NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF DIETHYLAMINE
(CAS No. 109-89-7)

IN F344/N RATS AND B6C3F1 MICE
(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

October 2011

NTP TR 566

NIH Publication No. 12-5908

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

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CONTENTS

SUMMARY

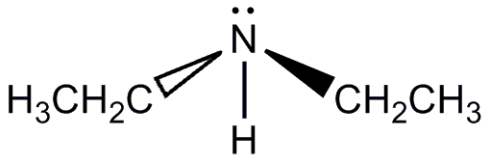
Background
Diethylamine is used in the production of a corrosion inhibitor and also of pesticides, insect repellants and rubber products. Diethylamine vapors are strong irritants to the eyes, nose, and throat of workers. We studied long-term exposure of lower concentrations of diethylamine to rats and mice to see if it caused cancer or other toxic effects.

Methods
We exposed groups of 50 male and female rats to atmospheres containing 31, 62.5, or 125 parts per million (ppm) of diethylamine. We also exposed groups of 50 male and female mice to atmospheres containing 16, 31, or 62.5 ppm diethylamine. Similar groups of 50 animals were exposed only to clean air in the same exposure chambers and served as the control groups. Animals were exposed 6 hours per day, 5 days per week for 2 years. Tissues from more than 40 sites were examined for every animal.

Results
The nose was the main site of injury for all groups of rats and mice exposed to diethylamine. A variety of lesions were observed in the nasal cavity, including atrophy, hyperplasia and metaplasia of the olfactory epithelium in rats and mice and hyperplasia and metaplasia of the respiratory epithelium in rats and squamous metaplasia of the respiratory epithelium in mice. Inflammation of the cornea was observed in some exposed male rats, and inflammation of the pleura and lung were observed in exposed female rats.

Conclusions
We conclude that exposure to diethylamine caused a spectrum of inflammatory lesions in the nose of male and female rats and mice and lesions in the eye of male rats and pleura and lung of female rats. There was no evidence that diethylamine caused any cancers in these studies.

ABSTRACT



DIETHYLAMINE

CAS No. 109-89-7

Chemical Formula: $C_4H_{11}N$ Molecular Weight: 73.14

Synonyms: Amine, diethyl-; DEA; diethamine; *N,N*-diethylamine; ethanamine, *N*-ethyl-; *N*-ethylethanamine

Diethylamine is used mainly as a chemical intermediate to produce the corrosion inhibitor *N,N*-diethylethanolamine and a lesser amount is used to produce pesticides and insect repellants and in rubber processing. Diethylamine was nominated for study by the National Institute of Environmental Health Sciences based upon its high production volume and ubiquitous natural occurrence in trace amounts and because of the lack of chronic toxicity and carcinogenicity data on the chemical. Male and female F344/N rats and B6C3F1 mice were exposed to diethylamine (approximately 99.9% pure) by inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in bacterial mutagenicity tester strains and mouse peripheral blood erythrocytes.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were exposed to diethylamine vapor at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 days. All rats survived to the end of the study. The mean body weights of 250 and 500 ppm males and females and 125 ppm males were significantly less than those of the chamber controls. Clinical findings included lethargy, nasal/eye discharge, abnormal breathing, thinness, eye abnormalities, and discolored urine. The thymus weights of males exposed to 125 ppm or greater and females exposed to 500 ppm were significantly less than those of the chamber controls. Focal eye lesions were noted at necropsy in four males and three females exposed to 500 ppm and one male exposed to 250 ppm. Crusty noses were observed in most 500 ppm males and females and in two 250 ppm males. Suppurative inflammation, necrosis of the turbinates (except in one 125 ppm female), and squamous metaplasia of the respiratory epithelium of the nose were present in all rats exposed to 125 ppm or greater. Ulcer of the respiratory epithelium and atrophy of the olfactory epithelium occurred in all rats exposed to 250 or 500 ppm, and ulcer of the nasopharyngeal duct was present in all 500 ppm rats. Suppurative inflammation of the cornea was present in most rats exposed to 500 ppm.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were exposed to diethylamine vapor at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 17 days. Two males and three females exposed to 500 ppm died during the first week of the study. The mean body weights of males and females exposed to 125 ppm or greater were significantly less than those of the chamber controls. Males and females exposed to 250 or 500 ppm lost weight during the study. Lethargy, abnormal breathing, and thinness were observed in most mice exposed to 250 or 500 ppm. Eye irritation and discharge, nasal discharge, and low fecal and urine output were noted in 500 ppm mice. Thymus weights of 250 and 500 ppm males and 125 ppm or greater females were significantly less than those of the chamber controls. Suppurative inflammation of the nose occurred in all males exposed to 250 or 500 ppm and all females exposed to 125 ppm or greater, and most males exposed to 125 ppm. Turbinate necrosis occurred in all exposed mice except one 31 ppm female. Squamous metaplasia of the respiratory epithelium and olfactory epithelial atrophy were seen in mice exposed to 125 ppm or greater. In the lung, the incidence of minimal chronic active inflammation of mainstem bronchi was significantly increased in 500 ppm males.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to diethylamine vapor at concentrations of 0, 8, 16, 32, 62, or 125 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. All rats survived to the end of the study. Mean body weights of all exposed groups were similar to those of the chamber control groups. There were significant exposure concentration-related decreases in sperm motility in 32, 62, and 125 ppm males; there were no significant differences in the lengths of estrous cycles between chamber control and exposed groups of females. Exposure-related nasal lesions were seen primarily in rats exposed to 62 or 125 ppm. These lesions included turbinate necrosis, suppurative inflammation, respiratory epithelial hyperplasia, squamous metaplasia of the respiratory epithelium, and olfactory epithelial atrophy.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were exposed to diethylamine vapor at concentrations of 0, 8, 16, 32, 62, or 125 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. The mean body weights of 125 ppm males and females were significantly less than those of the chamber controls. There were significant exposure concentration-related decreases in sperm motility in males exposed to 32, 62, or 125 ppm; the estrous cycle of 125 ppm females was significantly longer than that of the chamber controls but only by half a day. Histopathologic changes were noted primarily in the nasal cavity and involved both the respiratory and olfactory epithelium of males and females principally in the 62 or 125 ppm groups. These lesions included suppurative inflammation, squamous metaplasia of the respiratory epithelium, olfactory epithelial atrophy, and necrosis of the turbinates.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed to diethylamine vapor at concentrations of 0, 31, 62.5, or 125 ppm, 6 hours plus T_{90} (15 minutes) per day, 5 days per week for 105 weeks. Survival of exposed groups of rats was similar to that of the chamber control groups. Mean body weights of males and females exposed to 125 ppm were less than those of the chamber controls after week 57. Increased incidences of eye abnormality occurred in exposed males and females.

A spectrum of nonneoplastic lesions was observed in the respiratory and olfactory epithelium of the nose in exposed rats. The lesions included suppurative inflammation, ulceration of the respiratory epithelium, hyaline droplet accumulation in the glands of the respiratory epithelium, necrosis of the turbinates, squamous metaplasia of the respiratory epithelium, hyperplasia of the respiratory epithelium, atrophy of the olfactory epithelium, hyaline droplet accumulation in the respiratory and olfactory epithelium, basal cell hyperplasia of the olfactory epithelium, respiratory metaplasia of the olfactory epithelium, and goblet cell hyperplasia.

The incidence of chronic inflammation of the pleura was significantly increased in 125 ppm females. The incidences of histiocytic cellular infiltration of the alveolus of the lung were significantly increased in all exposed groups of females and the incidence of chronic inflammation was significantly increased in 125 ppm females.

In 125 ppm males, the incidence of suppurative inflammation of the cornea was significantly increased.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed to diethylamine vapor at concentrations of 0, 16, 31, or 62.5 ppm, 6 hours plus T_{90} (15 minutes) per day, 5 days

per week for 105 weeks. Survival of exposed groups of mice was similar to that of the chamber control groups. Mean body weights of males and females were similar to those of the chamber controls. Eye abnormality was observed in greater incidence in exposed groups of males than in the chamber controls, and torso/ventral ulcer/abscess was observed in six 62.5 ppm males compared to none in the chamber controls.

A similar spectrum of nonneoplastic lesions was seen in the nose of exposed mice as was seen in rats.

GENETIC TOXICOLOGY

Diethylamine was not mutagenic in either of two independent bacterial mutagenicity assays, each conducted with and without exogenous metabolic activation enzymes. Bacterial strains tested included *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvrA*/pKM101. In addition to the negative results the two bacterial assays, no significant increases in the frequencies of micronucleated erythrocytes were seen in peripheral blood of male or female B6C3F1 mice from the 3-month study.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of diethylamine in male or female F344/N rats exposed to 31, 62.5, or 125 ppm. There was *no evidence of carcinogenic activity* of diethylamine in male or female B6C3F1 mice exposed to 16, 31, or 62.5 ppm.

Exposure to diethylamine resulted in increased incidences of nonneoplastic lesions of the nose in male and female rats and mice, of the cornea in males rats, and of the pleura and lung in female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethylamine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in air	0, 31, 62.5, or 125 ppm	0, 31, 62.5, or 125 ppm	0, 16, 31, or 62.5 ppm	0, 16, 31, or 62.5 ppm
Body weights	125 ppm group 10% less than the chamber control group after week 57	125 ppm group 10% less than the chamber control group after week 57	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group
Survival rates	28/50, 21/50, 25/50, 36/50	31/50, 31/50, 30/50, 35/50	31/50, 38/50, 32/50, 37/50	32/50, 35/50, 36/50, 39/50
Nonneoplastic effects	Nose: glands, respiratory epithelium, accumulation, hyaline droplet (6/49, 45/50, 42/50, 45/50); goblet cell, hyperplasia (0/49, 0/50, 2/50, 13/50); inflammation, suppurative (5/49, 5/50, 10/50, 29/50); olfactory epithelium, accumulation, hyaline droplet (8/49, 49/50, 49/50, 42/50); olfactory epithelium, atrophy (2/49, 49/50, 50/50, 50/50); olfactory epithelium, hyperplasia, basal cell (0/49, 0/50, 22/50, 50/50); olfactory epithelium, respiratory metaplasia (2/49, 2/50, 2/50, 37/50); respiratory epithelium, accumulation, hyaline droplet (0/49, 29/50, 42/50, 11/50); respiratory epithelium, hyperplasia (5/49, 34/50, 35/50, 47/50); respiratory epithelium, metaplasia, squamous (0/49, 2/50, 6/50, 26/50); respiratory epithelium, ulcer (0/49, 0/50, 2/50, 22/50); turbinate, necrosis (0/49, 0/50, 1/50, 19/50) Eye: Cornea, inflammation, suppurative (0/49, 0/50, 1/50, 5/50)	Nose: glands, respiratory epithelium, accumulation, hyaline droplet (9/50, 46/49, 45/50, 44/50); goblet cell, hyperplasia (1/50, 0/49, 4/50, 20/50); inflammation, suppurative (6/50, 4/49, 15/50, 34/50); olfactory epithelium, accumulation, hyaline droplet (11/50, 49/49, 50/50, 48/50); olfactory epithelium, atrophy (1/50, 47/49, 48/50, 50/50); olfactory epithelium, hyperplasia, basal cell (0/50, 3/49, 29/50, 48/50); olfactory epithelium, respiratory metaplasia (3/50, 1/49, 2/50, 19/50); respiratory epithelium, accumulation, hyaline droplet (4/50, 48/49, 46/50, 39/50); respiratory epithelium, hyperplasia (7/50, 31/49, 41/50, 50/50); respiratory epithelium, metaplasia, squamous (1/50, 1/49, 5/50, 39/50); respiratory epithelium, ulcer (0/50, 0/49, 0/50, 34/50); turbinate, necrosis (0/50, 0/49, 0/50, 32/50) Pleura: inflammation, chronic (6/50, 14/50, 12/50, 21/50) Lung: alveolus, infiltration cellular, histiocyte (13/50, 24/50, 27/50, 35/50); inflammation, chronic (4/50, 11/50, 7/50, 24/50)	Nose: glands, respiratory epithelium, accumulation, hyaline droplet (5/50, 5/50, 16/50, 33/50); glands, respiratory epithelium, hyperplasia (42/50, 41/50, 44/50, 50/50); inflammation, suppurative (6/50, 5/50, 6/50, 14/50); olfactory epithelium, atrophy (9/50, 19/50, 50/50, 50/50); olfactory epithelium, respiratory metaplasia (14/50, 15/50, 44/50, 50/50); respiratory epithelium, accumulation, hyaline droplet (11/50, 6/50, 19/50, 30/50); respiratory epithelium, metaplasia, squamous (4/50, 7/50, 16/50, 34/50); turbinate, hyperostosis (5/50, 23/50, 50/50, 50/50)	Nose: glands, respiratory epithelium, accumulation, hyaline droplet (16/50, 28/49, 45/50, 42/50); glands, respiratory epithelium, inflammation, chronic active (8/50, 11/49, 16/50, 22/50); glands, respiratory epithelium, hyperplasia (43/50, 45/49, 47/50, 50/50); inflammation, suppurative (2/50, 1/49, 3/50, 9/50); olfactory epithelium, atrophy (8/50, 29/49, 49/50, 50/50); olfactory epithelium, respiratory metaplasia (4/50, 15/49, 48/50, 50/50); respiratory epithelium, accumulation hyaline droplet (20/50, 33/49, 47/50, 29/50); respiratory epithelium, metaplasia, squamous (0/50, 0/49, 13/50, 35/50); respiratory epithelium, necrosis (1/50, 0/49, 6/50, 16/50); turbinate, hyperostosis (4/50, 23/49, 49/50, 50/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethylamine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:		Negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S9; negative in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		Negative in males and females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on diethylamine on November 19, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 19, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of diethylamine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. D.L. Morgan, NIEHS, introduced the toxicology and carcinogenesis studies of diethylamine by describing its use as a chemical intermediate, the design of the inhalation studies, and the results of the short- and long-term studies. The proposed conclusions for the 2-year inhalation studies were *no evidence of carcinogenic activity* of diethylamine in male or female F344/N rats and *no evidence of carcinogenic activity* of diethylamine in male or female B6C3F1 mice.

Dr. Nagarkatti, the first principal reviewer, felt the studies answered all the important gaps in the database for this chemical and the report covered the background literature and study protocols adequately. She noted that exposure in inhalation chambers could result in exposure by other routes (e.g., dermal). She inquired about the occurrence of sporadic seizures in the control animals, about a possible link between thymic atrophy and immunosuppression, and whether other studies involving nitrosamine products were contemplated.

Dr. Morgan agreed that it was understood that whole body exposure would also entail some dermal and oral exposures in addition to inhalation. He noted that seizures had been noted in a number of studies involving singly housed Fischer rats. Extensive evaluation indicated no adverse effect of these seizures on the animals; nonetheless, the NTP has subsequently adopted a different strain for its studies.

Dr. G.P. Flake, NIEHS, noted that while the weights of the thymus glands were decreased in rats and mice in the short-term studies, it might not be possible to discern between a stress-induced reaction and immuno-suppression. Dr. Flake noted that the thymus is the most sensitive of the lymphoid organs to cortical hormones, but there was no histologic evidence of atrophy of the thymus or other lymphoid tissues in the 2-year studies. Dr. Morgan added that a number of studies have attempted without great success to demonstrate nitrosamine formation from diethylamine.

Dr. Cattley, the second principal reviewer, suggested including mention of a short-term study of the related dimethylamine. He also noted an increased incidence of corneal lesions and suggested they be included in the conclusions.

Dr. Sherley, the third principal reviewer, questioned the rationale for discounting the decreased incidences of mammary gland carcinomas in female rats and female mice and thought they should be mentioned in the conclusions.

