

**Table 4** Summary of the results of the newborn and young rat study of 2,4-di-*tert*-butylphenol

Newborn rat study				
Dose (mg/kg/day)	5	40	300	
Death	–	–	M: 2/12, F: 1/12	
Clinical signs	–	–	Decrease in locomotor activity bradypnea, hypothermia	
Body weight changes	–	–	9–25%↓	
Urinalysis	n.d.	n.d.	n.d.	
Hematological changes	–	–	–	
Blood biochemical changes	–	–	Various†	
Changes in relative organ weights	–	F: Liver 14%↑	Liver 39–51%↑, Kidney 37–41%↑ M: Spleen 24%↓	
Histopathological findings	–	F: Fatty degeneration in liver	Various changes in liver and kidney‡	
Developmental parameters	–	–	Slight delay in preputial separation	
Young rat study				
Dose (mg/kg/day)	5	20	75	300
Death	–	–	–	–
Clinical signs	–	–	–	–
Body weight changes	–	–	–	–
Urinalysis	–	–	–	UV↑ SG↓ OP↓
Hematological changes	–	–	–	Various§
Blood biochemical changes	–	–	F: Tcho↑ Pho↑	M: TB↑
Changes in relative organ weights	–	–	F: Liver 13%↑	F: Tcho↑ Pho↑ Liver 40–43%↑
Histopathological findings	n.d.	n.d.	–	Various changes in liver and kidney¶

Data on death are shown as no. of dead animals/no. of animals examined, according to sex. Statistically significant increases ( $P < 0.05$ ) in body weights, urinalysis and blood biochemical parameters, and relative organ weights are shown as ↑, while decreases are shown as ↓. Changes observed only in males or females are shown as 'M' or 'F', respectively, while neither 'M' nor 'F' is mentioned in the case of changes noted in both sexes. †Increase in total bilirubin and decrease in the A/G ratio in both sexes, increase in  $\gamma$ -GTP in males, and increase in total protein and BUN in females were noted. ‡Various changes were observed as shown in Table 3. §Various hematological changes were noted such as decrease in hemoglobin and hematocrit and increase in segmented neutrophils in females and prolongation of PT and APTT in males. ¶Various changes were observed as shown in Table 5. OP: osmotic pressure; Pho: phospholipid; SG: specific gravity; TB: total bilirubin; Tcho: total cholesterol; UV: urine volume; –: no change; n.d.: not determined.

or critical histopathological damage (Koizumi *et al.* 2001). We here tried to apply this UETL approach to the present study. For 2TBP, clinical signs such as decrease in locomotor activity and ataxic gait were noted in most of the animals given 200 mg/kg (newborn rats) and 500 mg/kg (young rats) (Table 2). Furthermore, a 8–17% lowering of body weight was observed at 200 mg/kg in newborn rats, but not in the young rat study. Therefore, equivalent toxic effects to these observed at 500 mg/kg in young rats might be expected to appear at 100–150 mg/kg in newborn animals. The UETLs were concluded to be 100–150 and 500 mg/kg/day in newborn and young rats, respectively. In the case of DTBP, clear toxicity was observed at the top dose of 300 mg/kg in both newborn and young rat studies (Table 4), but the level of severity was very different, for example, deaths were only noted in the newborn cases. It was considered difficult to estimate the UETLs from the results of main studies only. However, the most critical endpoint for toxicity, mortality, was also noted at 100 mg/kg and more, and 500 mg/kg, in the dose-finding studies of newborn and young rats, respectively. Therefore, it would be possible to estimate the appropriate UETLs as the minimum lethal dose by taking the results of the dose-finding

studies into consideration. The UETLs were concluded to be 100 mg/kg/day for the newborn, and 500 mg/kg/day for young rats, at which one out of eight rats was found dead in both cases. These analyzes of UETLs, considering equivalence in toxic degree, showed 3.3–5.0 times higher susceptibility of newborn rats to 2TBP and DTBP than young rats, consistent with our analytical results for NOAELs.

Higher susceptibility of newborn rats was also demonstrated in our previous analyzes of five phenols (4-nitrophenol, 2,4-dinitrophenol, 3-aminophenol, 3-methylphenol and 2,4,6-trinitrophenol) (Koizumi *et al.* 2001, 2002, 2003; Takahashi *et al.* 2004), considered mainly due to their poor metabolic and excretory capacity (Horster 1977; Cresteil *et al.* 1986). It has actually been reported that UDP-glucuronyltransferase and sulfotransferase activities, when 4-nitrophenol is used as the substrate, are lower in microsomes prepared from livers of newborn rats, and that the elimination rate of 2,4-dinitrophenol from serum of newborn rabbits is markedly slower than in young adults (Gehring & Buerge 1969; Matsui & Watanabe 1982). Unfortunately, there is no information on the toxicity mechanism and toxicokinetics of both 2TBP

**Table 5** Histopathological findings for the young rat study of 2,4-di-*tert*-butylphenol

Dose (mg/kg/day)	Grade	Scheduled-sacrifice group†			Recovery group	
		0	75	300	0	300
No. of animals examined (Male/Female)		6/6	6/6	6/6	6/6	6/6
Liver						
- Centrilobular hypertrophy of hepatocytes	+	0/0	0/0	4/4	0/0	0/0
Kidneys						
- Basophilic tubules	+	0/0	0/0	1/4	0/0	3/1
	++	0/0	0/0	4/0	0/0	2/0
	+++	0/0	0/0	1/1	0/0	1/0
- Granular casts	+	0/0	0/0	5/2	0/0	4/0
	++	0/0	0/0	1/1	0/0	0/0
- Proteinaceous casts	+	0/0	0/0	5/1	0/0	2/0
	++	0/0	0/0	1/0	0/0	0/0

