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↑ F: Tcho↑ Pho↑
Changes in relative organ weights	–	–	F: Liver 13%↑	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 γ-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

		Scheduled-sacrifice group†			Recovery group	
Dose (mg/kg/day)	Grade	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_{50} 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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BIOASSAY OF

1,5-NAPHTHALENEDIAMINE

FOR POSSIBLE CARCINOGENICITY

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

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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.

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I. INTRODUCTION

1,5-Naphthalenediamine (Figure 1) (NCI No. C03021), 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

*The CAS registry number is 2243-62-1

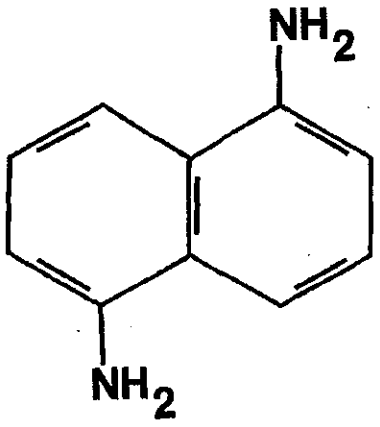

FIGURE 1
CHEMICAL STRUCTURE OF 1,5-NAPHTHALENEDIAMINE

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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 λ_{max} at 232, 328 and 498 nm with ϵ values of 62,800, 10,640 and 9, respectively. The literature (Sadtler Standard Spectra) indicates a λ_{max} at 328.5 nm with ϵ = 10,000 for 1,5-naphthalenediamine. The observed ϵ 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® (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® 15/40 denier Dacron® 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® 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® was used during the first 9 months of study. A second corncob bedding (Bed-o-Cobs®, 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® meal was supplied to rats for 12 months and mice for 11 months from Alpine® 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® 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.

*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)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	25	0	0	109
LOW DOSE	50	0.05 0	103	3
HIGH DOSE	50	0.10 0	103	3
FEMALE				
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)
MALE				
CONTROL	50	0	0	109
LOW DOSE	50	0.1 0	103	2
HIGH DOSE	50	0.2 0	103	2
FEMALE				
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