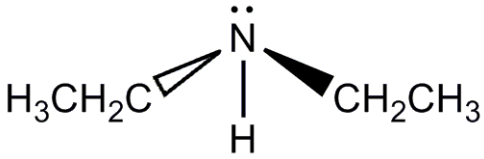
Dr. Morgan noted that generally conclusions concerning mammary gland are based on combined incidences of adenomas and carcinomas. In the present studies, no changes were seen in the incidences of mammary gland adenomas or fibroadenomas, and in contrast, the incidences of carcinomas were rather small, so little difference was seen in the overall combination.

Dr. J.R. Bucher, NIEHS, observed that in general, decreased tumor incidences receive less weight in consideration of study results, as the design of the studies is primarily to detect adverse effects with the goal of hazard identification. Only when tumor decreases are truly significant are they mentioned. He cautioned against misuse of the study results as evidence of protective or therapeutic effects of the chemicals studied.

Dr. Pino inquired if any conclusive association was being made concerning the occurrence of seizures in some study animals. Dr. Morgan replied that in an overview examination of a number of studies, no direct chemical association and no histopathologic lesions were detected in animals experiencing the seizures.

Dr. Cattley proposed the conclusions be accepted as written with the addition of lesions of the cornea in male rats. Dr. Sherley seconded the motion, which was approved unanimously with eight yes votes.

INTRODUCTION



DIETHYLAMINE

CAS No. 109-89-7

Chemical Formula: $C_4H_{11}N$ Molecular Weight: 73.14

Synonyms: Amine, diethyl-; DEA; diethamine; *N,N*-diethylamine; ethanamine, *N*-ethyl-; *N*-ethylethanamine

CHEMICAL AND PHYSICAL PROPERTIES

Diethylamine is a clear, alkaline liquid with a strong ammonia-like odor. The odor threshold for diethylamine is 0.14 ppm, which gives good warning of its presence. The boiling point is 56° C, and the vapor pressure is 195 mm Hg at 20° C (*CRC Handbook*, 1980). Diethylamine is soluble in water, ethanol, diethyl ether, acetone, aliphatic and aromatic hydrocarbons, mineral oils, and stearic and oleic acids (NIOSH, 1979). The hydrochloride salt of diethylamine is a crystalline solid with a melting point of 226° C and a boiling point range of 320° to 330° C (*Merck*, 1996). The salt is soluble in water, ethanol, and chloroform but is practically insoluble in diethyl ether. Diethylamine (pk_b=3.0) is more alkaline than ammonia (pk_b=4.76) (Beard and Noe, 1981). The more alkaline the compound, the lower the pk_b.

PRODUCTION, USE, AND HUMAN EXPOSURE

Manufacture of diethylamine is generally by high temperature and high pressure reaction of ammonia and an alcohol over a dehydration catalyst or dehydrogenation catalyst (*Merck*, 1996; SRI, 1997a). The reaction product is treated by continuous extraction and distillation to produce pure amine. In 1995, the United States produced 25 million pounds (SRI 1997a), exported 2.6 million pounds (SRI, 1997b), and imported 0.02 million pounds (SRI, 1997c) of diethylamine.

A majority of the diethylamine is used as a chemical intermediate to produce the corrosion inhibitor *N,N*-diethylethanolamine. In 1995, 12 million pounds (54% of total consumption) were used to produce *N,N*-diethylethanolamine. About 18% of total consumption was used to produce pesticides and insect repellants (4 million pounds), and about 2 million pounds (9% of total consumption) were used for rubber processing chemicals (SRI, 1997d). An unreported amount of diethylamine was used to produce pharmaceuticals (e.g., the alcohol antagonist disulfiram, the hypnotic flurazepam, the anesthetic lidocaine, and the antimalarial amodiaquin). Diethylamine is also used in the paint, lacquer, and varnish industries (BASF, 1995).

Data from the 1981 to 1983 National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health (NIOSH) estimate that 28,361 workers are potentially exposed to diethylamine in the United States (NIOSH, 1990). Occupational exposure to diethylamine vapor or liquid occurs primarily in the chemical industry, during the preparation of textile finishing agents, surfactants,

rubber processing chemicals, agricultural chemicals and pharmaceuticals. Worker exposure can also occur in the iron, steel, and metal industries where diethylamine is used as a corrosion inhibitor; in the polymer industry where diethylamine is used as a polymerization inhibitor; and in the dye industry where diethylamine is used as a catalyst and chemical intermediate (OSHA, 1981).

Patients taking the drug disulfiram for alcoholism may be exposed indirectly to diethylamine. After absorption in the stomach, disulfiram is reduced to diethyl dithiocarbamate, which breaks down to carbon disulfide and diethylamine; diethylamine is then excreted in the urine (Faiman *et al.*, 1984).

REGULATORY STATUS

The American Conference of Governmental Industrial Hygienists (2009) has recommended a threshold limit value 8-hour time-weighted average (TWA) for diethylamine of 5 ppm (15 mg/m^3) and a 15-minute short-term exposure limit of 15 ppm (45 mg/m^3). Occupational exposure to diethylamine is regulated as an air contaminant by the Occupational Safety and Health Administration; the 8-hour TWA permissible exposure limit is 25 ppm (75 mg/m^3) (NIOSH, 2005).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Very little information on the disposition and metabolism of diethylamine in experimental animals or humans was found in a review of the literature. The reaction of dietary amines with nitrite to produce nitrosamines, which are potential carcinogens, has been demonstrated both *in vitro* and *in vivo* (Sander, 1967; Sander *et al.*, 1968; Mirvish, 1975). However, in Wistar rats administered 600 ppm diethylamine and sodium nitrite in the diet, diethylnitrosamine could not be detected (Galea *et al.*, 1975). Diethylnitrosamine was not detected in blood or milk of goats fed kale containing 3% potassium nitrate followed by administration of a single oral dose of 200 mg/kg diethylamine hydrochloride (Juskiewicz and Kowalski, 1976). Monoamine oxidase is assumed to play an important role in the metabolism and detoxification of the aliphatic amines. Monoamine oxidase catalyzes the deamination of primary, secondary, and tertiary amines. Monoamine oxidase is widely distributed in tissues and is most concentrated in the liver, kidney, and intestinal mucosa (Beard and Noe, 1981). Traces of diethylamine (less than 0.5% of the dose) were detected in the urine of human volunteers following oral doses of triethylamine (Åkesson *et al.*, 1989). Diethylamine was also found in the gastro-intestinal tract after oral doses of triethylamine-*N*-oxide, the oxidative metabolite of triethylamine, indicating that triethylamine-*N*-oxide is dealkylated in the gastro-intestinal tract to diethylamine. There was no evidence that diethylamine produced from triethylamine-*N*-oxide was subsequently metabolized to *N*-nitrosodiethylamine in the stomach.

TOXICITY

Experimental Animals

The acute toxicity of diethylamine has been investigated in various animal species by a variety of exposure methods. The LC_{50} in rats was reported as 4,000 ppm for a 4-hour inhalation exposure, and the oral LD_{50} ranges from 540 to 1,000 mg/kg in rats and 500 to 650 mg/kg in mice (Sax, 1996). Severe toxicity leading to death occurred in Sherman rats administered a single dose of 500, 1,000, or 2,000 mg/kg diethylamine by gavage (Union Carbide, 1950). In albino rats, mice, and guinea pigs administered diethylamine hydrochloride by gavage (dose not provided), toxicity was characterized by a decrease in motor activity and excitability (Saratikov *et al.*, 1984). No gross lesions were observed at necropsy.

The dermal LD_{50} ranges from 580 to 820 mg/kg in rabbits (Sax, 1996). Dermal contact with neat diethylamine for 24 hours caused mild irritation of rabbit skin (Union Carbide, 1950; Smyth *et al.*, 1951). Instillation of 100 µL of 2% diethylamine into the eyes of rabbits caused redness, swelling, and corneal damage (Jacobs and Martens, 1989). Severe ocular irritation was also reported after inhalation exposure of rabbits to 50 ppm (150 mg/m^3) diethylamine for 7 hours per day, 5 days per week, for up to 6 weeks (Brieger and Hodes, 1951) and in rats exposed to 250 ppm (750 mg/m^3) 6.5 hours per day, 5 days per week, for up to 6 months (Lynch *et al.*, 1986).

Acute inhalation exposure (60 to 600 ppm for 15 minutes) of male OF1 mice produced expiratory bradypnea indicative of upper airway irritation (Gagnaire *et al.*, 1989). The calculated concentration resulting in a 50% decrease in respiratory rate (RD_{50}) was 202 ppm. Exposure of groups of six rats each to 1,000, 2,000, 4,000, or 8,000 ppm or saturated diethylamine for 4 hours resulted in the death of one rat at 2,000 ppm, three rats at 4,000 ppm, and all six rats in

the 8,000 ppm and saturated vapor groups (Union Carbide, 1950). Irritation of the nose and eyes, tremors, and poor coordination were observed after 4 hours of exposure to 4,000 ppm. Exposure to 8,000 ppm caused convulsions, bloody discharge from the nose, and severe irritation of the ears and feet. Exposure to saturated vapor caused extreme congestion of the lungs, liver, kidneys, and spleen, as well as convulsions, loud rales, and corneal opacity (Union Carbide, 1950).

In a subchronic inhalation study, male and female F344/N rats and B6C3F1 mice were exposed to 250 or 1,000 ppm diethylamine for 6 hours per day, 5 days per week, for up to 16 days (NIOSH, 1987). Acute ulcerative necrotizing rhinitis and squamous metaplasia of the nasal mucosa were present in all exposed mice, with turbinate atrophy present in some animals. Squamous metaplasia of the tracheal mucosa was observed in the rats exposed to 1,000 ppm. Acute peribronchiolar pneumonia was also observed in the lungs of several rats exposed to 1,000 ppm and one rat exposed to 250 ppm. No treatment-related effects were observed in the heart, coronary arteries, or aorta. The nasal cavity was also the target site in male and female Fischer 344 rats exposed to 500 ppm diethylamine for 10 days (NIOSH, 1984). A moderate to marked necrotizing inflammation of the nasal mucosa was reported.

Early interest in the pharmacology of the simple aliphatic amines was initially stimulated by their structural relationship with epinephrine. The aliphatic amine hydrochlorides were observed to have sympathomimetic activity (increased blood pressure) when given intravenously (Barger and Dale, 1911). Repeated administration of the amines resulted in cardiac depression and vasodilatation (Ahlquist, 1945). Brieger and Hodes (1951) reported that triethylamine caused significant cardiac muscle degeneration in rabbits. Diethylamine was reported to cause a slight, questionable increase in cardiac degeneration in rabbits. Lynch *et al.* (1986) exposed male and female Fischer 344 rats to 25 or 250 ppm diethylamine 6.5 hours per day, 5 days per week, for up to 6 months to more fully investigate the potential cardiac toxicity reported by Brieger and Hodes (1951). Rats exposed to 250 ppm had decreased body weights and nasal cavity lesions including squamous metaplasia, rhinitis, and lymphoid hyperplasia. There were no treatment-related effects in rats exposed to 25 ppm diethylamine. Measurements of cardiotoxicity were all negative in exposed rats. Electrocardiograms (ECGs) were recorded from 10 rats per sex per group just prior to terminal sacrifice. There were no changes in ECGs or cardiac-related clinical chemistry indices. There was no histological evidence of cardiac muscle degeneration. In a comparable inhalation study of triethylamine (Lynch *et al.*, 1990) male and female rats were exposed to 0, 25, or 247 ppm triethylamine vapor 6 hours per day, 5 days per week, for up to 28 weeks. No physiologic or pathologic evidence of cardiotoxicity was seen in rats exposed to either concentration of triethylamine.

The potential immunotoxicity of diethylamine was investigated in a two-stage sensitization test using female CF1 (BR) albino mice (USEPA, 1987). A 0.1 mL dose of a 1.0% (v/v in 70% ethanol) diethylamine solution was applied to the abdomen of mice on days 0, 1, 2, and 3 of the study. The mice were challenged on day 10 by applying 0.01 mL of a 50% (v/v in 70% ethanol) diethylamine solution to the dorsal and ventral surfaces of the left ear. Animals were challenged again on day 17 using the dorsal and ventral surfaces of the right ear. Mice were examined on study days 11, 12, 18, and 19 for changes in ear thickness of at least 20%, and no changes were detected.

Humans

Diethylamine is toxic if inhaled or swallowed or if it comes in contact with the eyes or skin. Severe eye damage has been reported after exposure to diethylamine vapor or contact with the liquid; in a case of accidental exposure of the eyes to liquid diethylamine, severe corneal damage with some permanent visual impairment was reported (OSHA, 1981). Long-term ocular exposure to diethylamine vapor may cause corneal edema resulting in temporary foggy vision and the appearance of halos around lights.

The acute effects of diethylamine vapor on the nasal cavity were evaluated in adult volunteers (Lundqvist *et al.*, 1992). Exposure of five men to 25 ppm diethylamine for 15 minutes did not cause changes in nasal volume or nasal airway resistance. In a subsequent experiment, the acute sensory effects of diethylamine were evaluated in five men during exposure to increasing concentrations from 0 to 12 ppm for 1 hour. Subjective sensations including perceived odor intensity and sensory irritation, termed nose and eye irritation, were registered on a linear scale. A moderate to strong olfactory response and distinct nasal and eye irritation were reported.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of diethylamine in experimental animals or humans was found in the literature.

CARCINOGENICITY

Experimental Animals

No studies evaluating the carcinogenicity of diethylamine or other aliphatic amines were found in the literature. Diethylamine is the only aliphatic amine evaluated in a chronic carcinogenicity study by the NTP.

Because dietary amines can potentially react with salivary nitrite to produce carcinogenic nitrosamines, the coadministration of diethylamine and sodium nitrite has been investigated in several studies. Galea *et al.* (1975) treated Wistar rats with sodium nitrite (15 mg/day) and diethylamine (15 mg/day) in the diet for up to 217 days. Hyperplasia of Kupffer cells, small periportal inflammatory infiltrates, and chronic interstitial nephritis were observed in treated rats. Nitrosodiethylamine was not detected in the stomachs of treated rats.

There were no significant increases in the incidences of liver neoplasms in male C57 × C3H mice administered a single 50 mg/kg dose of diethylamine hydrochloride by gavage on postnatal day 15 and held for 110 weeks after treatment (Rijhsinghani *et al.*, 1982). However, when diethylamine treatment was followed by a single 50 mg/kg dose of sodium nitrite, there was a significant increase in the incidence of liver tumors compared to that in mice administered diethylamine hydrochloride alone. In a three-generation reproductive toxicity study, F_0 rats were administered sodium nitrite (100 mg/kg; 1,000 µmol/kg) in drinking water beginning at 30 days of age (Druckrey *et al.*, 1963). The F_1 and F_2 offspring of these rats were given either sodium nitrite (100 mg/kg) in drinking water and diethylamine hydrochloride (500 mg/kg in feed) concurrently or sodium nitrite (100 mg/kg) alone in drinking water for life. No treatment-related effects were reported.

No liver neoplasms were detected in male English short-hair guinea pigs after administration of 4.0 g/L diethylamine hydrochloride in drinking water for 2.5 years or after concurrent administration of diethylamine hydrochloride and sodium nitrite (Sen *et al.*, 1975). Treated animals receiving both chemicals were administered either a low mix consisting of 2.0 g/L diethylamine hydrochloride plus 0.4 g/L sodium nitrite for 2.5 years or a high mix consisting of 4.0 g/L diethylamine hydrochloride plus 0.8 g/L sodium nitrite for 18 months after which plain water was given for 12 months.

Humans

No epidemiology studies or case reports examining diethylamine exposure and cancer risk in humans were found in the literature.