†No histopathological examination was conducted for the 5 and 20 mg/kg scheduled-sacrifice groups. +, mild; ++, moderate; +++, marked.

and DTBP; however, the immature functions involved in the toxicokinetics in newborn rats would be implicated in the higher susceptibility, as in the case of five phenols previously analyzed. While there are very little data on toxicokinetics of environmental chemicals in the newborn, relatively plentiful information has been reported in humans for pharmaceuticals which are clinically applied during the early postnatal period. Recently, Ginsberg *et al.* (2002) conducted comparative analysis of pharmacokinetic parameters for 45 drugs in both children and adults, and showed half-lives in children aged two months or under to generally be two-fold longer than in adults.

As for the susceptibility of the newborn to toxicity of chemicals, although it is generally important to take the sensitivity of target organs and tissues themselves (toxicodynamics) into consideration besides toxicokinetics, there are insufficient data on differences between newborn and young/adult animals. For appearance of toxicity, which is the outcome of toxicokinetics and toxicodynamics, some comparative studies have relied on LD<sub>50</sub> values (Goldenthal 1971; Sheehan & Gaylor 1990). However, it is not considered that information on acute toxicity at lethal dosage is appropriate when considering the susceptibility of newborn in risk assessment, because dose-response curves could differ, as mentioned above. With prolonged, subtoxic doses, which are basis for TDI or ADI, our series of comparative studies constitute the first systematic assessment, providing an important base for development of new methods of risk assessment of susceptibility of the newborn.

In conclusion, clinical signs and effects on the liver were observed for 2TBP, and hepatic and renal toxicity for DTBP. Although there were no clear differences in toxicity profiles between the newborn and young rats for both chemicals, the toxicity levels differed markedly. The susceptibility of the newborn to these chemicals appears to be 4–5 times higher than that of young animals.

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#### REFERENCES

- Adams J (1986) Methods in behavioral teratology. In: Riley EP, Vorhees CV (eds). *Handbook of Behavioral Teratology*. Plenum Press, New York, pp. 67–97.
- Ashby J, Lefevre PA (2000) The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. *J Appl Toxicol* 20: 35–47.
- Bartlett MS (1937) Properties of sufficiency and statistical tests. *Proc R Soc Lond Ser A* 160: 268–282.
- Chemical Products' Handbook (2004) *14504 No Kagakushohin*. [Chemical Products of 14504]. The Chemical Daily Co., Ltd, Tokyo. (In Japanese).
- Cresteil T, Beaune P, Celier C, Leroux JP, Guengerich FP (1986) Cytochrome P-450 isoenzyme content and monooxygenase activities in rat liver: Effect of ontogenesis and pretreatment by phenobarbital and 3-methylcholanthrene. *J Pharmacol Exp Ther* 236: 269–276.
- Dourson M, Charney G, Scheuplein R (2002) Differential sensitivity of children and adults to chemical toxicity. II. Risk and regulation. *Regul Toxicol Pharmacol* 35: 448–467.
- Dunnnett CW (1964) New tables for multiple comparisons with a control. *Biometrics* 20: 482–491.
- Fisher RA (1922) On the interpretation of chi-square from contingency tables and the calculation of P. *J R Stat Soc* 85: 87–94.
- Fukuda N, Ito Y, Yamaguchi M *et al.* (2004) Unexpected nephrotoxicity induced by tetrabromobisphenol A in newborn rats. *Toxicol Lett* 150: 145–155.
- Gaytan F, Bellido C, Aguilar R, Aguilar E (1988) Balanopreputial separation as an external sign of puberty in the rat: Correlation with histologic testicular data. *Andrologia* 20: 450–453.
- Gehring PJ, Buerge JF (1969) The distribution of 2,4-dinitrophenol relative to its cataractogenic activity in ducklings and rabbits. *Toxicol Appl Pharmacol* 15: 574–592.
- Ginsberg G, Hattis D, Sonawane B *et al.* (2002) Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol Sci* 66: 185–200.
- Goldenthal EI (1971) A compilation of LD50 values in newborn and adult animals. *Toxicol Appl Pharmacol* 18: 185–207.
- Hirata-Koizumi M, Kusuoka O, Nishimura N *et al.* (2005) Susceptibility of newborn rats to hepatotoxicity of 1,3-dibromopropane and 1,1,2,2-tetrabromomethane, compared with young rats. *J Toxicol Sci* 30: 29–42.
- Hollander M, Wolfe DA (1973) *Nonparametric Statistical Methods*. John Wiley and Sons, New York.

