMMATION, CHRONIC		1 (3%)	
FIBROSIS		1 (3%)	
#PANCREATIC ACINUS	(15)	(32)	(33)
ATROPHY, NOS		1 (3%)	
#ESOPHAGUS	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
INFLAMMATION, CHRONIC	11 (73%)	4 (13%)	4 (12%)
#KIDNEY/PELVIS	(15)	(32)	(33)
HYPERPLASIA, EPITHELIAL		1 (3%)	4 (12%)
ENDOCRINE SYSTEM			
#THYROID	(15)	(33)	(33)
CYSTIC FOLLICLES	9 (60%)	4 (12%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(15)	(33)	(33)
HYPERPLASIA, NODULAR			1 (3%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN		1 (3%)	
*EAR CANAL	(15)	(33)	(33)
EPIDERMAL INCLUSION CYST		1 (3%)	
INFLAMMATICN, CHRONIC SUPPURATIV		2 (6%)	1 (3%)
HYPERKERATOSIS		2 (6%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(15)	(33)	(33)
INFLAMMATION, CHRONIC			1 (3%)
ALL CTHER SYSTEMS			
NONE			
SPECIAL MCRPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY		2	2

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	33
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE	1 (7%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	1 (3%)	
#LUNG/BRONCHUS	(15)	(32)	(32)
BRONCHIECTASIS	1 (7%)	1 (3%)	
#LUNG	(15)	(32)	(32)
EPIDERMAL INCLUSION CYST		2 (6%)	
EDEMA, NOS			1 (3%)
HEMORRHAGE			1 (3%)
INFLAMMATION, INTERSTITIAL		1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE			2 (6%)
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)	1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		13 (41%)	2 (6%)
METAPLASIA, SQUAMOUS		2 (6%)	
#LUNG/ALVEOLI	(15)	(32)	(32)
METAPLASIA, SQUAMOUS		2 (6%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(13)	(32)	(31)
ATROPHY, NOS	4 (31%)	1 (3%)	2 (6%)
#SPLEEN	(15)	(33)	(33)
HEMATOPOIESIS	1 (7%)	3 (9%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(32)	(33)
INFLAMMATION, INTERSTITIAL		1 (3%)	
HYPERPLASIA, NODULAR		1 (3%)	8 (24%)
HYPERPLASIA, NOS			1 (3%)
#LIVER/PERIPORTAL	(15)	(32)	(33)
FIBROSIS		1 (3%)	
*BILE DUCT	(15)	(33)	(33)
HYPERPLASIA, NOS		5 (15%)	12 (36%)
HYPERPLASIA, CYSTIC		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(32)	(33)
HYDRONEPHROSIS		1 (3%)	
INFLAMMATION, CHRONIC	5 (33%)		
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(22)	(27)
HYPERPLASIA, CHROMOPHOBE-CELL	2 (15%)		
#THYROID	(14)	(33)	(32)
CYSTIC FOLLICLES	4 (29%)	7 (21%)	
HYPERPLASIA, FOLLICULAR-CELL		7 (21%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(33)
CYST, NOS	1 (7%)		
#UTERUS	(15)	(33)	(32)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
#UTERUS/ENDOMETRIUM	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE		1 (3%)	4 (13%)
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	5 (15%)	6 (19%)
HYPERPLASIA, NOS		2 (6%)	1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CYSTIC	2 (13%)		3 (9%)
NERVOUS SYSTEM			
#BRAIN	(14)	(33)	(33)
INFLAMMATION, NECROTIZING			1 (3%)
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV		2 (6%)	1 (3%)
*LENS CAPSULE	(15)	(33)	(33)
MINERALIZATION		1 (3%)	
*EAR CANAL	(15)	(33)	(33)
EPIDERMAL INCLUSION CYST			1 (3%)
HYPERKERATOSIS			2 (6%)
*MIDDLE EAR	(15)	(33)	(33)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*EPICARDIUM	(15)	(33)	(33)
THROMBOSIS, NOS		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ACCIDENTAL DEATH			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		2	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED 4,4′-THIODIANILINE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED 4,4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	14	34	26
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	26
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(23)
INFLAMMATION, SUPPURATIVE		2 (6%)	1 (4%)
#LUNG	(14)	(34)	(23)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)	6 (18%)	2 (9%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	6 (43%)	1 (3%)	1 (4%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (7%)	1 (3%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(14)	(34)	(25)
HEMATOPOIESIS		1 (3%)	
#MESENTERIC L. NODE	(3)	(1)	(1)
INFLAMMATION, SUPPURATIVE		1 (100%)	
HYPERPLASIA, LYMPHOID	2 (67%)		1 (100%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(24)
HYPERPLASIA, NODULAR		1 (3%)	
*BILE DUCT	(14)	(34)	(26)
HYPERPLASIA, NOS		1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(25)
INFLAMMATION, CHRONIC		2 (6%)	
ENDOCRINE SYSTEM			
#THYROID	(14)	(33)	(23)
HYPERPLASIA, FOLLICULAR-CELL		29 (88%)	4 (17%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(14)	(34)	(26)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY			3