GENETIC TOXICITY

Published data provide no evidence of diethylamine-associated genotoxicity, although data from mammalian cell studies are limited. The genetic toxicity of diethylamine has been investigated in several prokaryotic systems. Incubation with diethylamine (140 mg/L; 1,900 mM) for 1 hour in the absence of metabolic activation did not induce the lambda prophage in *Escherichia coli* strain K-12 (Thomson and Woods, 1975). Several studies reported that diethylamine was not mutagenic in *Salmonella typhimurium*. Cotruvo *et al.* (1978) first concluded that neither diethylamine (220 µmol/plate) nor ozonated diethylamine (ozonated in water for 1 hour at pH 11.1) induced gene mutations in *S. typhimurium*; testing was conducted in strains TA98, TA100, TA1535, TA1536, TA1537, and TA1538 with and without rat liver S9. Zeiger *et al.* (1987) exposed *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 to a dose range of 33 to 3,333 µg/plate (0.5 to 46 µmol/plate) using the preincubation method in either the presence or absence of 10% induced rat or hamster liver metabolic enzymes (Appendix E); no increases in revertant colonies were observed with any strain or activation condition at any dose level of diethylamine. Similarly, diethylamine did not induce *his* gene mutations in *S. typhymurium* strains TA98, TA100, or TA1538 using the plate incorporation method in either the presence or absence of rat liver S9 (Khudoley *et al.*, 1987).

The potential ability of diethylamine to cause DNA damage in an *in vivo* mammalian test system was investigated by Loury *et al.* (1987). Male F344/N rats were administered 500 mg/kg diethylamine by gavage, and when evaluated 12 hours later, there was no evidence of unscheduled DNA synthesis in their kidney cells.

STUDY RATIONALE

Diethylamine was nominated by the National Institute of Environmental Health Sciences for chronic toxicity and carcinogenicity testing based upon its high production volume and because of the lack of chronic toxicity and carcinogenicity data on the chemical. Diethylamine was also of interest because of the ability of secondary amines to form carcinogenic nitrosamines. Studies of diethylamine were designed to complement planned studies of two structurally-related aliphatic amines, triethylamine (a tertiary amine), and isopropyl amine (a primary amine). Chronic studies of triethylamine and isopropylamine were not conducted because their subchronic toxicities were the same as diethylamine, and there was greater interest in a chronic study of diethylamine because of its potential to form nitrosamines. Inhalation was chosen as the route of exposure in the 2-week, 3-month, and 2-year studies of diethylamine because inhalation is a major route of human exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Diethylamine

Diethylamine was obtained from Alkyl Amines Chemicals, Ltd. (Maharashtra, India) in one lot (BE/07/01) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Research Triangle Institute (RTI) (Research Triangle Park, NC), the study laboratory at Battelle Toxicology Northwest (Richland, WA), Galbraith Laboratories, Inc. (Knoxville, TN), and Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO) (Appendix I). Reports on analyses performed in support of the diethylamine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid with a strong ammonia odor, was identified as diethylamine using infrared and nuclear magnetic resonance spectroscopy and gas chromatography (GC) coupled with mass spectrometry.

Karl Fischer titration indicated 275 ppm water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for diethylamine. GC indicated one major peak and no impurities with areas greater than 0.1% of the total peak area. The overall purity of lot BE/07/01 was determined to be approximately 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Periodic reanalyses of the bulk chemical were performed during the 2-week, 3-month, and 2-year studies using GC, and no degradation of the bulk chemical was detected.

VAPOR GENERATION AND EXPOSURE SYSTEM

Diethylamine was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor into a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate and nitrogen flow rate. The pressure in the distribution manifold was kept fixed to ensure constant flow through the manifold and into all chambers as the flow of vapor to each chamber was adjusted.

Individual Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. A metering valve with a flow indicator at the manifold controlled the flow rate to each chamber. To initiate exposure, the chamber exposure valves were rotated to allow the vapor to flow to each exposure chamber inlet duct where it was further diluted with HEPA®-filtered, conditioned air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector was used with and without animals in the exposure chambers to ensure that diethylamine vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

VAPOR CONCENTRATION MONITORING

Summaries of the chamber vapor concentrations are given in Tables I2 through I4. Chamber and room concentrations of diethylamine were monitored by an on-line gas chromatograph. Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 30 (2-year studies) minutes during each 6-hour exposure period using stream-select and gas sampling valves in a separate heated valve oven. The sample lines composing each sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum

regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of diethylamine in nitrogen supplied by a standard generator. The on-line gas chromatograph was calibrated by a comparison of chamber concentration data to data from grade samples that were collected with acrylic ester adsorbent gas sampling tubes, extracted with methylene chloride containing triethylamine as an internal standard, and analyzed using an off-line gas chromatograph. Known values of chamber atmosphere were sampled at a constant flow rate ensured by a calibrated orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of diethylamine and the internal standard (triethylamine) in methylene chloride.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. A T_{90} value of 12 minutes was selected for the 2-week and 3-month studies. Due to the reactivity of diethylamine with large groups of exposed rats and mice, a T_{90} value of 15 minutes was used for the 2-year studies.

The uniformity of vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week and 3-month studies and approximately quarterly during the 2-year studies. The vapor concentration was measured using the on-line gas chromatograph with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. Chamber concentration uniformity was maintained throughout the studies.

The persistence of diethylamine in the chambers after vapor delivery ended was determined by monitoring the vapor concentration in the 500 ppm chambers in the 2-week studies, the 125 ppm chambers in the 3-month studies, and the 125 ppm (rats) and 62.5 ppm (mice) chambers in the 2-year studies, with (all studies) and without (3-month and 2-year studies) animals present in the chambers. In the 2-week studies, the concentration decreased to 1% of the target concentration within 31 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 29 minutes without animals present and within 59 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 23 (rat) and 16 (mice) minutes without animals present and within 98 (rats) and 42 (mice) minutes with animals present.

Samples of the test atmosphere from the distribution lines and the low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies and also at the beginning and end of one generation day during the 2-week, 3-month, and 2-year studies. The atmosphere samples were collected with adsorbent gas sampling tubes containing an acrylic ester, followed by a tube containing activated coconut charcoal, and extracted with methylene chloride. Additional samples were collected from the generator reservoir, and all of the samples were analyzed using GC to measure the stability and purity of diethylamine in the generation and delivery system.

No evidence of degradation of diethylamine was noted in any part of the exposure system. Two impurity peaks with areas greater than 0.1% of the total peak areas were noted in some of the samples collected from the exposure chambers in the 3-month and 2-year studies. Additional collections of test atmosphere samples determined that only one of these impurity peaks was reproducible, and it was identified as *N,N*-diethyl-formamide using GC coupled with mass spectrometry. Parallel sampling with acetonitrile-filled bubblers and sorbent collection tubes demonstrated that the presence of *N,N*-diethylformamide in the samples was most likely due to artifact formation on the sorbent. No impurity peaks were resolved in the generator reservoir samples.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 13 days and were approximately 5 to 6 weeks old on the first day of the studies. Before the studies began, four male and six female rats and five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the

protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of five male and five female rats and mice were exposed to diethylamine by inhalation at concentrations of 0, 31, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 12 (rats) or 13 (mice) days. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded twice daily. The animals were weighed on days 1, 6, and 13 and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations of the nose of all rats and mice and of the eye and lung of all chamber control and 500 ppm rats and mice were performed; the eye and lung were examined to a no-effect level in groups of animals exposed to lower concentrations of diethylamine (Table 1).

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to diethylamine and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 to 5 weeks old. Animals were quarantined for 11 or 12 days and were approximately 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed on five male and five female sentinel rats and mice at week 2 and five male and five female chamber control rats and mice at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to diethylamine by inhalation at concentrations of 0, 8, 16, 32, 62, or 125 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital plexus of clinical pathology rats on days 3 and 23 and from core study rats at study termination for hematology and clinical chemistry analyses and from the retroorbital sinus of mice at study termination for hematology analyses. At all time points, the animals were anesthetized with a 70% CO_2/air mixture. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Erythrocyte, leukocyte and platelet counts, hemoglobin concentrations, packed cell volume, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were determined on an Abbott Cell-Dyn 3700 hematology analyzer (Abbott Diagnostics Systems, Abbott Park, IL). Manual hematocrits were performed using a microcentrifuge (Heraeus Haemofuge, Hanau, Germany) and a Damon/IEC capillary reader (International Equipment, Co., Needham Heights, MA) for comparison to the Abbott Cell-Dyn 3700 values for packed cell volume. Platelet and erythrocyte morphology were determined using blood smears stained with a Romanowsky-type aqueous stain in a Wescor 7100 aerospray slide stainer (Wescor, Inc., Logan, UT). Leukocyte differential data were measured with the Abbott Cell-Dyn 3700 hematology analyzer. When population flags appeared, manual differentials were determined using the blood smears. Manual leukocyte differential counts were based on a minimum of 100 white cells. If the manual and automated differential were within ±10 cells for the two major leukocyte cell types, the instrument differential was accepted. If they did not agree, another manual differential was performed; if those agreed, the manual differential was accepted. Reticulocytes were stained with New Methylene Blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood for serum chemistry analyses was placed in tubes without anticoagulant, allowed to clot, centrifuged, and the serum was separated. Urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total bile acids, and creatine kinase were determined using a Roche Hitachi 912 system (Roche Diagnostic, Corp., Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats and mice exposed to 0, 32, 62, and 125 ppm. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the

females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study rats and mice in the 0 and 125 ppm groups; target organs were examined to a no-effect level. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to diethylamine by inhalation at concentrations of 0, 31, 62.5, or 125 ppm for rats and 0, 16, 31, or 62.5 ppm for mice, 6 hours plus T_{90} (15 minutes) per day, 5 days per week, for 105 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Chambers, racks, and cages were rotated weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks through week 93; body weights were recorded initially, then weekly for the first 13 weeks, and then every 4 weeks through week 93; clinical findings and body weights were recorded every 2 weeks thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1. Zymbal's glands, which are not routinely examined microscopically in NTP studies, were trimmed for microscopic examination after being identified as a potential target tissue in the rat.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report slides, paraffin blocks, residual wet tissues, and pathology date were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the nose of all animals and the eye, lung, and testes of rats.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

Three standard sections are taken through the nose in NTP studies, and these are referred to as Levels I, II, and III. Proceeding from anterior to posterior, Level I is taken immediately posterior to the upper incisor teeth; Level II is taken through the level of the incisive papilla anterior to the first palatal ridge; and Level III is taken through the middle of the second molar teeth (Figure 1). The mucosa of the nasal passages in Levels I and II is lined by respiratory and transitional epithelium, except for the ventral meatus of Levels I and II (squamous epithelium) and the dorsal meatus of Level II (olfactory epithelium). Level III is lined almost entirely by olfactory epithelium, except for the ventral meatus, which is lined by respiratory epithelium.

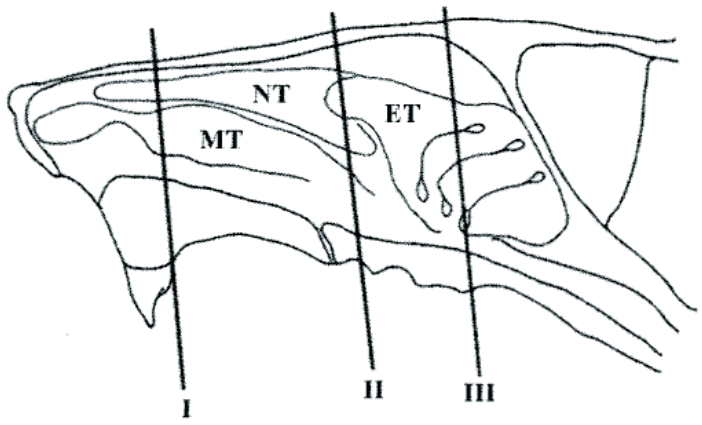


FIGURE 1
Rodent Nasal Cavity Diagram Illustrating the Levels of Sections
Level I: Immediately posterior to incisor teeth
Level II: At incisive papilla anterior to first palatal ridge
Level III: Section taken through the second molar
MT=maxilloturbinate; NT=nasoturbinate; ET=ethmoid turbinates

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Diethylamine

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies 13 days	Rats: 11 or 12 days Mice: 11 days	12 days
Average Age When Studies Began 5 to 6 weeks	6 to 7 weeks	5 to 6 weeks
Date of First Exposure August 12, 2002	Rats: December 16 (males) or 17 (females), 2002 Mice: December 16, 2002	Rats: August 25, 2003 Mice: August 18, 2003
Duration of Exposure 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days	6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks	6 hours plus T_{90} (15 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure Rats: August 27, 2002 Mice: August 28, 2002	Rats: March 17 (males) or 18 (females), 2003 Mice: March 19 (males) or 20 (females), 2003	Rats: August 24, 2005 Mice: August 18, 2005
Necropsy Dates Rats: August 28, 2002 Mice: August 29, 2002	Rats: March 18 (males) or 19 (females), 2003 Mice: March 20 (males) or 21 (females), 2003	Rats: August 22-25, 2005 Mice: August 15-19, 2005
Average Age at Necropsy 8 to 9 weeks	19 to 20 weeks	109 to 111 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Diethylamine

2-Week Studies	3-Month Studies	2-Year Studies
Diet NTP-2000 irradiated wafers (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods, changed weekly	Same as 2-week studies	Same as 2-week studies
Water Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly and rotated daily	Same as 2-week studies, except rotated weekly	Same as 3-month studies
Cage Board Techboard® untreated paper cage pan liner (Sheperd Specialty Papers, Inc., Kalamazoo, MI), changed daily	Same as 2-week studies	Same as 2-week studies
Chamber Air Supply Filters Single HEPA (open stock); Charcoal (RSE, Inc., New Baltimore, MI); Purafil (Environmental Systems, Lynnwood, WA)	Same as 2-week studies	Same as 2-week studies, except single HEPA filter changed annually
Chambers Stainless steel (Lab Products, Inc., Seaford, DE), changed weekly, excreta pans changed daily	Same as 2-week studies	Same as 2-week studies
Chamber Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour
Exposure Concentrations 0, 31, 62.5, 125, 250, or 500 ppm	0, 8, 16, 32, 62, or 125 ppm	Rats: 0, 31, 62.5, or 125 ppm Mice: 0, 16, 31, or 62.5 ppm
Type and Frequency of Observation Observed twice daily; animals were weighed on days 1, 6, and 13 and at the end of the studies; clinical findings were recorded twice daily.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.	Observed twice daily; animals were weighed initially and then weekly for the first 13 weeks; clinical findings were recorded every 4 weeks for the first 13 weeks; afterwards, body weights and clinical findings were recorded every 4 weeks through week 93; then every 2 weeks, and at the end of the studies.
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Diethylamine

2-Week Studies	3-Month Studies	2-Year Studies
Clinical Pathology		
None	Blood was collected from the retroorbital plexus of clinical pathology rats on days 3 and 23 and from core study rats at the end of the studies for hematology and clinical chemistry and from the retroorbital sinus of mice at study termination for hematology analyses. ***Hematology:*** hematocrit; packed cell volume, hemoglobin, hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids	None
Histopathology		
Histopathology was performed on 0 and 500 ppm rats and mice. In addition to gross lesions and tissue masses, the eye and lung of rats and mice exposed to lower concentrations of diethylamine were examined to a no-effect level and the nose was examined in all rats and mice.	Complete histopathology was performed on 0 and 125 ppm core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland (rats only).