- Horster M (1977) Nephron function and perinatal homeostasis. *Ann Rech Vet* **8**: 468–482.
- Jensh RP, Brent RL (1988) The effects of prenatal X irradiation on the appearance of reflexes and physiologic markers in the neonatal rat. *Radiat Res* **116**: 416–426.
- Koizumi M, Nishimura N, Enami T *et al.* (2002) Comparative toxicity study of 3-aminophenol in newborn and young rats. *J Toxicol Sci* **27**: 411–421.
- Koizumi M, Noda A, Ito Y *et al.* (2003) Higher susceptibility of newborn than young rats to 3-methylphenol. *J Toxicol Sci* **28**: 59–70.
- Koizumi M, Yamamoto Y, Ito Y *et al.* (2001) Comparative study of toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. *J Toxicol Sci* **26**: 299–311.
- Landrigan PJ, Kimmel CA, Correa A, Eskenazi B (2004) Children's health and the environment: Public health issues and challenges for risk assessment. *Environ Health Perspect* **112**: 257–265.
- Matsui M, Watanabe HK (1982) Developmental alteration of hepatic UDP-glucuronosyltransferase and sulphotransferase towards androsterone and 4-nitrophenol in Wistar rats. *Biochem J* **204**: 441–447.
- MHLW (2001a) 2-tert-Butylphenol (88-18-6). In: Ministry of Health, Labor and Welfare (ed). *Toxicity Testing Reports of Environmental Chemicals*, Vol. 8. Chemical Investigation Promoting Council, Tokyo, pp. 208–218.
- MHLW (2001b) 2,4-Di-tert-butylphenol (96-76-4). In: Ministry of Health, Labor and Welfare (ed). *Toxicity Testing Reports of Environmental Chemicals*, Vol. 8. Chemical Investigation Promoting Council, Tokyo, pp. 372–386.
- NAS (National Academy of Sciences) (1993) *Pesticides in the Diets of Infants and Children*. National Academy Press, Washington, DC.
- New Chemical Index (2001) *Shin Kagaku Index*. [New Chemical Index.] The Chemical Daily Co., Ltd, Tokyo. (In Japanese).
- NIHS (1994) *Glossary of Terms for Toxicity Testing*. Biological Safety Research Center, National Institute of Health Sciences, Tokyo. (In Japanese).
- OECD (2004) Draft guidance document on reproductive toxicity testing and assessment, OECD environment, health and safety publications series on testing and assessment No. 43. November 10, 2004 (1st Version).
- Scheuplein R, Charnley G, Dourson M (2002) Differential sensitivity of children and adults to chemical toxicity. I. Biological basis. *Regul Toxicol Pharmacol* **35**: 429–447.
- Shafer TJ, Meyer DA, Crofton KM (2005) Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. *Environ Health Perspect* **113**: 123–136.
- Sheehan DM, Gaylor DW (1990) Analysis of the adequacy of safety factors. *Teratology* **41**: 590–591.
- Steel RD (1959) A multiple comparison rank sum test: Treatment versus control. *Biometrics* **15**: 560–572.
- Takahashi M, Ogata H, Izumi H *et al.* (2004) Comparative toxicity study of 2,4,6-trinitrophenol (picric acid) in newborn and young rats. *Cong Anom* **44**: 204–214.
- Zheng Q, Olivier K, Won YK, Pope CN (2000) Comparative cholinergic neurotoxicity of oral chlorpyrifos exposures in preweanling and adult rats. *Toxicol Sci* **55**: 124–132.

BIOASSAY OF  
1,5-NAPHTHALENEDIAMINE  
FOR POSSIBLE CARCINOGENICITY

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REPORT ON THE BIOASSAY OF 1,5-NAPHTHALENEDIAMINE  
FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of 1,5-naphthalenediamine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 1,5-naphthalenediamine was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

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

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the

statistical analysis was performed by Mr. W. W. Belew (5,8) and Mr. R. M. Helfand (5), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5,10), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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## SUMMARY

A bioassay of 1,5-naphthalenediamine for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 1,5-Naphthalenediamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low dietary concentrations utilized in the chronic bioassay were, respectively, 0.1 and 0.05 percent for rats and 0.2 and 0.1 percent for mice. The compound was administered in the diet for 103 weeks, followed by up to 4 weeks of observation. Fifty mice of each sex and 25 rats of each sex were placed on test as controls. These animals were observed for up to 110 weeks.

There were no significant positive associations between the administered concentrations of 1,5-naphthalenediamine and mortality in either sex of rats or mice. In all groups adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

Among dosed female rats, a statistically significant increase in endometrial stromal polyps was observed. Several of these tumors underwent malignant transformation to endometrial stromal sarcomas. The incidence of female rats having either adenoma or carcinoma of the clitoral gland was statistically significant. No neoplasms were observed at significantly increased incidences in dosed male rats. Based on lack of clinical signs or weight loss, the male rats may have been able to withstand a higher dose.

In mice, dose-related increases in thyroid neoplasms were observed in both sexes. The incidence of thyroid C-cell carcinomas was significant for high dose female mice. The combined incidences of papillary adenomas, follicular-cell adenomas and papillary cystadenomas of the thyroid were significant for mice of both sexes. The incidence of hepatocellular carcinomas and the incidence of alveolar/bronchiolar adenomas were each significant for dosed female mice.

Under the conditions of this bioassay, 1,5-naphthalenediamine was carcinogenic in female Fischer 344 rats, causing clitoral and uterine neoplasms. 1,5-Naphthalenediamine was also carcinogenic for B6C3F1 mice, producing thyroid neoplasms in males and neoplasms of the thyroid, liver, and lung in females. Insufficient evidence was provided for the carcinogenicity of the compound in male Fischer 344 rats.



TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION	1
II. MATERIALS AND METHODS	4
A. Chemicals	4
B. Dietary Preparation	5
C. Animals	5
D. Animal Maintenance	6
E. Selection of Initial Concentrations	9
F. Experimental Design	10
G. Clinical and Histopathologic Examinations	13
H. Data Recording and Statistical Analyses	14
III. CHRONIC TESTING RESULTS: RATS	20
A. Body Weights and Clinical Observations	20
B. Survival	20
C. Pathology	23
D. Statistical Analyses of Results	26
IV. CHRONIC TESTING RESULTS: MICE	39
A. Body Weights and Clinical Observations	39
B. Survival	39
C. Pathology	42
D. Statistical Analyses of Results	44
V. DISCUSSION	54
VI. BIBLIOGRAPHY	56
APPENDIX A	A-1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 1,5-NAPHTHALENEDIAMINE	
APPENDIX B	B-1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 1,5-NAPHTHALENEDIAMINE	
APPENDIX C	C-1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 1,5-NAPHTHALENEDIAMINE	
APPENDIX D	D-1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 1,5-NAPHTHALENEDIAMINE	

## I. INTRODUCTION

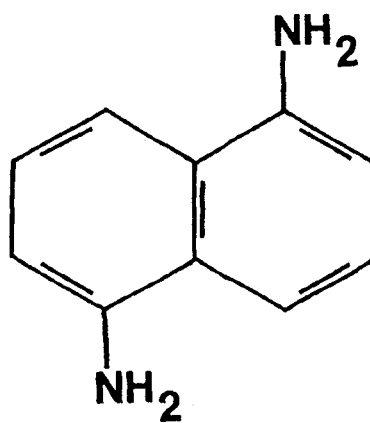
1,5-Naphthalenediamine (Figure 1) (NCI No. CO3021), a bicyclic aromatic amine used in the dye industry, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amines are one class of chemicals believed to contribute to the increased cancer risk in this industry (Wynder et al., 1963). The structural similarity of 1,5-naphthalenediamine to both the human bladder carcinogen 2-naphthylamine (International Agency for Research on Cancer [IARC], 1974) and the suspected carcinogen 1-naphthylamine (IARC, 1974) was an additional factor in its selection for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1,5-naphthalenediamine.\* It is also known as 1,5-diaminonaphthalene.

1,5-Naphthalenediamine can be used as an oxidation base (Colour Index [C.I.] 76595), an intermediate in the synthesis of the dye Naphthylene Red (C.I. 21650) (Society of Dyers and Colourists, 1956), and in the production of a black trisazo dye for cotton (Taube, 1973). 1,5-Naphthalenediamine has also been used as a precursor for 1,5-naphthalenediisocyanate (Hirai and Yamamoto, 1975); as an intermediate in the synthesis of drugs for the symptomatic treatment of asthma or

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\*The CAS registry number is 2243-62-1



**FIGURE 1**  
**CHEMICAL STRUCTURE OF 1,5-NAPHTHALENEDIAMINE**

2

rhinitis (Hall, 1976); as a component of piperazine-modified aromatic polyamides (Fujiwara et al., 1974); and as a modifier for phenolic resins used in rapid curing compounds (Freeman et al., 1974); however, these uses appear to be purely experimental.

Specific production data for 1,5-naphthalenediamine are not available; however, the exclusion of this compound from the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies that it is not produced in commercial quantities (in excess of 1000 pounds or \$1000 in value annually).

The potential for exposure to 1,5-naphthalenediamine may be greatest for workers in the dye industry and persons engaged in chemical research with this compound.

## II. MATERIALS AND METHODS

### A. Chemicals

1,5-Naphthalenediamine was purchased from Carroll Products, Wood River Junction, Rhode Island by the NCI for Mason Research Institute, Worcester, Massachusetts, and chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point of 190° to 191°C suggested a compound of high purity based on its narrow range and its close proximity to the value (190°C) reported in the literature (Pollock and Stevens, 1965). Elemental analysis was consistent with  $C_{10}H_{10}N_2$ , the molecular formula for 1,5-naphthalenediamine. However, nonaqueous amine group titration was approximately 89 to 90 percent of that expected on a theoretical basis. Vapor-phase chromatography revealed one homogeneous peak, but thin-layer chromatography utilizing two solvent systems (acetone:ammonium hydroxide and methylethylketone:formic acid), each visualized with 254 nm and 367 nm light, indicated the presence of one nonmotile impurity. Nuclear magnetic and infrared analyses were consistent with the structure of the compound. Ultraviolet analysis showed  $\lambda_{max}$  at 232, 328 and 498 nm with  $\epsilon$  values of 62,800, 10,640 and 9, respectively. The literature (Sadtler Standard Spectra) indicates a  $\lambda_{max}$  at 328.5 nm with  $\epsilon = 10,000$  for 1,5-naphthalenediamine. The observed  $\epsilon$  at 328 nm was 10,640 (6 percent greater than expected).

Throughout this report the term 1,5-naphthalenediamine is used to represent this compound.

## B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox<sup>®</sup> (Allied Mills, Inc., Chicago, Illinois). 1,5-Naphthalenediamine was administered to the dosed animals as a component of the diet. Under an exhaust hood, proper amounts of the chemical were removed from the stock bottle. The compound was blended in an aluminum bowl with an aliquot of the ground feed. Once visual homogeneity was attained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender, along with the remainder of the meal and blended for 20 minutes. Prepared diets were placed in double plastic bags and stored in the dark at 4°C. The mixture was used for 1 week only.