AUTO/NECROPSY/HISTO PERF			2
AUTOLYSIS/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 FED 4, 4'-THIODIANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	35	35
ANIMALS MISSING			3
ANIMALS NECROPSIED	12	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	34	31
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(12)	(33)	(30)
INFLAMMATION, SUPPURATIVE	2 (17%)		
#LUNG	(12)	(34)	(28)
BRONCHOPNEUMONIA SUPPURATIVE	1 (8%)	3 (9%)	
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA	10 (83%)	1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (8%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(12)	(33)	(30)
ANGIECTASIS		1 (3%)	
HEMATOPOIESIS	1 (8%)		1 (3%)
#MESENTERIC L. NODE	(3)	(1)	(2)
INFLAMMATION, SUPPURATIVE	1 (33%)	1 (100%)	2 (100%)
HYPERPLASIA, LYMPHOID	1 (33%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*BILE DUCT	(12)	(34)	(32)
HYPERPLASIA, NOS			4 (13%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*RECTUM	(12)	(34)	(32)
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
URINARY SYSTEM			
#URINARY BLADDER	(12)	(34)	(30)
HYPERPLASIA, EPITHELIAL		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(11)	(33)	(30)
CYSTIC FOLLICLES		4 (12%)	
HYPERPLASIA, FOLLICULAR-CELL		31 (94%)	26 (87%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(12)	(31)	(30)
INFLAMMATION, SUPPURATIVE	1 (8%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
HYPERPLASIA, CYSTIC	8 (67%)	2 (6%)	
#OVARY	(12)	(31)	(30)
CYST, NOS	1 (8%)		
HEMORRHAGE			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY			3
NECROPSY PERF/NO HISTO PERFORMED			1
AUTOLYSIS/NO NECROPSY	2	1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED 4,4′-THIODIANILINE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Skin: Squamous-cell Papilloma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	48	--
Skin: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	5/33 (15)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure fom Linear Trend[e]	P = 0.007		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.613	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	48	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Squamous-cell Carcinoma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	54	--
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	3/33 (9)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.039		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.291	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	50	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/15 (0)	4/33 (12)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.450	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	50	--
Liver: Hepatocellular Carcinoma[b]	0/15 (0)	21/33 (64)	10/33 (30)
P Values[c,d]	N.S.	P < 0.001	P = 0.014
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.352	1.450
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	53

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/15 (0)	23/33 (70)	12/33 (36)
P Values[c,d]	N.S.	P < 0.001	P = 0.005
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.713	1.790
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	53
Colon: Adenocarcinoma, NOS[b]	0/15 (0)	6/32 (19)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.010		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.803	0.026
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	65

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/15 (0)	28/33 (85)	32/33 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.685	5.827
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	42	32
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/15 (0)	30/33 (91)	32/33 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		5.156	5.827
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	42	32

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Ear Canal: Squamous-cell Carcinoma[b]	0/15 (0)	5/33 (15)	6/33 (18)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.613	0.778
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	44	25
Ear Canal: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	15/33 (45)	8/33 (24)
P Values[c,d]	N.S.	P = 0.001	P = 0.037
Departure from Linear Trend[e]	P = 0.002		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		2.305	1.112
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	35	25