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Diethylamine

2-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from male animals in the 0, 32, 62, and 125 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 days prior to the end of the studies from females exposed to 0, 32, 62, or 125 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B3, C1, C3, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, and tooth) before microscopic evaluation the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of

site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of diethylamine was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

2-WEEK STUDY

All rats survived to the end of the study (Table 2). The final mean body weights and body weight gains of 250 and 500 ppm males and females and 125 ppm males were significantly less than those of the chamber controls. As percentages of chamber controls, the mean body weights of males were more severely affected than the mean body weights of females. Lethargy, nasal/eye discharge, abnormal breathing, and thinness were observed in all animals in the 250 and 500 ppm groups. Eye abnormalities occurred in four males and four females in the 500 ppm group. A reddish-brown discoloration of the urine of undetermined etiology was observed in the cage pans throughout the study. Mean absolute organ weights were significantly decreased compared to the chamber controls in the 250 and 500 ppm groups, except for testis (250 ppm), male lung (250 ppm), female lung (250 and 500 ppm), and female kidney (250 ppm) (Table G1). Relative organ weights that were significantly increased included the heart of both sexes at 500 ppm, the heart of females at 250 ppm, the kidney of both sexes (125 ppm or greater males, 62.5 ppm or greater females), the liver of females at 250 and 500 ppm, and the testis of the 250 and 500 ppm groups. Significant decreases were noted in the absolute and relative thymus weights of both sexes (125 ppm or greater males, 500 ppm females). Focal eye lesions were noted at necropsy in four males and three females exposed to 500 ppm and one male exposed to 250 ppm. Crusty noses were observed in four females and three males in the 500 ppm group and two males in the 250 ppm group.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Inhalation Study of Diethylamine

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	104 ± 1	173 ± 4	69 ± 3	
31	5/5	102 ± 1	171 ± 5	69 ± 4	99
62.5	5/5	102 ± 1	171 ± 4	70 ± 4	99
125	5/5	102 ± 1	158 ± 3*	56 ± 3**	91
250	5/5	101 ± 2	128 ± 3**	27 ± 1**	74
500	5/5	102 ± 1	103 ± 2**	1 ± 2**	59
Female					
0	5/5	87 ± 2	124 ± 3	37 ± 3	
31	5/5	85 ± 2	124 ± 3	39 ± 2	99
62.5	5/5	88 ± 3	125 ± 3	37 ± 1	101
125	5/5	86 ± 2	120 ± 2	33 ± 2	96
250	5/5	86 ± 3	101 ± 2**	16 ± 2**	82
500	5/5	85 ± 2	86 ± 3**	1 ± 2**	69

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

Suppurative inflammation was present in Level I and II nasal sections of most rats exposed to 62.5 ppm or greater but was most prominent in Level I (Table 3). Inflammation was graded as marked in most rats exposed to 250 or 500 ppm, mild or moderate in rats exposed to 125 ppm, and minimal or mild in rats exposed to 62.5 ppm. The severity of inflammation was graded on the basis of the extent of infiltration of the nasal mucosa by neutrophils and the volume of exudates in the nasal lumen. Multifocal ulceration of the respiratory epithelium occurred in nasal Level I and to a lesser extent in Level II of all rats exposed to 250 or 500 ppm. The epithelial lining of the nasopharyngeal duct in nasal Level III was ulcerated in all rats exposed to 500 ppm. Ulceration was graded on the basis of the number and depth of areas of lost surface epithelium. Necrosis of the tips of the nasal and maxillary turbinates with sloughing of the mucosa and loss or necrosis of the underlying turbinate bone was present in all males and females exposed to 125 ppm or greater (except one 125 ppm female). Turbinate necrosis was graded by the degree of tissue loss and was considered to be mild or minimal if the bony tissue appeared necrotic but the overlying mucosa was intact.

Squamous metaplasia of the respiratory epithelium was observed at the Level I nasal area in all male rats exposed to 62.5 ppm or greater and all female rats exposed to 125 ppm or greater. The severity of the squamous metaplasia increased with increasing exposure concentration with minimal severity in the 62.5 ppm males to moderate severity in the 500 ppm groups. Grading was based upon the thickness of the squamous epithelium and the extent of mucosal surface area involved. Atrophy of the olfactory epithelium lining the dorsal meatus and adjacent medial septum and lateral wall of Level II was present in all rats exposed to 250 or 500 ppm. This lesion was characterized by reduction in thickness of the epithelium, and the severity was graded on the basis of the extent of surface area affected and the degree of thinning of the mucosa.

Minimal or mild suppurative inflammatory infiltrate of the cornea was present in all 500 ppm females, three 500 ppm males, and one 250 ppm male (Table 3). This inflammation extended into the iris/ciliary body in two males exposed to 500 ppm. Grading of the inflammation was based on the extent and the intensity of the inflammation.

Exposure Concentration Selection Rationale: Because exposure to 250 or 500 ppm diethylamine for 16 days caused significantly decreased body weights in rats, 125 ppm was selected as the highest exposure concentration for both sexes in the 3-month study. Although nasal lesions were present in rats exposed to 125 ppm for 16 days, these lesions were generally mild and were not likely to compromise the 3-month study. Diethylamine exposure concentrations of 0, 8, 16, 32, 62, and 125 ppm were selected for both sexes of rats in the 3-month study.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Week Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
Nose[a]	5	5	5	5	5	5
Inflammation, Suppurative[b]	0	0	5** (1.6)[c]	5** (2.8)	5** (3.8)	5** (4.0)
Respiratory Epithelium, Ulcer	0	0	1 (1.0)	1 (2.0)	5** (3.6)	5** (3.6)
Nasopharyngeal Duct, Ulcer	0	0	0	0	0	5** (3.2)
Turbinates, Necrosis	0	0	0	5** (2.2)	5** (3.8)	5** (3.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	5** (1.0)	5** (2.2)	5** (3.0)	5** (3.0)
Olfactory Epithelium, Atrophy	0	0	0	0	5** (3.0)	5** (2.8)
Eye	5	0	0	5	5	5
Cornea, Inflammation, Suppurative	0			0	1 (1.0)	3 (3.0)
Female						
Nose	5	5	5	5	5	5
Inflammation, Suppurative	0	0	2 (1.0)	5** (2.2)	5** (3.8)	5** (4.0)
Respiratory Epithelium, Ulcer	0	0	0	0	5** (4.0)	5** (3.8)
Nasopharyngeal Duct, Ulcer	0	0	0	0	0	5** (3.4)
Turbinates, Necrosis	0	0	0	4* (2.0)	5** (3.0)	5** (3.8)
Respiratory Epithelium, Metaplasia, Squamous	0	0	2 (1.0)	5** (2.2)	5** (2.8)	5** (3.0)
Olfactory Epithelium, Atrophy	0	0	0	0	5** (2.8)	5** (2.8)
Eye	5	0	0	0	5	5
Cornea, Inflammation, Suppurative	0				0	5** (1.2)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-MONTH STUDY

All rats survived to the end of the study (Table 4). Final mean body weights and body weight gains of all exposed groups were similar to those of the chamber control groups (Table 4 and Figure 2). The only clinical finding was a single occurrence of a torso lateral ulcer/abscess in a 125 ppm male.

There were no exposure-related changes in hematology or clinical chemistry endpoints (Table F1).

The relative kidney weights of all groups of exposed females were increased and were significantly greater than those of the chamber controls, except in the 32 ppm group (Table G2). The relative liver weight of 125 ppm males was significantly increased.

There was a dose-related decrease seen in the motility of sperm from male rats with the values of those exposed to 32, 62, or 125 ppm diethylamine being significantly lower (5%-26%) than those of the chamber controls; no significant differences were observed in the estrous cyclicity of female rats administered 32, 62, or 125 ppm diethylamine when compared to the chamber controls (Table H1 and H2).

TABLE 4
Survival and Body Weights of Rats in the 3-Month Inhalation Study of Diethylamine

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	111 ± 3	347 ± 6	236 ± 5	
8	10/10	107 ± 3	344 ± 4	236 ± 4	99
16	10/10	109 ± 3	357 ± 6	249 ± 5	103
32	10/10	110 ± 3	350 ± 7	240 ± 7	101
62	10/10	109 ± 3	355 ± 7	246 ± 8	102
125	10/10	111 ± 3	338 ± 6	227 ± 6	97
Female					
0	10/10	93 ± 1	204 ± 6	111 ± 5	
8	10/10	92 ± 1	199 ± 3	108 ± 3	98
16	10/10	93 ± 1	197 ± 3	104 ± 3	97
32	10/10	90 ± 1	200 ± 4	111 ± 3	98
62	10/10	89 ± 1	202 ± 3	113 ± 3	99
125	10/10	93 ± 1	201 ± 5	108 ± 5	99

[a] Number of animals surviving at 3 months/number initially in group

[b] Weights and weight changes are given as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test.

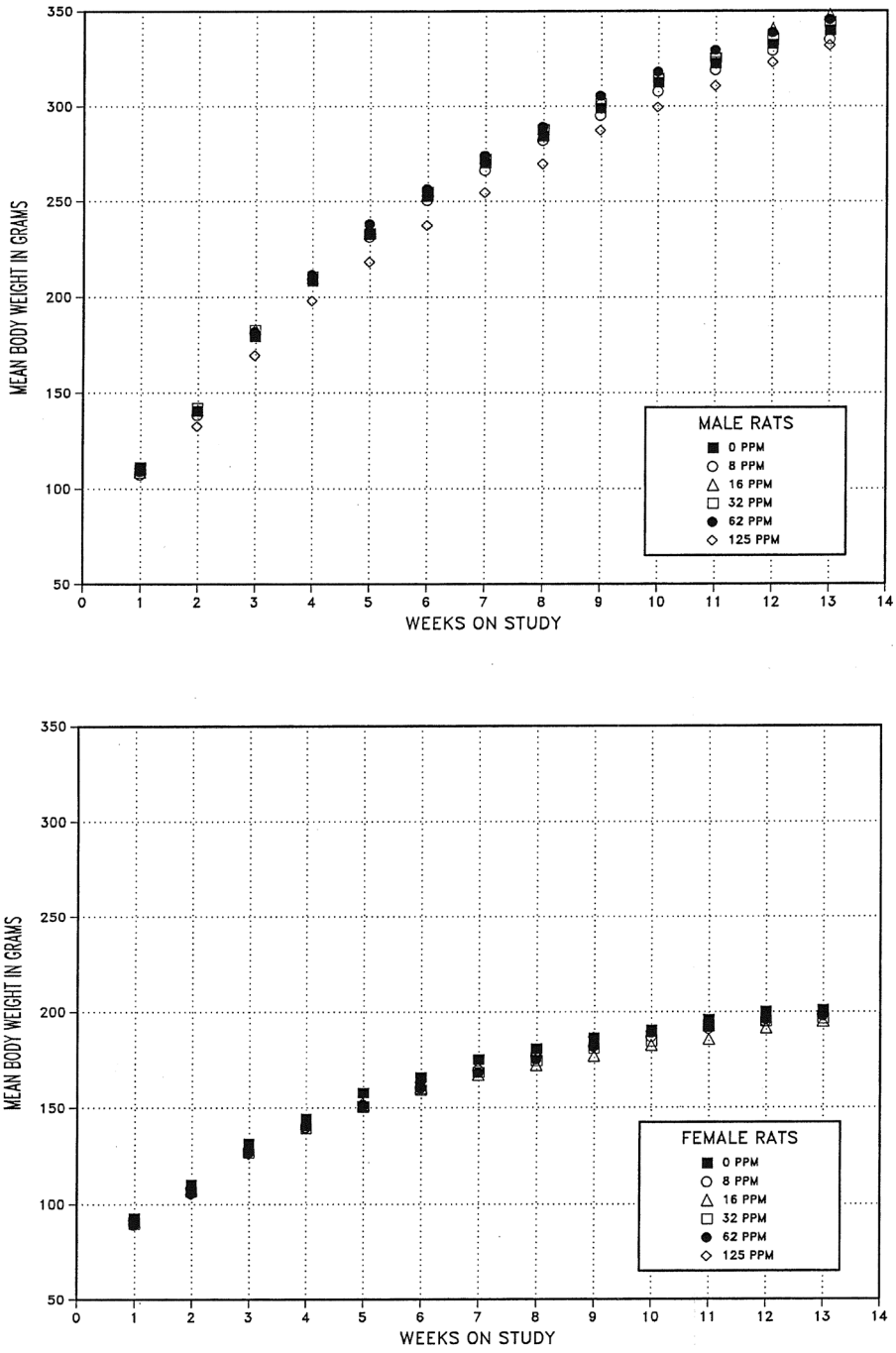


FIGURE 2
Growth Curves for Rats Exposed to Diethylamine by Inhalation for 3 Months

Exposure-related histopathology findings in rats were limited to the nose. Suppurative inflammation of minimal to mild severity was observed in all males and most females in the 125 ppm groups; a few 62 ppm males and females and one 16 ppm female also exhibited inflammation (Table 5). Suppurative inflammation was characterized by increased numbers of neutrophils in the nasal tissue and was most obvious on the tips of the nasal turbinates. Necrosis of the nasal turbinates in Level I was noted in one male and one female exposed to 125 ppm and consisted of sloughing of the respiratory epithelium with exposure and partial necrosis of the underlying bone. Respiratory epithelial hyperplasia was present in all 125 ppm males, in most 125 ppm females and 62 ppm males and females, and in a few 16 and 32 ppm males and one 16 ppm female. Hyperplastic respiratory epithelium contained three or more layers of epithelial cell nuclei with loss of the normal orderly, polarized, arrangement. Nonkeratinizing squamous metaplasia of the respiratory epithelium occurred in all 125 ppm males, five 125 ppm females, one 62 ppm male, and one 16 ppm male. Both hyperplasia and metaplasia of the respiratory epithelium were most commonly observed and most severe on the tips of the nasal turbinates and on the lateral wall of the dorsal half of nasal Level I. Olfactory epithelial atrophy was present in all 125 ppm males and females, most 62 ppm males and females, and two 32 ppm females. Atrophy was most pronounced in the dorsal meatus of nasal Level II and only rarely affected the olfactory epithelium of nasal Level III. The atrophy was characterized by decreased height of the olfactory epithelium associated with a decreased number of olfactory cell nuclei, and was accompanied by a reduction in the number of nerves and Bowman's glands in the underlying lamina propria.

There were no inflammatory changes of the eye as had been observed in the 2-week study at higher concentrations.

TABLE 5
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm		32 ppm		62 ppm		125 ppm	
Male										
Number Examined Microscopically	10	10	10		10		10		10	
Inflammation, Suppurative[a]	0	0	0		0		2	(2.5)[b]	10**	(1.6)
Turbinate, Necrosis	0	0	0		0		0		1	(2.0)
Respiratory Epithelium, Hyperplasia	0	0	3	(1.0)	3	(1.0)	9**	(1.3)	10**	(2.3)
Respiratory Epithelium, Metaplasia, Squamous	0	0	1	(1.0)	0		1	(2.0)	10**	(2.1)
Olfactory Epithelium, Atrophy	0	0	0		0		7**	(1.1)	10**	(1.9)
Female										
Number Examined Microscopically	10	10	10		10		10		10	
Inflammation, Suppurative	0	0	1	(2.0)	0		3	(1.0)	7**	(1.4)
Turbinate, Necrosis	0	0	0		0		0		1	(1.0)
Respiratory Epithelium, Hyperplasia	0	0	1	(1.0)	0		9**	(1.2)	9**	(1.9)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0		0		0		5*	(1.6)
Olfactory Epithelium, Atrophy	0	0	0		2	(1.0)	9**	(1.2)	10**	(2.2)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Exposure Concentration Selection Rationale: Chemical-related microscopic lesions were present only in the nasal cavity of rats exposed to diethylamine for 3 months. Lesions consisted of turbinate necrosis, suppurative inflammation, hyperplasia and squamous metaplasia of the respiratory epithelium, and atrophy of the olfactory epithelium. Turbinate necrosis was limited to one male and one female exposed to 125 ppm. The lesions were not considered severe enough to compromise a 2-year study. Exposure concentrations of 0, 31, 62.5, and 125 ppm diethylamine were selected for the 2-year study in rats.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 3). Survival of exposed groups was similar to that of the chamber control groups.