## C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All animals were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Dosed and control animals were received in separate shipments. Upon arrival, a sample of animals was examined for parasites and other signs of disease. All animals appeared to have parasites. They were treated with 3.0 gm of piperazine adipate per liter of drinking water, ad libitum, for 3 days, followed by 3 days of plain tapwater and 3 subsequent days of piperazine adipate administration. During this period, new cages

with fresh bedding were provided daily. Animals were held in quarantine by species for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

#### D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek<sup>®</sup> 15/40 denier Dacron<sup>®</sup> filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 14 months of study rats were housed in galvanized-steel wire-mesh cages suspended over newspapers. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, rats were held in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. SAN-I-CEL<sup>®</sup> corncob bedding (Paxton Processing Company, Paxton, Illinois) was used for the first 2 months that rats were housed in polycarbonate cages. For the remainder of the study, Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was used. Stainless steel cage racks were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate shoe box type cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc., Garfield, New Jersey). Nonwoven fiber filter bonnets were used over cage lids. Control mice were housed ten per cage for the first month of study and five per cage thereafter. Dosed mice were held five per cage throughout the study. Clean cages, lids, and bedding were provided twice per week. SAN-I-CEL<sup>®</sup> was used during the first 9 months of study. A second corncob bedding (Bed-o-Cobs<sup>®</sup>, The Andersons Cob Division, Maumee, Ohio) was used for the next 8 months. Aspen bedding was used for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available from 250 ml polycarbonate water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, water was supplied as needed between changes. Food and water were available ad libitum.

Wayne Lab-Blox<sup>®</sup> meal was supplied to rats for 12 months and mice for 11 months from Alpine<sup>®</sup> aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) containing stainless steel baffles. After that period, meal was supplied from stainless steel gangstyle food hoppers (Scientific Cages, Inc., Bryan, Texas). During the 2-year period of chemical administration, dosed animals were supplied



with meal containing the appropriate concentrations of 1,5-naphthalenediamine. Control animals had untreated meal available. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine<sup>®</sup> feed cups.

All rats utilized in the 1,5-naphthalenediamine bioassay were housed in a room with other rats receiving diets containing\* acetylaminofluorene (53-96-3); sodium nitrite (76-32-00-0); L-arginine glutamate (4320-30-3); N-butylurea (592-31-4); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 4-nitroanthranilic acid (619-17-0); N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); aniline hydrochloride (142-04-1); and p-anisidine hydrochloride (20265-97-8).

Dosed mice were in a room with mice intubated with m-cresidine (102-50-1); and with other mice receiving diets containing N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4) and 1H-benzotriazole (95-14-7). Control mice were in a room with other mice receiving diets containing hydrazobenzene (530-50-7); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); tris(2,3-dibromopropyl)phosphate (126-72-7); N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4); aniline hydrochloride (142-04-1); and 2-chloro-o-phenylenediamine sulfate.

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\*CAS registry numbers are given in parentheses.

#### E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 1,5-naphthalenediamine for administration to dosed animals in the chronic studies, subchronic toxicity studies were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 1,5-Naphthalenediamine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups and five of the six mouse groups in concentrations of 0.03, 0.1, 0.3, 1.0, and 3.0 percent. The sixth group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 8 weeks.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of 20 percent relative to controls was selected as the high concentration for the chronic bioassay.

Deaths were recorded for all groups of rats receiving concentrations of 0.3 percent or more. Mean body weight depression was approximately 19 and 9 percent, respectively, in males and females dosed with 0.1 percent 1,5-naphthalenediamine. The concentration of 1,5-naphthalenediamine selected for administration as the high dose in the rat chronic bioassay was 0.1 percent.

Deaths were recorded for all groups of mice receiving concentrations of 0.3 percent or more and in the group of female mice

receiving 0.03 percent. Mean body weight depression was approximately 22 and 3 percent, respectively, in males and females dosed with 0.3 percent. Males receiving 0.1 percent experienced mean body weight depression of approximately 3 percent, while females receiving the same concentration had a greater mean body weight than the controls. The concentration of 1,5-naphthalenediamine selected for administration as the high dose in the mouse chronic bioassay was 0.2 percent.

#### F. Experimental Design

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

Rats were all approximately 7 weeks old at the time they were placed on test. Dosed rats were born approximately 1 month earlier than controls and were started on test 1 month earlier than controls. The dietary concentrations of 1,5-naphthalenediamine administered were 0.10 and 0.05 percent. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. The dosed rats were supplied with feed containing 1,5-naphthalenediamine for a total of 103 weeks, followed by a 3- to 4-week observation period.

All mice were approximately 7 weeks old at the time they were placed on test. Dosed mice were born approximately 1 month earlier

TABLE 1  
 DESIGN SUMMARY FOR FISCHER 344 RATS  
 1,5-NAPHTHALENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	1,5-NAPHTHALENE- DIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
CONTROL	25	0	0	109
LOW DOSE	50	0.05 0	103	3
HIGH DOSE	50	0.10 0	103	3
<u>FEMALE</u>				
CONTROL	25	0	0	110
LOW DOSE	50	0.05 0	103	3
HIGH DOSE	50	0.10 0	103	4

TABLE 2  
 DESIGN SUMMARY FOR B6C3F1 MICE  
 1,5-NAPHTHALENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	1,5-NAPHTHALENE- DIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
CONTROL	50	0	0	109
LOW DOSE	50	0.1 0	103	2
HIGH DOSE	50	0.2 0	103	2
<u>FEMALE</u>				
CONTROL	50	0	0	109
LOW DOSE	50	0.1 0	103	2
HIGH DOSE	50	0.2 0	103	3

than controls and were started on test 1 month earlier than controls. The dietary concentrations of 1,5-naphthalenediamine administered were 0.2 and 0.1 percent. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. The dosed mice were supplied with feed containing 1,5-naphthalenediamine for a total of 103 weeks, followed by a 2- to 3-week observation period.