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

[a]Treated groups received doses of 1,500 or 3,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	2/32 (6)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.147	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	63	--
Liver: Hepatocellular Carcinoma[b]	0/15 (0)	5/32 (16)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.023		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.632	0.025
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	69

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/15 (0)	6/32 (19)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.803	0.291
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	65
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	24/33 (73)	32/32 (100)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.025		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.660	6.059
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	46	44

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Ear Canal: Squamous-cell Carcinoma[b]	0/15 (0)	1/33 (3)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.026	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	58	--
Ear Canal: Squamous-cell Papilloma or Carcinoma[b]	0/15 (0)	6/33 (18)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.778	0.291
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	46	60

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Preputial Gland: Adenoma, NOS[b]	0/15 (0)	2/33 (6)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.142	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	63	--
Uterus: Adenocarcinoma, NOS[b]	0/15 (0)	31/33 (94)	23/32 (72)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		5.446	3.840
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	56	44

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed 4,4′-Thiodianiline in the Diet[a]

---

[a]Treated groups received doses of 1,500 or 3,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS

IN MICE FED 4,4′-THIODIANILINE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/13 (8)	32/34 (94)	22/24 (92)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		12.235	11.917
Lower Limit		2.762	2.601
Upper Limit		260.907	257.538
Weeks to First Observed Tumor	88	54	54
Liver: Hepatocellular Adenoma or Carcinoma[b]	4/13 (31)	33/34 (97)	23/24 (96)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		3.154	3.115
Lower Limit		1.633	1.555
Upper Limit		4.281	4.280
Weeks to First Observed Tumor	88	54	50

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Kidney: Tubular-cell Adenoma or Adenocarcinoma[b]	0/14 (0)	2/34 (6)	0/25 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.130	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	70	--
Thyroid: Follicular-cell Carcinoma[b]	0/14 (0)	15/33 (45)	20/23 (87)
P Values[c,d]	P < 0.001	P = 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		2.163	4.504
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	54

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/14 (0)	22/33 (67)	20/23 (87)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.041		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		3.316	4.504
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	54

[a]Treated groups received doses of 2,500 or 5,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P < 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when P < 0.05 for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4′-Thiodianiline in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/12 (0)	32/34 (94)	30/31 (97)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		4.451	4.712
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	54	40
Kidney: Tubular-cell Adenocarcinoma[b]	0/12 (0)	2/34 (6)	0/31 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.113	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	78	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4′-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/11 (0)	3/33 (9)	15/30 (50)
P Values[c,d]	P < 0.001	N.S.	P = 0.002
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.220	1.926
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	63	40
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/11 (0)	11/33 (33)	18/30 (60)
p Values[c,d]	P < 0.001	P = 0.025	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		1.228	2.363
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	59	40

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed 4,4'-Thiodianiline in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Adenoma, NOS[b]	0/11 (0)	0/33 (0)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite
Lower Limit		--	0.119
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	56

[a]Treated groups received doses of 2,500 or 5,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Review of the Bioassay of 4,4'-Thiodianiline* 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 4,4'-Thiodianiline was reviewed.

The primary reviewer agreed with the conclusion in the report that 4,4'-Thiodianiline was carcinogenic in both rats and mice, under the conditions of test. He noted that the subchronic study was performed in a different species of rat and mouse than used in the chronic phase. From the appearance of the weight curves, the primary reviewer said that it was likely that the MTD was exceeded in both species. Despite this deficiency, 4,4'-Thiodianiline was clearly carcinogenic in the treated animals. He concluded that his only reservation concerning the probability of a human carcinogenic risk from 4,4-Thiodianiline would be that its effect was elicited at highly toxic levels in the treated animals.

The secondary reviewer noted the inadequate number of animals used in the study. Irrespective of the deficiency, he said that it was clear that 4,4'-Thiodianiline was carcinogenic in the treated animals.

It was moved that the report on 4,4'-Thiodianiline be accepted as written and that it be presumed to pose a carcinogenic risk to humans. 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.

*U.S. GOVERNMENT PRINTING OFFICE: 1978 260-899/3039 1-3

DHEW Publication No. (NIH) 78-847