TABLE 6
Survival of Rats in the 2-Year Inhalation Study of Diethylamine

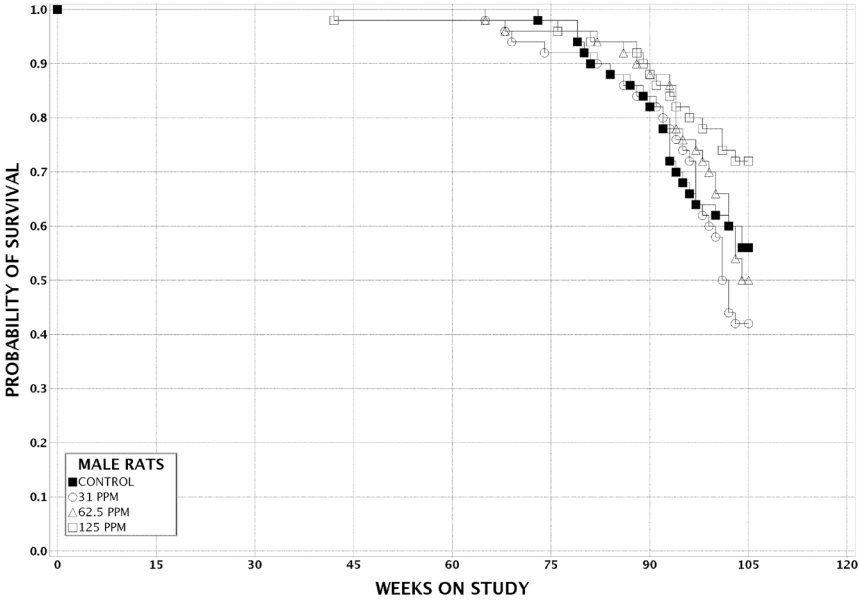
	Chamber Control	31 ppm	62.5 ppm	125 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	19	24	20	13
Natural deaths	3	5	5	1
Animals surviving to study termination	28	21	25	36
Percent probability of survival at end of study[a]	56	42	50	72
Mean survival (days)[b]	686	678	693	698
Survival analysis[c]	P=0.038N	P=0.333	P=0.927	P=0.136N
Female				
Animals initially in study	50	50	50	50
Moribund	17	15	18	15
Natural deaths	2	4	2	0
Animals surviving to study termination	31	31	30[d]	35[d]
Percent probability of survival at end of study	62	62	60	68
Mean survival (days)	687	702	689	693
Survival analysis	P=0.640N	P=1.000	P=1.000	P=0.682N

[a] Kaplan-Meier determinations

[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).

[c] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

[d] Includes one animal that died during the last week of the study



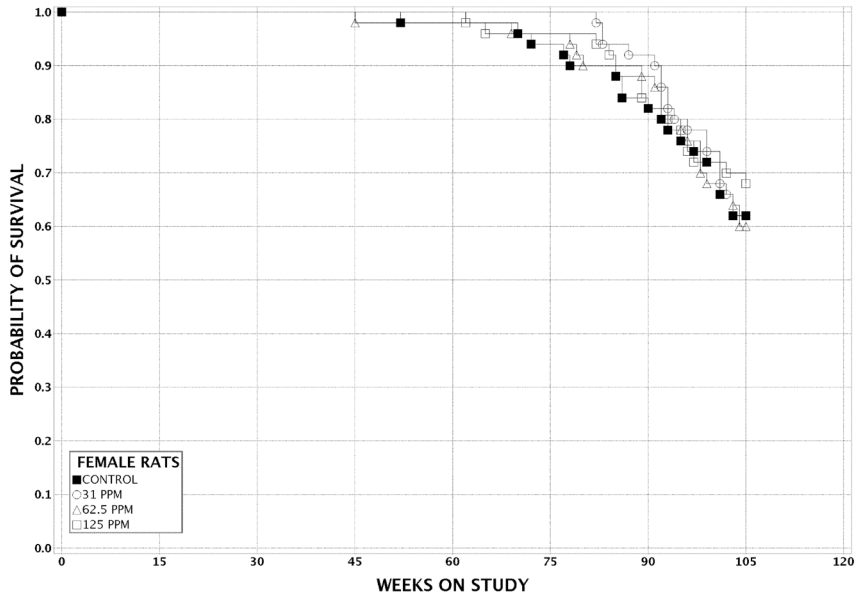


FIGURE 3
Kaplan-Meier Survival Curves for Rats Exposed to Diethylamine by Inhalation for 2 Years

Body Weights and Clinical Findings

Mean body weights of males and females exposed to 125 ppm were less than those of the chamber controls after week 57 (Figure 4 and Tables 7 and 8). Increased incidences of eye abnormality occurred in exposed males and females (males: chamber control, 0/50; 31 ppm, 3/50; 62.5 ppm, 1/50; 125 ppm, 3/50; females: 1/50; 3/50; 7/50; 5/50). Lethargy was more common in 125 ppm males than in the chamber controls.

Clonic seizures, usually observed during routine animal care, were noted in a few chamber control and exposed males (1/50, 2/50, 7/50, 9/50) and females (2/50, 7/50, 11/50, 13/50). More females (33) than males (19) developed seizures. There was an increased incidence of seizures with increasing exposure concentration in both males and females. The seizures were initially observed during week 23. No evidence of brain lesions was found to account for the cause or effect of the seizures. Similar, sporadic seizures have been observed in F344/N rats in other NTP inhalation or dermal studies at three different laboratories. In all of these studies, the single common factor was that the animals were housed individually. No such episodes have been observed in concurrent dosed feed, gavage, or drinking water studies in which rats are group housed. In the individually housed animals, most seizures were observed early in the day when technical and maintenance activities were commencing following the animals' dark cycle. No deaths were associated with the seizures, and there were no correlations with body weight, feed consumption or composition, or histopathological lesions in this or the other studies. Thus, these transient events were not considered to have affected the toxicologic or carcinogenicity evaluations of this study.

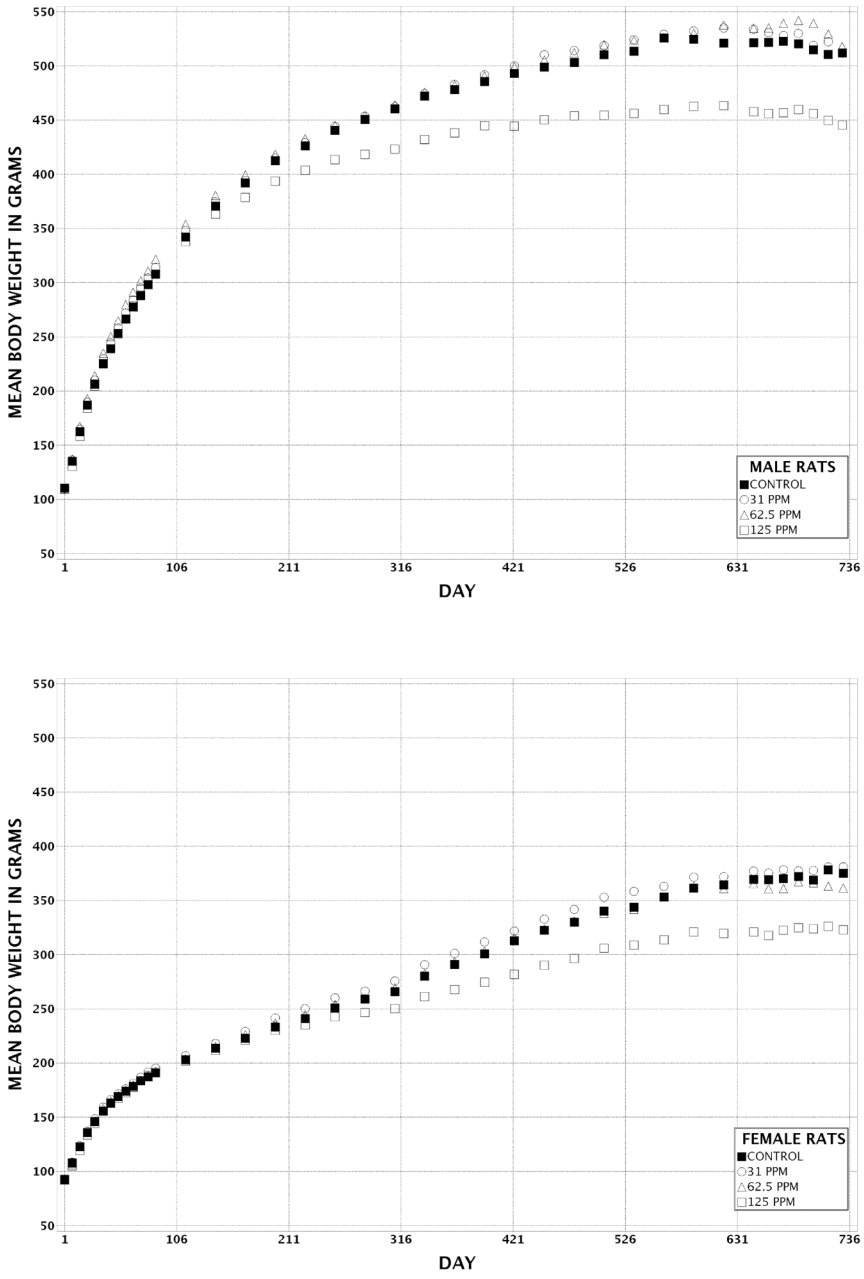


FIGURE 4
Growth Curves for Rats Exposed to Diethylamine by Inhalation for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Diethylamine

Days on Study	Chamber Control		31 ppm			62.5 ppm			125 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	110	50	110	100	50	111	100	50	110	99	50
8	135	50	137	101	50	137	102	50	131	97	50
15	163	50	165	101	50	167	103	50	158	97	50
22	187	50	190	102	50	193	103	50	184	99	50
29	206	50	210	102	50	214	104	50	205	99	50
37	226	50	230	102	50	235	104	50	225	100	50
44	239	50	244	102	50	250	105	50	239	100	50
51	253	50	258	102	50	265	105	50	253	100	50
58	267	50	272	102	50	280	105	50	267	100	50
65	278	50	284	102	50	291	105	50	279	100	50
72	288	50	295	103	50	302	105	50	289	100	50
79	298	50	304	102	50	311	104	50	298	100	50
86	308	50	314	102	50	322	104	50	308	100	50
114	342	50	348	102	50	354	103	50	338	99	50
142	370	50	375	101	50	381	103	50	363	98	50
170	392	50	394	101	50	400	102	50	379	97	50
198	413	50	415	101	50	418	101	50	394	96	50
226	426	50	429	101	50	433	102	50	404	95	50
254	441	50	444	101	50	445	101	50	414	94	50
282	451	50	454	101	50	454	101	50	418	93	50
310	461	50	462	100	50	464	101	50	423	92	49
338	472	50	475	101	50	476	101	50	432	92	49
366	478	50	483	101	50	482	101	50	438	92	49
394	486	50	492	101	50	491	101	50	445	92	49
422	493	50	500	101	50	499	101	50	445	90	49
450	499	50	510	102	50	505	101	50	450	90	49
478	503	50	514	102	47	512	102	48	454	90	49
506	510	49	519	102	47	520	102	48	455	89	49
534	514	49	524	102	46	524	102	48	456	89	48
562	526	45	529	101	46	526	100	48	460	88	47
590	525	44	532	102	44	530	101	47	463	88	47
618	521	43	535	103	42	538	103	45	463	89	45
646	521	38	534	102	39	535	103	43	458	88	42
660	522	34	531	102	38	535	103	39	456	87	41
674	523	33	528	101	33	539	103	37	457	87	40
688	520	32	530	102	30	542	104	35	460	88	39
702	515	31	519	101	26	539	105	33	456	89	38
716	511	30	522	102	21	530	104	28	450	88	36
Mean for weeks											
1-13	228		232	102		237	104		227	99	
14-52	419		422	101		425	101		396	95	
53-103	510		519	102		522	102		454	89	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Diethylamine

Days on Study	Chamber Control		31 ppm			62.5 ppm			125 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	93	50	93	100	50	92	99	50	93	100	50
8	108	50	109	101	50	107	100	50	105	98	50
15	123	50	124	101	50	123	100	50	120	97	50
22	136	50	137	101	50	136	100	50	134	98	50
29	146	50	149	102	50	147	101	50	144	99	50
37	156	50	159	102	50	158	102	50	156	100	50
44	163	50	166	102	50	165	101	50	163	100	50
51	169	50	172	102	50	171	101	50	168	99	50
58	174	50	176	101	50	175	101	50	173	99	50
65	178	50	181	102	50	180	101	50	178	100	50
72	184	50	187	102	50	185	100	50	184	100	50
79	187	50	192	102	50	189	101	50	189	101	50
86	191	50	195	102	50	192	101	50	192	100	50
114	203	50	207	102	50	204	101	50	202	100	50
142	214	50	218	102	50	215	101	50	212	99	50
170	223	50	229	103	50	226	101	50	221	99	50
198	233	50	242	104	50	237	102	50	231	99	50
226	241	50	250	104	50	245	102	50	235	98	50
254	251	50	260	104	50	254	101	50	243	97	50
282	259	50	266	103	50	260	100	50	247	95	50
310	266	50	276	104	50	270	101	49	250	94	50
338	280	50	291	104	50	283	101	49	261	93	50
366	291	49	301	104	50	294	101	49	268	92	50
394	301	49	312	104	50	303	101	49	275	91	50
422	313	49	322	103	50	316	101	49	282	90	50
450	323	49	333	103	50	324	100	49	291	90	48
478	330	49	342	104	50	331	100	49	297	90	48
506	340	47	353	104	50	338	99	48	306	90	48
534	344	47	359	104	50	342	100	48	309	90	48
562	353	45	363	103	50	353	100	45	314	89	48
590	361	44	372	103	47	363	101	45	321	89	45
618	364	42	372	102	46	361	99	44	320	88	42
646	370	39	377	102	41	366	99	40	321	87	41
660	369	39	375	102	40	361	98	40	318	86	40
674	370	37	378	102	39	361	98	38	323	87	37
688	372	36	377	101	37	367	99	34	325	87	36
702	369	36	378	102	35	366	99	33	324	88	36
716	378	31	381	101	32	363	96	33	326	86	35
Mean for weeks											
1-13	154		157	102		155	101		154	99	
14-52	241		249	103		244	101		234	97	
53-103	347		356	103		344	99		308	89	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the nose, lung and pleura, and eye. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Nose: A spectrum of nonneoplastic lesions was observed in the respiratory and olfactory epithelium of exposed rats. The incidences of suppurative inflammation were significantly increased in 125 ppm males and females and in 62.5 ppm females compared to the chamber controls (Tables 9, A3, and B3); these lesions were noted particularly in Levels II and III. The inflammation was characterized by infiltrates of neutrophils in the mucosa and aggregates of neutrophils in the lumen of the nasal cavity, which were sometimes associated with a foreign body such as food material or colonies of bacteria. In other cases, the suppuration was associated with areas of ulceration of the respiratory epithelium or necrosis of the turbinates (Plate 1). The incidences of ulceration of the respiratory epithelium were significantly increased in 125 ppm males and females. Ulcers were observed in Levels I and II and consisted of areas in which the respiratory epithelium had been completely lost to the level of the underlying lamina propria. Significantly increased incidences of necrosis of the turbinates occurred in 125 ppm males and females. The necrosis of the naso- and/or maxilloturbinates was mild to moderate in average severity in the 125 ppm males and moderate in average severity in the 125 ppm females. The necrosis of the turbinate bone was often accompanied by necrosis or ulceration of the overlying respiratory epithelium and was characterized by loss of bone matrix, amphophilic staining, and loss of osteocytes within the lacunae, and sometimes by fragmentation and separation of the necrotic bone.