#### G. Clinical and Histopathologic Examinations

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

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

and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

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

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

#### H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical

observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined



histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups,  $k$ , are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to  $0.05/k$ . In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose

relationship. Significant departures from linearity ( $P < 0.05$ , two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a  $P < 0.025$  one-tailed test when the control incidence is not zero,  $P < 0.050$  when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity,

the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

### III. CHRONIC TESTING RESULTS: RATS

#### A. Body Weights and Clinical Observations

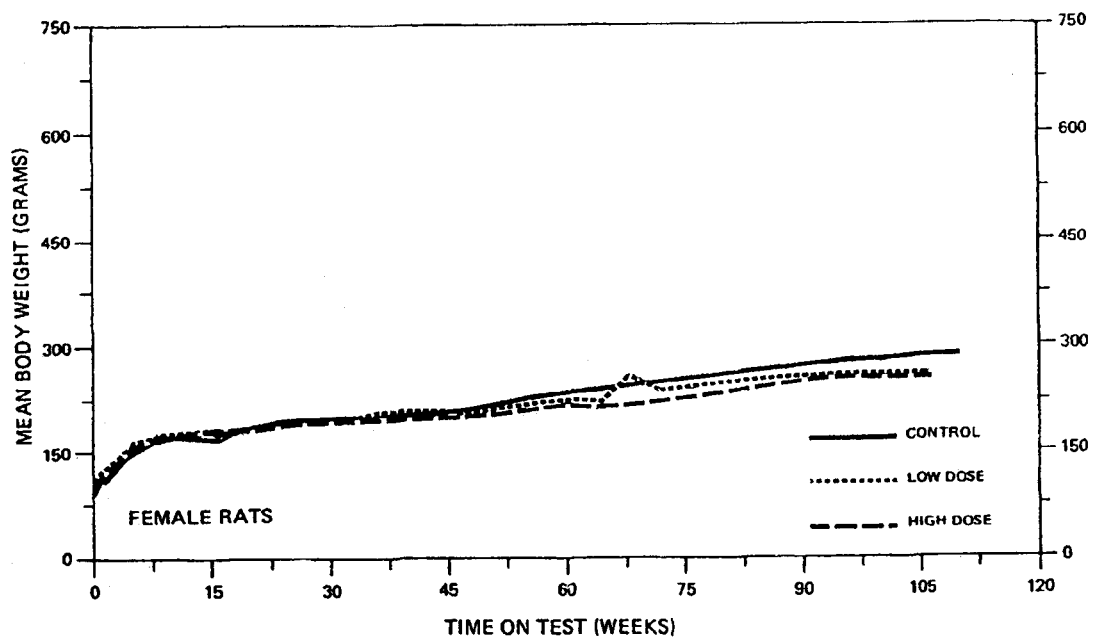
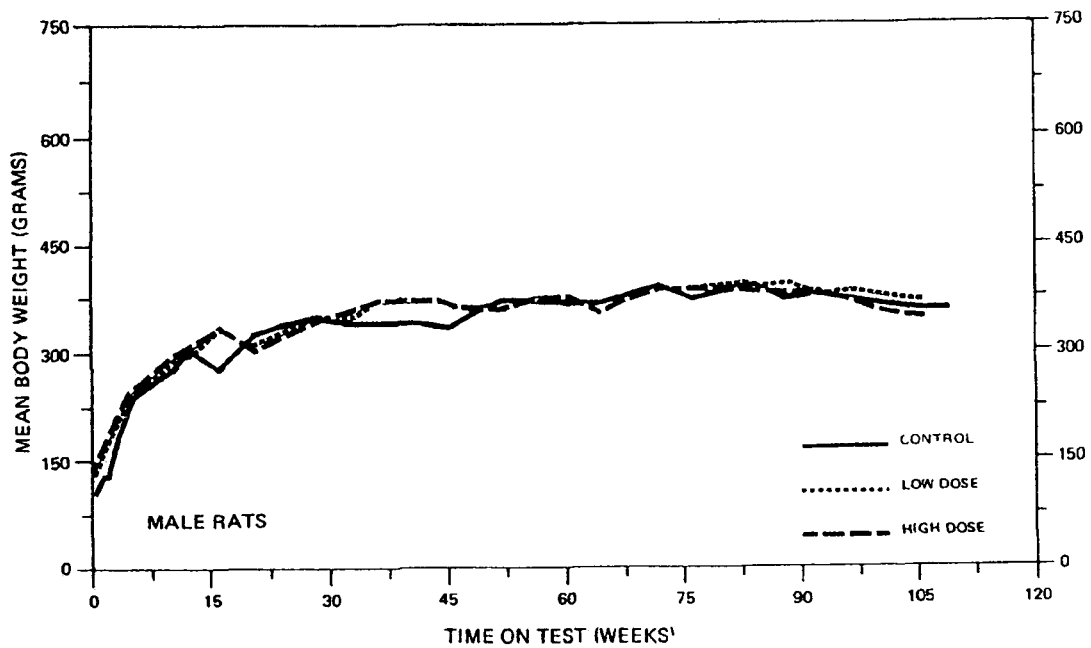
There was no appreciable depression in mean body weight when dosed rats were compared with their respective controls (Figure 2).

Subcutaneous masses were observed in 2 high dose, 3 low dose, and 1 control males, and in 12 high dose, 3 low dose, and 2 control females. Crusted cutaneous masses occurred in 4 high dose males, 1 low dose male, 2 low dose females, and 1 control female, while firm nodular growths were detected in 1 high dose, 2 low dose, and 2 control males, and in 1 low dose female. Swelling of the eyes was exhibited by 2 high dose males, 2 high dose females, and 2 low dose females and swelling of the nose by 1 low dose male. Only 1 control female experienced crusted lesions in the vaginal area while 4 low dose and 9 high dose females were so effected. Alopecia was recorded for 1 low dose female, emaciation was observed in 1 male and 1 female control, and 1 female control exhibited abdominal distention.

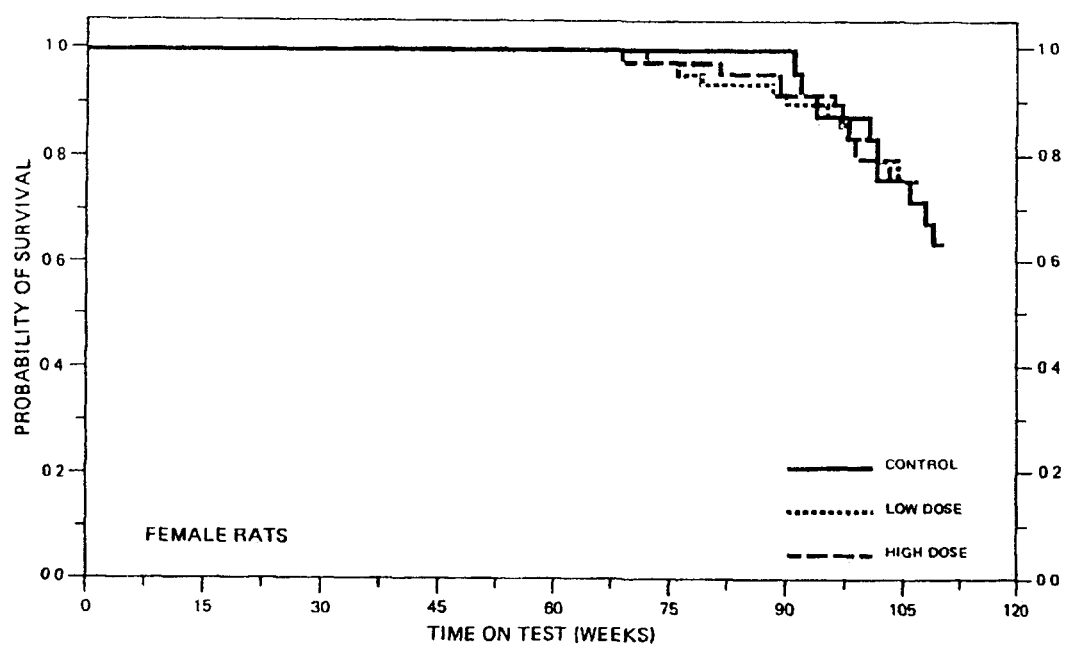
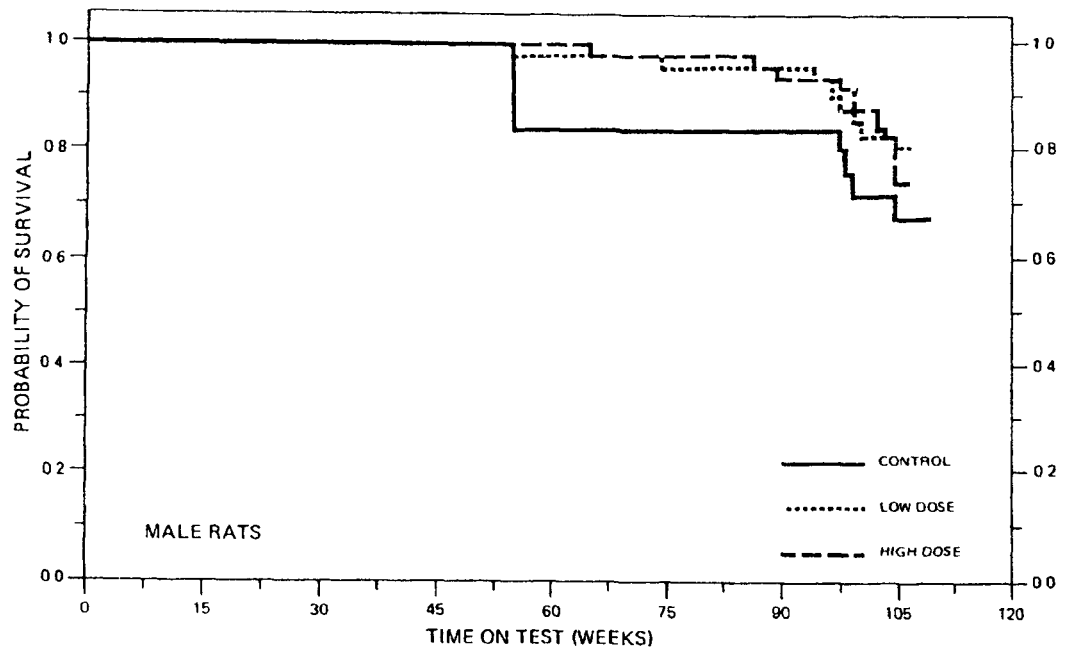
#### B. Survival

The estimated probabilities of survival for male and female rats in the control and 1,5-naphthalenediamine-dosed groups are shown in Figure 3. There was no significant positive association between dosage and mortality for either male or female rats.