Incidences of squamous metaplasia of the respiratory epithelium were significantly increased in 125 ppm males and females and in 62.5 ppm males compared to the chamber controls (Tables 9, A3, and B3). These incidences were generally of mild severity and in Levels I and II. The metaplastic epithelium was often noted overlying hyperostotic nasal turbinates or adjacent to zones of ulceration or necrosis. Hyperplasia of the respiratory epithelium in Levels I and II was found in all 125 ppm females, and hyperplasia occurred in most exposed males and most 31 and 62.5 ppm females. Hyperplastic epithelium exhibited increased cellularity with crowding of epithelial cells, which resulted in increased thickness of the mucosa and occasional small papillary proliferations. Hyperplasia of the respiratory epithelium occurred both in the presence and absence of inflammation and was often accompanied by goblet cell hyperplasia and hyaline droplet accumulation. Hyperplasia of the glands beneath the respiratory epithelium was noted in most chamber control and exposed rats; however, the severity of the hyperplasia of the respiratory epithelial glands increased with increasing exposure concentration in both sexes (Plate 2). The hyperplastic glands were enlarged, often dilated, and were found most often in the transition zone from respiratory epithelium to olfactory epithelium lining the nasal septum, in the dorsal meatus of Level I, and along the medial aspect of the nasal turbinates in Level II. Hyaline droplet accumulation of the respiratory epithelial glands was present in the glandular cell cytoplasm of most exposed rats, but in only a few of the chamber controls.

Prominent histologic changes were also observed in the olfactory epithelium. Atrophy of the olfactory epithelium occurred in most exposed males and females and the severity increased with increasing exposure concentration (Plate 3) (Tables 9, A3, and B3). The atrophy was most pronounced in the dorsal meatus of Level II and was characterized by diminished numbers of neuronal cells with thinning of the epithelium and associated reduction in the olfactory nerve bundles and Bowman's glands and ducts in the subjacent lamina propria. Hyaline droplet accumulation in the olfactory epithelium was noted in most exposed males and females, and cytoplasmic vacuolization of the olfactory epithelium was observed in a few males and females in the areas of atrophy. Basal cell hyperplasia occurred concomitantly with olfactory epithelial atrophy in most 125 ppm males and females and in many 62.5 ppm males and females (Plate 4). The hyperplastic basal cells formed a thickened basal zone either in a normal linear arrangement or in small clusters of cells. In most 125 ppm males and in many 125 ppm females the olfactory epithelium was replaced by metaplastic respiratory epithelium in focal areas, typically in association with intense inflammation.

Hyperostosis of the nasal and/or maxillary turbinate bones in Level I was observed in three males and two females in the 125 ppm groups (Tables 9, A3, and B3). The hyperostotic bone was thickened by an increase in the bone matrix accompanied by an increased concentration of parallel cement lines.

TABLE 9
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control		31 ppm		62.5 ppm		125 ppm	
Male								
Number Examined Microscopically	49		50		50		50	
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet[a]	6	(1.0)[b]	45**	(1.2)	42**	(1.6)	45**	(1.5)
Glands, Respiratory Epithelium, Hyperplasia	44	(1.0)	46	(1.2)	46	(1.7)	48	(1.7)
Goblet Cell, Hyperplasia	0		0		2	(1.5)	13**	(2.2)
Inflammation, Suppurative	5	(1.6)	5	(1.6)	10	(1.7)	29**	(2.6)
Olfactory Epithelium, Accumulation, Hyaline Droplet	8	(1.0)	49**	(2.4)	49**	(2.1)	42**	(1.7)
Olfactory Epithelium, Atrophy	2	(1.5)	49**	(1.5)	50**	(1.8)	50**	(2.3)
Olfactory Epithelium, Hyperplasia, Basal Cell	0		0		22**	(1.8)	50**	(2.4)
Olfactory Epithelium, Respiratory Metaplasia	2	(1.5)	2	(1.0)	2	(1.5)	37**	(1.6)
Olfactory Epithelium, Vacuolization Cytoplasmic	0		2	(4.0)	8**	(3.8)	1	(4.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0		29**	(1.2)	42**	(1.4)	11**	(1.5)
Respiratory Epithelium, Hyperplasia	5	(1.6)	34**	(1.2)	35**	(1.3)	47**	(1.9)
Respiratory Epithelium, Metaplasia, Squamous	0		2	(1.0)	6*	(1.8)	26**	(2.1)
Respiratory Epithelium, Necrosis	0		0		1	(1.0)	4	(1.3)
Respiratory Epithelium, Ulcer	0		0		2	(2.5)	22**	(3.3)
Turbinate, Hyperostosis	0		0		0		3	(2.3)
Turbinate, Necrosis	0		0		1	(2.0)	19**	(2.9)

TABLE 9
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control		31 ppm		62.5 ppm		125 ppm	
Female								
Number Examined Microscopically	50		49		50		50	
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet	9	(1.0)	46**	(1.6)	45**	(1.7)	44**	(1.6)
Glands, Respiratory Epithelium, Hyperplasia	45	(1.0)	49*	(1.7)	48	(1.9)	49	(2.1)
Goblet Cell, Hyperplasia	1	(2.0)	0		4	(1.8)	20**	(2.5)
Inflammation, Suppurative	6	(2.0)	4	(1.5)	15*	(1.5)	34**	(2.9)
Olfactory Epithelium, Accumulation, Hyaline Droplet	11	(1.3)	49**	(2.6)	50**	(2.6)	48**	(2.4)
Olfactory Epithelium, Atrophy	1	(1.0)	47**	(1.9)	48**	(2.3)	50**	(2.7)
Olfactory Epithelium, Hyperplasia, Basal Cell	0		3	(1.0)	29**	(1.7)	48**	(2.9)
Olfactory Epithelium, Respiratory Metaplasia	3	(1.7)	1	(2.0)	2	(1.0)	19**	(1.7)
Olfactory Epithelium, Vacuolization Cytoplasmic	0		1	(4.0)	4	(3.8)	3	(3.3)
Respiratory Epithelium, Accumulation, Hyaline Droplet	4	(1.0)	48**	(1.9)	46**	(1.2)	39**	(1.4)
Respiratory Epithelium, Hyperplasia	7	(1.4)	31**	(1.2)	41**	(1.4)	50**	(2.4)
Respiratory Epithelium, Metaplasia, Squamous	1	(1.0)	1	(1.0)	5	(1.4)	39**	(2.3)
Respiratory Epithelium, Necrosis	0		0		1	(1.0)	4	(1.8)
Respiratory Epithelium, Ulcer	0		0		0		34**	(3.1)
Turbinate, Hyperostosis	0		0		0		2	(3.0)
Turbinate, Necrosis	0		0		0		32**	(3.0)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Lung and Pleura: The incidences of minute foci of chronic inflammation and fibrosis of the pleura and subpleural lung were increased in 31 and 125 ppm females (Tables 10 and B3). In a few animals, the chronic inflammation and fibrosis were limited to either the pleura or to the alveolar wall interstitium of the subpleural lung. The minimal to mild inflammatory cell component of these fibrotic foci consisted primarily of lymphocytes and macrophages and was often accompanied by focal aggregates of histiocytes in the adjacent alveolar spaces. Small isolated aggregates of alveolar histiocytes were also identified in some rats in the absence of pleural or subpleural inflammatory lesions. Although the incidences of the foci of chronic lung inflammation, chronic pleural inflammation, and alveolar histiocytic aggregates increased with increasing exposure concentration and were statistically significant, the lesions were minute in size and the severity of the lesions did not increase with increasing exposure concentration.

Eye: Suppurative inflammation of the cornea occurred in five 125 ppm males and one 62.5 ppm male and was not found in the chamber controls (Tables 10 and A3). The incidence in the 125 ppm males was significantly increased. Suppurative inflammation of the cornea was observed in two 31 ppm, two 62.5 ppm, and one 125 ppm females, but no chamber control females (Tables 10 and B3). Chronic inflammation of the cornea was noted in three 125 ppm males but did not occur in chamber control males.

Cataracts occurred in three 31 ppm and five 125 ppm males compared to the single occurrence in chamber

TABLE 10
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control		31 ppm		62.5 ppm		125 ppm	
Male								
Eye[a]	49		50		50		50	
Cornea, Inflammation, Suppurative[b]	0		0		1	(2.0)[c]	5*	(2.4)
Cornea, Inflammation, Chronic	0		0		0		3	(1.7)
Lens, Cataract	1	(2.0)	3	(4.0)	1	(3.0)	5	(2.6)
Retina, Atrophy	1	(2.0)	3	(4.0)	1	(3.0)	3	(3.3)
Female								
Lung	50		50		50		50	
Alveolus, Infiltration Cellular, Histiocyte	13	(1.2)	24*	(1.3)	27**	(1.3)	35**	(1.4)
Inflammation, Chronic	4	(1.5)	11	(1.3)	7	(1.4)	24**	(1.3)
Pleura	50		50		50		50	
Inflammation, Chronic	6	(1.2)	14*	(1.2)	12	(1.3)	21**	(1.3)
Eye	50		50		50		50	
Cornea, Inflammation, Suppurative	0		2	(2.5)	2	(2.5)	1	(3.0)
Lens, Cataract	3	(2.0)	2	(4.0)	5	(3.6)	4	(3.5)
Retina, Atrophy	4	(2.5)	2	(4.0)	8	(3.3)	6	(3.0)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

control males. Cataracts occurred in five 62.5 ppm and four 125 ppm females compared to three in chamber control females (Tables 10, A3, and B3).

The incidences of cataracts in rats were not significantly increased nor were there exposure concentration-related trends. The average severity of the cataracts in exposed males and females was increased compared to chamber controls.

Retinal atrophy occurred in more 31 and 125 ppm males than in male chamber controls, and in more 62.5 and 125 ppm females than in female chamber controls (Tables 10, A3, and B3). The incidences of retinal atrophy were not significantly increased in males or females nor were there exposure concentration-related trends. The severity of retinal atrophy in exposed males and females was increased compared to chamber controls. Retinal atrophy was characterized by variable loss of cells in the inner and outer nuclear layers, progressing to an almost complete loss of all retinal layers in the most severely affected eyes. It was noted that most of the males and females with retinal were the same animals as those with cataracts.

Other Findings: Zymbal's glands, which are not routinely examined microscopically in NTP studies, were trimmed for microscopic examination after being identified as a potential target tissue in the rat. In males, Zymbal's gland carcinoma occurred in the 31 and 125 ppm groups (0/50; 3/50, 0/50, 2/50; Tables A1 and A2) and adenoma or carcinoma (combined) occurred in the chamber control, 31 ppm, and 125 ppm groups (2/50, 4/50, 0/50, 2/50); but the incidences did not reach statistical significance. Two carcinomas occurred in 31 ppm females (0/50, 2/50, 0/50, 0/50; Table B1). Since the incidences of neoplasms in exposed animals were not statistically different from those in the chamber controls and did not increase in an exposure concentration-related manner, the incidences are not likely related to exposure.

The incidence of hyperplasia of the pars distalis in the pituitary gland was significantly increased in 125 ppm females compared to the chamber controls (9/50, 10/50, 7/50, 18/50; Table B3).

In the skin, the incidence of fibroma, fibrous histiocytoma, fibrosarcoma, or sarcoma (combined) was significantly increased in 31 ppm males (chamber control, 2/50; 31 ppm, 8/50; 62.5 ppm, 2/50; 125 ppm, 0/50; Tables A1 and A2). The incidence exceeded the historical control range for inhalation studies, but not for all study routes combined [inhalation studies: 28/349 (8% ± 3%), range 4%-12%; all routes: 154/1,398 (11% ± 4%), range 4%-22%]. The increase was primarily due to the increased incidence of fibroma (1/50, 4/50, 2/50, 0/50; Tables A1 and A2). Since there was no exposure concentration-related trend, and the increased incidence in 31 ppm males was largely due to benign fibromas, these lesions were not considered to be chemical related. Significantly decreased incidences of mammary gland carcinoma (9/50, 3/50, 2/50, 2/50) and adenoma or carcinoma (combined) (10/50, 3/50, 2/50, 2/50) occurred in all exposed groups of females (Tables B1 and B2). The incidences of carcinoma in the 62.5 and 125 ppm groups were less than the historical control range for inhalation studies but within the range for all routes combined [inhalation studies: 41/350 (12% ± 6%), range 6%-20%; all routes: 74/1,350 (6% ± 5%), range 0%-20%]. The biological significance of this effect was uncertain because the concurrent chamber control incidence was at the upper end of the historical control ranges.

MICE

2-WEEK STUDY

Two males and three females exposed to 500 ppm died during the first week of the study (Table 11). The final mean body weights and mean body weight gains of males and females exposed to 125 ppm or greater were significantly less than those of the chamber controls. Males and females exposed to 250 or 500 ppm lost weight during the study. Lethargy and abnormal breathing were observed in all mice exposed to 250 or 500 ppm; thinness was observed in most of these mice. Eye irritation was observed in all 500 ppm mice and eye discharge was observed in three 500 ppm males and two 500 ppm females. Nasal discharge was observed and low fecal and urine output were noted in 500 ppm mice. No exposure-related gross lesions were seen in early death or terminal sacrifice mice.

TABLE 11
Survival and Body Weights of Mice in the 2-Week Inhalation Study of Diethylamine

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.3 ± 0.3	28.3 ± 0.4	5.0 ± 0.2	
31	5/5	23.5 ± 0.4	26.7 ± 0.5	3.1 ± 0.3*	94
62.5	5/5	23.1 ± 0.2	27.4 ± 0.3	4.3 ± 0.3*	97
125	5/5	23.3 ± 0.3	24.6 ± 0.4**	1.3 ± 0.4**	87
250	5/5	22.8 ± 0.6	20.8 ± 0.6**	–2.0 ± 0.2**	73
500	3/5[c]	23.4 ± 0.3	16.6 ± 1.2**	–6.6 ± 1.0**	59
Female					
0	5/5	19.6 ± 0.3	22.3 ± 0.2	2.6 ± 0.1	
31	5/5	19.4 ± 0.6	22.5 ± 0.5	3.1 ± 0.5	101
62.5	5/5	20.1 ± 0.4	22.6 ± 0.3	2.5 ± 0.3	102
125	5/5	19.4 ± 0.4	20.3 ± 0.4**	0.9 ± 0.3**	91
250	5/5	20.0 ± 0.4	18.0 ± 0.3**	–2.0 ± 0.4**	81
500	2/5[d]	19.6 ± 0.5	15.2 ± 0.5**	–4.4 ± 0.6**	68

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Days of death: 4, 6
[d] Day of deaths: 6

Absolute heart weights were significantly less than those of the chamber controls in all exposed groups of males and 125 ppm or greater females; the relative heart weights of 31 and 250 ppm females were significantly less than those of the chamber controls (Table G3). Absolute and relative liver weights of 125 ppm or greater males and 250 ppm females, and the absolute liver weight of 125 and 500 ppm females were significantly less than those of the chamber controls. Absolute and relative thymus weights were significantly less than those of the chamber controls in 250 and 500 ppm males and 125 ppm or greater females. Absolute lung weights of 250 and 500 ppm males were significantly less than those of the chamber controls; the relative lung weights of 250 and 500 ppm males and 125 ppm or greater females were significantly greater than those of the chamber controls. Absolute right kidney weights of 250 ppm males and females and 500 ppm males were significantly less than those of the chamber controls; relative right kidney weights of 31, 125, 250, and 500 ppm females were significantly greater than those of the chamber controls. Relative right testis weights of 250 and 500 ppm males were significantly greater than those of the chamber controls.