Adequate numbers of male rats were at risk from late-developing tumors with 74 percent (37/50) of the high dose, 80 percent (40/50) of the low dose and 68 percent (17/25) of the control surviving on



**FIGURE 2**  
**GROWTH CURVES FOR 1,5-NAPHTHALENEDIAMINE CHRONIC STUDY RATS**



**FIGURE 3**  
**SURVIVAL COMPARISONS OF 1,5-NAPHTHALENE-DIAMINE CHRONIC STUDY RATS**

test until the termination of the study. No lesions were reported for the 4 control rats that died in week 55.

With 76 percent (38/50) of the high dose, 76 percent (38/50) of the low dose and 64 percent (16/25) of the control rats surviving on test until the termination of the study, adequate numbers of females were at risk from late-developing tumors.

C. Pathology

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

The incidence of liver neoplasms in male and female rats administered 1,5-naphthalenediamine in the diet appeared to be increased relative to controls. In female rats, tumors of the clitoral gland, uterus, and C-cell neoplasms of the thyroid appeared to be related to compound administration. The incidences of these tumors are as follows:

	MALES			FEMALES		
	Con- trol	Low Dose	High Dose	Con- trol	Low Dose	High Dose
<u>LIVER</u> (Number of animals with tissues examined histopathologically)	(25)	(49)	(49)	(24)	(50)	(49)
Neoplastic Nodule	1	3	2	0	3	4
Hepatocellular Carcinoma	0	4	2	0	1	0
<u>PREPUTIAL/CLITORAL GLAND</u> (Number of animals necropsied)	(25)	(49)	(50)	(24)	(50)	(50)
Carcinoma	0	0	1	1	3	8
Adenoma	0	0	1	0	0	5



	MALES			FEMALES		
	Con- trol	Low Dose	High Dose	Con- trol	Low Dose	High Dose
<u>UTERUS AND ENDOMETRIUM</u>						
(Number of animals with tissues examined histopathologically)	-	-	-	(24)	(49)	(48)
Adenocarcinoma				1	2	4
Endometrial Stromal Polyp				2	14	20
Endometrial Stromal Sarcoma				1	2	2
<u>THYROID</u>						
(Number of animals with tissues examined histopathologically)	(21)	(47)	(47)	(21)	(49)	(48)
C-Cell Adenoma	0	2	5	0	7	3
C-Cell Carcinoma	2	3	3	1	5	1

Neoplasms of the clitoral (preputial) gland were presented grossly as round, fluctuant cystic subcutaneous lesions in the genital area, which on section were filled with pasty green material. On microscopic examination, the cyst contents consisted of desquamated epithelial cells, frequently mixed with leukocytes from secondary inflammation. The inner portion of the cyst wall was lined by hyperkeratinized squamous epithelium often thrown into papillary folds. Peripheral to this was a zone of large, round glandular cells at least a few of which had coarse, brightly eosinophilic cytoplasmic granules. If the peripheral border appeared smooth and intact, the lesion was classified as an adenoma. If there was disorganization of the glandular structure and invasion into the surrounding stroma, the tumor was called a carcinoma.

Thyroid C-cell tumors were observed in dosed female rats at incidences increased relative to controls (4/48 [8 percent] high dose, 12/49 [24 percent] low dose, 1/21 [5 percent] controls). C-cell adenomas were discrete masses of these cells, often containing small cysts lined by flat epithelium and containing colloid-like material. In C-cell carcinomas, the tumor cells often assumed a spindle shape and tended to invade surrounding tissue.

Uterine horns containing neoplasms were usually grossly enlarged. The neoplasms themselves were varicolored, polypoid, frequently gelatinous masses projecting into the uterine cavity. Endometrial stromal polyps had a fibrous connective tissue core richly supplied with large vessels. The surface of the polyps was covered with well-differentiated endometrium which often formed glands in the superficial portion of the polyps. These tumors frequently became necrotic at the tip and exhibited hemorrhage and secondary inflammation. In a few rats, the connective tissue stroma of these lesions underwent malignant transformation characterized by increased cellularity, mitoses, and formation of plump, pleomorphic nuclei. Such tumors were classified as stromal sarcomas. A uterine adenocarcinoma was a collection of fairly well-differentiated glands arranged back-to-back with no obvious intervening stroma. Nuclei of the glands were markedly pleomorphic with frequent mitoses. There was invasion into the myometrium and sometimes into extra uterine structures.

There were instances in this study, as noted in the summary tables, where neoplastic lesions occurred only in dosed animals, or with increased frequency when compared to the control group. No pulmonary neoplasms were found in the controls; alveolar/bronchiolar tumors were seen in dosed rats of both sexes. There was only one urinary tract neoplasm in a female control; a few more occurred in dosed rats, both male and female. No gliomas of the brain were seen in controls; a few gliomas were found in dosed rats of both sexes. These neoplasms occurred in such small numbers that a conclusive interpretation as to their significance is not possible.

Rats in all groups exhibited a variety of nonneoplastic inflammatory and degenerative changes, and none were associated with administration of the compound.

Based upon the results of this pathologic examination, 1,5-naphthalenediamine was carcinogenic to female Fischer 344 rats since feeding of the compound was associated with adenomas and carcinomas of the clitoral gland. In addition, 1,5-naphthalenediamine feeding appeared to be associated with increased incidences of thyroid, liver and uterine neoplasms in female rats and liver neoplasms in male rats.

#### D. Statistical Analyses of Results

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