In the nose, suppurative inflammation occurred in all males exposed to 250 or 500 ppm, all females exposed to 125 ppm or greater, and most males exposed to 125 ppm (Table 12). Turbinate necrosis occurred in all exposed mice except one 31 ppm female. The necrosis was characterized by partial to complete loss of maxillo- and/or nasoturbinates in Level I with necrosis of respiratory epithelium and underlying bone. The nasal septum was also necrotic in some mice. Squamous metaplasia of the respiratory epithelium was seen on the nasal septum, turbinates, and/or lateral walls in Level I of most 125 and 250 ppm males and females. Incidences of olfactory epithelial atrophy were significantly increased in 125 and 250 ppm males and in 125 ppm or greater females. Necrosis of individual olfactory epithelial cells was observed in three females and one male exposed to 500 ppm. These changes primarily involved the dorsal meatus of Level II.

In the lung, minimal chronic active inflammation of mainstem bronchi at their bifurcation was noted in four males and two females in the 500 ppm groups (Table 12). The inflammatory infiltrate consisted primarily of mononuclear cells with a few neutrophils and was associated with minimal necrosis of overlying individual epithelial cells in one male and one female in the 500 ppm groups.

Exposure Concentration Selection Rationale: Because exposure to 250 and 500 ppm diethylamine for 17 days caused mortality in mice and body weight losses exceeding 18%, a high concentration of 125 ppm was selected for both sexes of mice in the 3-month study. Although nasal lesions were present in mice exposed to 125 ppm for 17 days, these lesions were generally mild and were considered not likely to compromise the 3-month study. Diethylamine exposure concentrations of 0, 8, 16, 32, 62 and 125 ppm were selected for both sexes of mice in the 3-month study.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Week Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
Nose[a]	5	5	5	5	5	5
Inflammation, Suppurative[b]	0	0	0	3 (1.3)[c]	5** (2.0)	5** (2.4)
Turbinates, Necrosis	0	5** (1.0)	5** (1.0)	5** (1.8)	5** (3.8)	5** (3.6)
Olfactory Epithelium, Atrophy	0	0	0	5** (1.8)	4* (2.0)	2 (1.5)
Olfactory Epithelium, Necrosis	0	0	0	0	0	1 (2.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	4* (2.0)	5** (2.2)	3 (1.7)
Lung	5	0	0	0	5	5
Bronchus, Inflammation, Chronic, Active	0				0	4* (1.0)
Female						
Nose	5	5	5	5	5	5
Inflammation, Suppurative	0	0	0	5** (1.8)	5** (1.8)	5** (2.0)
Turbinates, Necrosis	0	4* (1.0)	5** (1.0)	5** (2.2)	5** (3.4)	5** (3.8)
Olfactory Epithelium, Atrophy	0	0	1 (1.0)	5** (2.0)	5** (2.0)	4* (2.3)
Olfactory Epithelium, Necrosis	0	0	0	0	0	3 (1.7)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	5** (1.8)	5** (1.8)	2 (2.0)
Lung	5	0	0	0	5	5
Bronchus, Inflammation, Chronic, Active	0				0	2 (1.0)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-MONTH STUDY

All mice survived to the end of the study (Table 13). The final mean body weights and body weight gains of 125 ppm males and females were significantly less than those of the chamber controls (Table 13 and Figure 5). There were no clinical findings related to diethylamine exposure.

There were no exposure-related changes in hematology endpoints (Table F2).

The absolute weights of the liver, right kidney, and thymus of 125 ppm males; heart, liver, and right kidney of 125 ppm females; and thymus of 62 and 125 ppm females were significantly less than those of the chamber controls (Table G4). The relative weights of the heart, right kidney, lung, and right testis of 125 ppm males and the lung of 125 ppm females were significantly greater than those of the chamber controls. No gross lesions were observed at necropsy.

There was a dose-related decrease seen in the motility of sperm from male mice with values of those exposed to 32, 62, or 125 ppm diethylamine being significantly lower (7-15%) than those of the chamber controls; except for a slight (0.5 day), but statistically significant increase in estrous cycle length, no significant differences were observed in the estrous cyclicity of female mice administered 32, 62, or 125 ppm diethylamine when compared to the chamber controls (Tables H3 and H4).

TABLE 13
Survival and Body Weights of Mice in the 3-Month Inhalation Study of Diethylamine

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.8 ± 0.3	39.3 ± 0.8	15.5 ± 0.8	
8	10/10	23.6 ± 0.2	38.4 ± 1.0	14.8 ± 0.9	98
16	10/10	23.3 ± 0.3	37.8 ± 0.3	14.5 ± 0.3	96
32	10/10	24.1 ± 0.3	39.6 ± 0.9	15.5 ± 0.7	101
62	10/10	24.1 ± 0.2	39.3 ± 0.8	15.2 ± 0.6	100
125	10/10	22.9 ± 0.6	30.8 ± 0.5**	7.8 ± 0.7**	78
Female					
0	10/10	19.6 ± 0.2	32.6 ± 1.4	13.0 ± 1.3	
8	10/10	19.8 ± 0.3	31.9 ± 1.5	12.1 ± 1.3	98
16	10/10	19.8 ± 0.3	34.3 ± 1.4	14.6 ± 1.2	105
32	10/10	19.6 ± 0.4	32.5 ± 1.1	12.9 ± 1.0	100
62	10/10	19.8 ± 0.2	31.7 ± 1.0	11.9 ± 1.1	97
125	10/10	19.7 ± 0.3	27.3 ± 0.3**	7.6 ± 0.3**	84

** Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test

[a] Number of animals surviving at 3 months/number initially in group

[b] Weights and weight changes are given as mean ± standard error.

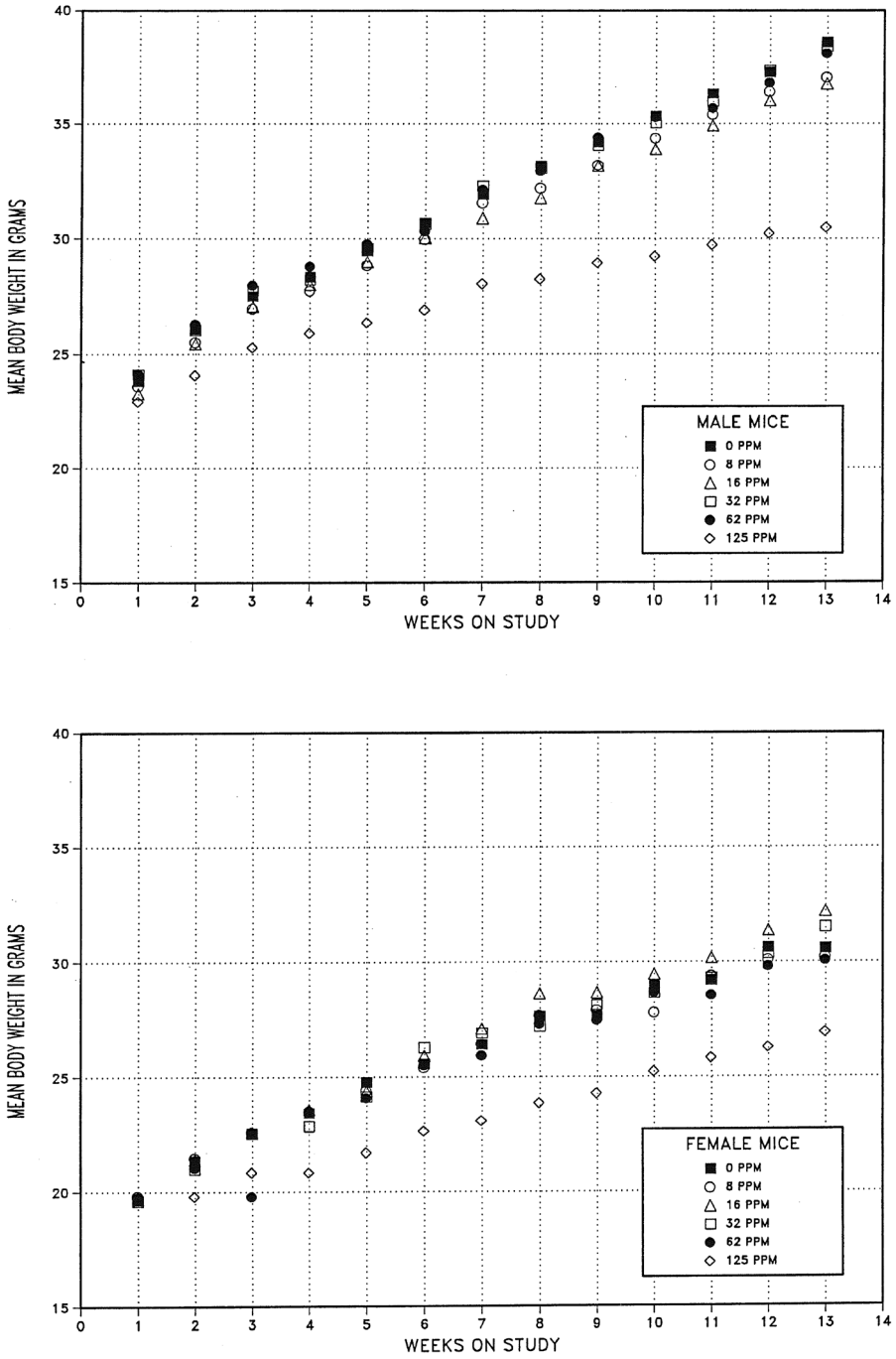


FIGURE 5
Growth Curves for Mice Exposed to Diethylamine by Inhalation for 3 Months

Histopathologic changes were noted primarily in the nasal cavity and involved both the respiratory and olfactory epithelium of males and females principally in the 62 and 125 ppm groups. Incidences of minimal to mild suppurative inflammation were significantly increased in 125 ppm males and females and also occurred in a few 62 ppm females (Table 14). The 125 ppm mice exhibited significantly increased incidences of mild to moderate necrosis of the maxillo- and/or naso-turbinates in Level I. Significantly increased incidences of respiratory epithelial squamous metaplasia, generally mild, were noted on the septum, turbinates, and/or the lateral walls in Level I of 125 ppm males and females. Minimal squamous metaplasia was also present in one male and one female in the 62 ppm groups. The incidences of olfactory epithelial atrophy were significantly increased in 32 ppm or greater males and females. The atrophy was noted primarily in the dorsal meatus of Level II and sometimes in Level III.

Exposure Concentration Selection Rationale: A high concentration of 125 ppm diethylamine was considered inappropriate for a long-term study in mice because of the significant reductions in body weight gain observed at this concentration in the 3-month study. Final mean body weights were significantly reduced in male (–22%) and female (–16%) mice exposed to 125 ppm for 3 months. There were no significant changes in body weight gains or organ weights (except for the thymus in females) of mice exposed to 62 ppm. Microscopic changes in mice exposed to 62 ppm were observed only in the nasal cavity. Nasal lesions consisted of minimal to mild suppurative inflammation, turbinate necrosis, squamous metaplasia of the respiratory epithelium, and olfactory epithelial atrophy. The severities of these lesions were not considered severe enough to compromise a 2-year study. Nasal cavity changes were minimal in the 32 ppm group. Based upon these results, exposure concentrations of 0, 16, 31, and 62.5 ppm were selected for the 2-year study in mice.

TABLE 14
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Suppurative[a]	0	0	0	0	0	8** (1.5)[b]
Olfactory Epithelium, Atrophy	0	0	0	4* (1.0)	9** (2.2)	10** (2.9)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	1 (1.0)	9** (2.2)
Turbinate, Necrosis	0	0	0	0	0	7** (2.4)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Suppurative	0	0	0	0	3 (1.0)	8** (1.0)
Olfactory Epithelium, Atrophy	0	0	0	9** (1.1)	10** (2.4)	10** (2.8)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	1 (1.0)	9** (1.8)
Turbinate, Necrosis	0	0	0	0	0	6** (2.7)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 6). Survival of exposed groups of mice was similar to that of the chamber control groups.

TABLE 15
Survival of Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	15	10	12	11
Natural deaths	4	2	6	2
Animals surviving to study termination	31	38	32	37
Percent probability of survival at end of study[a]	62	76	64	74
Mean survival (days)[b]	686	703	687	701
Survival analysis[c]	P=0.416N	P=0.174N	P=0.943N	P=0.267N
Female				
Animals initially in study	50	50	50	50
Moribund	13	11	7	10
Natural deaths	5	4	7	1
Animals surviving to study termination	32	35	36	39
Percent probability of survival at end of study	64	70	72	78
Mean survival (days)	684	688	716	717
Survival analysis	P=0.113N	P=0.755N	P=0.364N	P=0.150N

[a] Kaplan-Meier determinations

[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).

[c] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

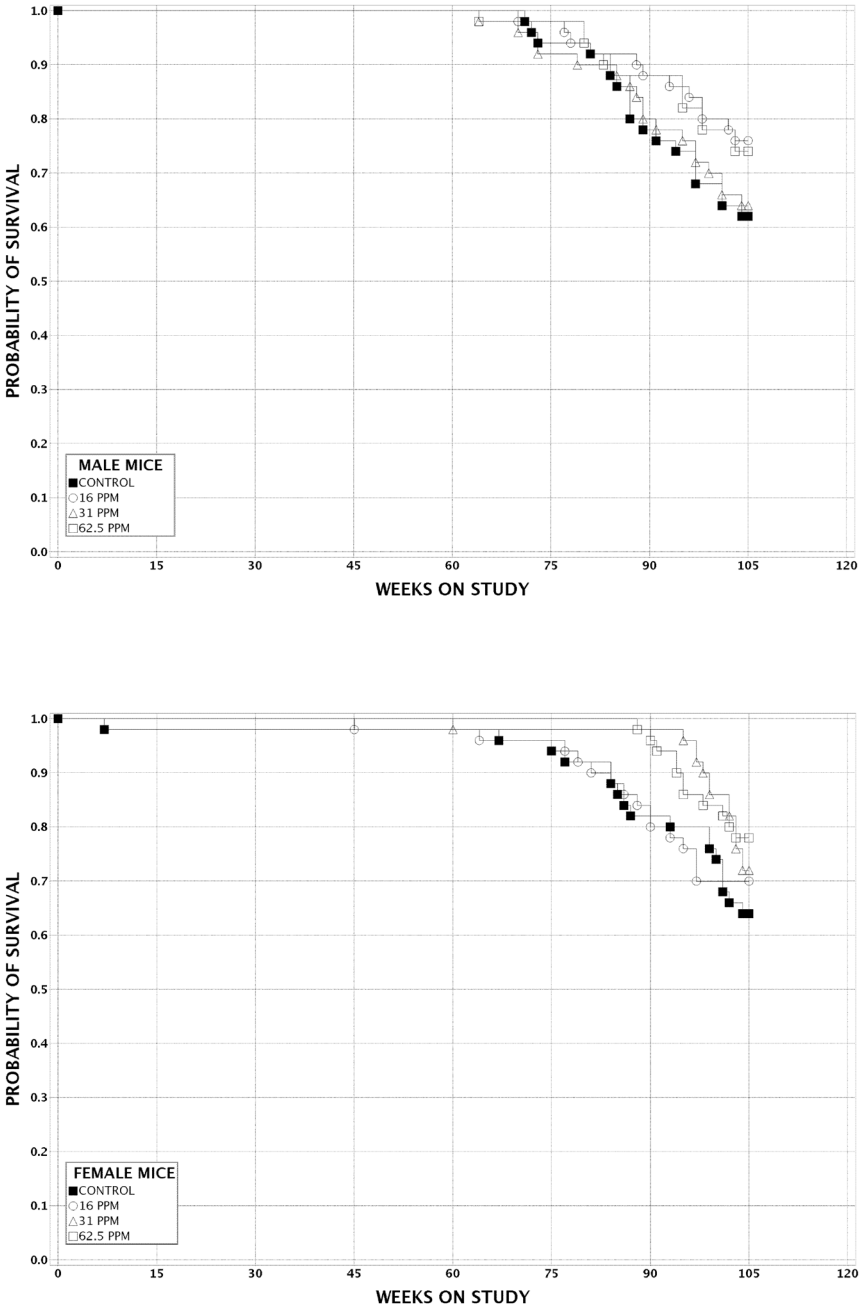


FIGURE 6
Kaplan-Meier Survival Curves for Mice Exposed to Diethylamine by Inhalation for 2 Years

Body Weights and Clinical Findings

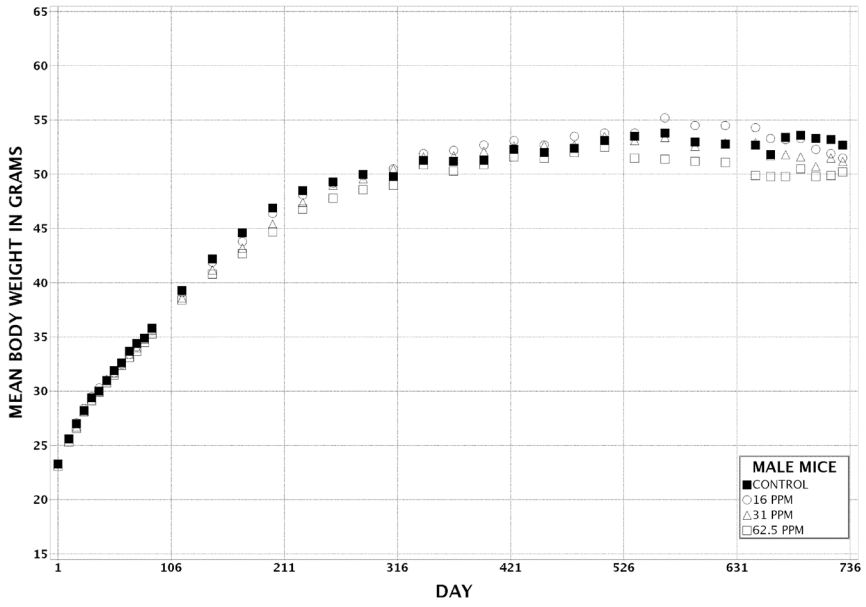
Mean body weights of males and females were similar to those of the chamber controls throughout the study (Tables 16 and 17 and Figure 7). Greater incidences of eye abnormality were observed in exposed groups of males compared to the chamber controls, and torso/ventral ulcer/abscess was observed in six 62.5 ppm males compared to none in the chamber controls.

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Diethylamine

Days on Study	Chamber Control		16 ppm			31 ppm			62.5 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.3	50	23.3	100	50	23.3	100	50	23.1	99	50
11	25.6	50	25.6	100	50	25.6	100	50	25.3	99	50
18	27.0	50	27.1	100	50	26.7	99	50	26.6	99	50
25	28.2	50	28.4	101	50	28.1	100	50	28.1	99	50
32	29.4	50	29.5	100	50	29.2	99	50	29.1	99	50
39	30.0	50	30.3	101	50	29.9	100	50	29.9	100	50
46	31.0	50	31.0	100	50	31.1	100	50	30.8	99	50
53	31.9	50	31.7	100	50	31.7	100	50	31.5	99	50
60	32.6	50	32.5	100	50	32.4	100	50	32.4	99	50
67	33.7	50	33.4	99	50	33.4	99	50	33.1	98	50
74	34.4	50	34.1	99	50	34.0	99	50	33.7	98	50
81	34.9	50	34.9	100	50	34.8	100	50	34.5	99	50
88	35.8	50	35.7	100	50	35.6	99	50	35.3	99	50
116	39.3	50	39.1	100	50	38.6	98	50	38.4	98	50
144	42.2	50	41.9	99	50	41.2	98	50	40.8	97	50
172	44.6	50	43.8	98	50	43.2	97	50	42.7	96	50
200	46.9	50	46.4	99	50	45.4	97	50	44.7	95	50
228	48.5	50	48.1	99	50	47.4	98	50	46.8	97	50
256	49.3	50	49.2	100	50	49.0	99	50	47.8	97	50
284	50.0	50	49.9	100	50	49.6	99	50	48.6	97	50
312	49.8	50	50.5	102	50	50.5	102	50	49.0	98	50
340	51.3	50	51.9	101	50	51.6	101	50	50.9	99	50
368	51.2	50	52.2	102	50	51.7	101	50	50.3	98	50
396	51.3	50	52.7	103	50	52.1	102	50	50.9	99	50
424	52.3	50	53.1	102	50	52.6	101	50	51.6	99	50
452	52.0	50	52.7	101	50	52.7	101	49	51.5	99	49
480	52.4	50	53.5	102	50	52.7	101	49	52.0	99	49
508	53.1	48	53.8	101	49	53.5	101	46	52.5	99	49
536	53.5	47	53.8	101	48	53.1	99	46	51.5	96	49
564	53.8	46	55.2	103	46	53.4	99	45	51.4	96	46
592	53.0	43	54.5	103	46	52.6	99	44	51.2	97	44
620	52.8	39	54.5	103	44	52.9	100	41	51.1	97	44
648	52.7	38	54.3	103	43	52.9	101	39	49.9	95	44
662	51.8	37	53.3	103	43	51.7	100	39	49.8	96	41
676	53.4	34	53.2	100	42	51.8	97	37	49.8	93	41
690	53.6	34	53.3	100	40	51.6	96	35	50.5	94	39
704	53.3	33	52.3	98	40	50.7	95	35	49.8	93	39
718	53.2	32	51.9	98	38	51.5	97	33	49.9	94	37
Mean for weeks											
1-13	30.6		30.6	100		30.4	100		30.3	99	
14-52	46.9		46.8	100		46.3	99		45.5	97	
53-103	52.7		53.4	101		52.3	99		50.9	97	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Diethylamine

Days on Study	Chamber Control		16 ppm			31 ppm			62.5 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.8	50	19.9	100	50	19.8	100	50	19.5	99	50
11	21.5	50	21.3	99	50	21.7	101	50	21.3	99	50
18	22.4	50	22.2	99	50	22.6	101	50	22.2	99	50
25	23.4	50	23.5	100	50	23.8	102	50	23.3	100	50
32	24.4	50	24.6	101	50	24.9	102	50	24.6	101	50
39	25.0	50	25.3	101	50	25.8	103	50	25.4	102	50
46	26.2	50	26.2	100	50	26.7	102	50	26.2	100	50
53	26.9	49	26.8	100	50	27.5	102	50	27.0	100	50
60	27.2	49	27.6	101	50	28.0	103	50	27.7	102	50
67	27.7	49	27.6	100	50	28.8	104	50	28.1	102	50
74	28.5	49	28.6	101	50	29.5	104	50	28.6	100	50
81	28.5	49	29.3	103	50	30.1	105	50	29.2	102	50
88	29.1	49	29.6	102	50	30.6	105	50	30.1	104	50
116	31.4	49	32.5	103	50	33.6	107	50	32.7	104	50
144	34.3	49	34.7	101	50	36.0	105	50	35.2	102	50
172	36.5	49	37.1	102	50	38.1	104	50	36.9	101	50
200	39.1	49	39.6	101	50	40.5	103	50	39.5	101	50
228	41.5	49	41.6	100	50	43.1	104	50	41.8	101	50
256	43.4	49	44.1	102	50	45.5	105	50	42.6	98	50
284	45.6	49	46.0	101	50	47.1	104	50	43.9	96	50
312	46.3	49	47.4	103	50	48.4	105	50	44.2	96	50
340	49.2	49	50.9	104	49	51.2	104	50	47.6	97	50
368	50.9	49	52.0	102	49	52.3	103	50	48.9	96	50
396	52.6	49	53.9	102	49	54.4	103	50	49.9	95	50
424	55.5	49	56.2	101	49	56.6	102	49	51.8	93	50
452	56.8	49	58.3	103	48	58.3	103	49	53.0	93	50
480	59.0	48	59.6	101	48	59.1	100	49	54.9	93	50
508	59.9	48	61.0	102	48	60.9	102	49	56.3	94	50
536	60.4	46	61.5	102	47	61.3	102	49	56.6	94	50
564	60.6	46	61.7	102	45	62.6	103	49	56.8	94	50
592	60.5	43	61.8	102	44	62.1	103	49	57.0	94	50
620	60.7	41	61.7	102	42	61.7	102	49	57.0	94	49
648	59.8	41	60.7	102	40	61.1	102	49	55.2	92	47
662	58.1	40	58.8	101	38	59.6	103	48	53.7	92	44
676	57.3	40	58.1	102	36	58.9	103	46	53.4	93	43
690	56.8	38	58.4	103	35	58.5	103	43	54.1	95	42
704	56.8	36	57.4	101	35	57.1	101	43	53.1	94	41
718	57.1	33	56.2	98	35	55.8	98	38	52.8	92	40
Mean for weeks											
1-13	25.4		25.6	101		26.1	103		25.6	101	
14-52	40.8		41.5	102		42.6	105		40.5	100	
53-103	57.7		58.6	102		58.8	102		54.0	94	



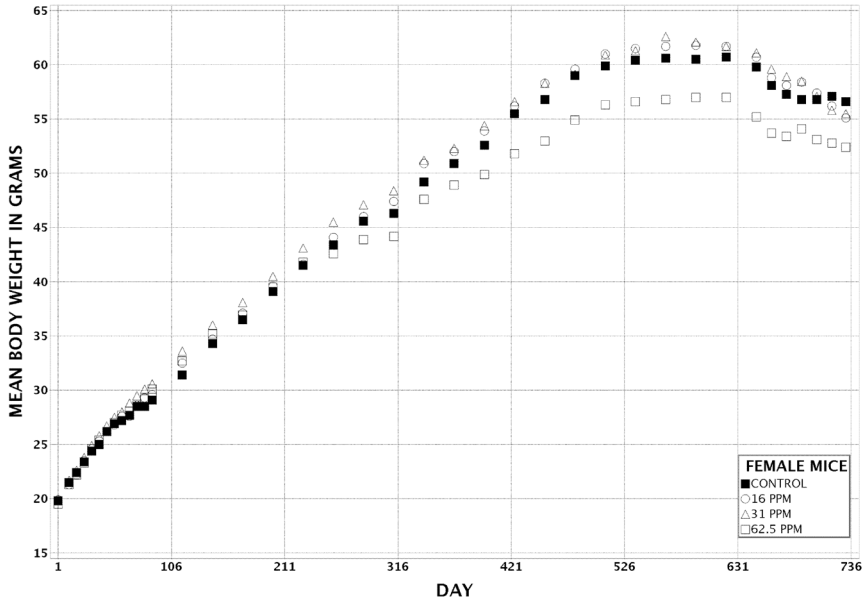


FIGURE 7
Growth Curves for Mice Exposed to Diethylamine by Inhalation for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the nose. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

Nose: A spectrum of nonneoplastic lesions occurred in the respiratory and olfactory epithelium. The incidences of suppurative inflammation were significantly increased in 62.5 ppm males and females (Tables 18, C3, and D3). The suppurative inflammation consisted primarily of aggregates of neutrophils in the lumen of the nasal cavity. In some cases a foreign body such as feed material and/or colonies of bacteria was present in the areas of suppuration, whereas in other animals the suppuration was associated with areas of necrosis and ulceration of the epithelium or turbinates. The incidence of necrosis of the respiratory epithelium was significantly increased in 62.5 ppm females; this lesion occurred in all groups of males and females except 16 ppm females. Necrosis varied from a few necrotic cells to full thickness detachment of necrotic epithelium from the basement membrane. Turbinate necrosis and respiratory epithelial ulceration were noted in a few 62.5 ppm males and females. Necrosis of turbinate bone usually occurred at the tips of the naso- and maxillo-turbinates, and in some cases the necrotic bone was separated from the remaining viable turbinate bone. A significantly increased incidence of chronic active inflammation of the respiratory epithelial glands occurred in 62.5 ppm females. Increased incidences of chronic active inflammation of respiratory epithelial glands also occurred in all exposed groups of males and 16 and 31 ppm females, but without statistical significance. This inflammation was usually minimal and characterized by a mixed, primarily mononuclear, infiltrate in the lamina propria around the glands and small numbers of neutrophils within the lumens of the glands. Significantly increased incidences of squamous metaplasia of the respiratory epithelium occurred in 31 and 62.5 ppm males and females. The squamous metaplastic epithelium consisted of two or more layers of keratinized or nonkeratinized flattened to polygonal cells and was often found overlying hyperostotic nasal turbinates or in areas of necrosis, ulceration, and suppuration in Level I. Incidences of hyperplasia of the respiratory epithelial glands were significantly increased in 62.5 ppm males and females, and severities generally increased with increasing exposure concentration. The hyperplastic glands were enlarged and often dilated and were most often found in the transition zone from respiratory epithelium to olfactory epithelium lining the nasal septum, along the medial aspect of the nasal turbinates in Level II, and in the dorsal meatus of Level I. Significantly increased incidences of hyaline droplet accumulation in the cytoplasm of the respiratory epithelial glandular cells occurred in all exposed groups of females and in 31 and 62.5 ppm males. Significantly increased incidences of hyaline droplet accumulation in the respiratory surface epithelium also occurred in 62.5 ppm males and in 16 and 31 ppm females compared to the chamber controls. Cytoplasmic vacuolization in focal areas of the respiratory epithelium was noted in a few 16 and 31 ppm males and females and in one 62.5 ppm female. The vacuoles were single to multiple, varied in size from approximately 2 to 15 microns in diameter, and tended to be most numerous in the apical portion of the cytoplasm.

The incidences of atrophy of the olfactory epithelium were significantly increased in all exposed groups of males and females, and atrophy was present in almost all 31 and 62.5 ppm mice (Tables 18, C3, and D3). Atrophy was most pronounced in the dorsal meatus of Level II, and was characterized by loss of neuronal cells with thinning of the epithelium, and loss of olfactory nerve bundles and Bowman's glands and ducts in the underlying lamina propria. Respiratory metaplasia of the olfactory epithelium was significantly increased in 31 and 62.5 ppm males and in all exposed groups of females. This metaplasia was manifested by a transformation of the normal multicell, layered, olfactory epithelium to a ciliated, pseudostratified epithelium similar to the respiratory epithelium of the anterior nasal cavity (Plates 5 and 6). Incidences of cytoplasmic vacuolization of focal areas in the olfactory epithelium were significantly increased in 16 ppm males and females. Focal necrosis of olfactory epithelium was noted in two 16 ppm males and minimal focal necrosis was seen in two 31 ppm and one 62.5 ppm females.

Incidences of hyperostosis of the naso- and maxillo-turbinates were significantly increased in all exposed groups of males and females, and hypertostosis was present in nearly all 31 and 62.5 ppm mice (Tables 18, C3, and D3). The severity of this lesion increased with increasing exposure concentration and was characterized by an increased amount of bone matrix, which resulted in thickened bone with a markedly increased concentration of cement lines (Plates 7 and 8).

Other Findings: The incidence of mild cardiomyopathy in the heart was significantly increased in 31 ppm males compared to that of the chamber controls; however, neither the incidences nor the severities increased with increasing exposure concentration [chamber control, 0/50; 16 ppm, 1/50 (2.0); 31 ppm, 8/50 (2.0); 62.5 ppm, 1/50 (2.0); Table C3]. No

TABLE 18
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control		16 ppm		31 ppm		62.5 ppm	
Male								
Number Examined Microscopically	50		50		50		50	
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet[a]	5	(1.0)[b]	5	(1.0)	16**	(1.3)	33**	(1.5)
Glands, Respiratory Epithelium, Inflammation, Chronic Active	6	(1.0)	9	(1.1)	8	(1.3)	11	(1.1)
Glands, Respiratory Epithelium, Hyperplasia	42	(1.1)	41	(1.2)	44	(1.1)	50**	(1.6)
Inflammation, Suppurative	6	(1.7)	5	(1.6)	6	(1.7)	14*	(1.1)
Olfactory Epithelium, Atrophy	9	(1.0)	19*	(1.3)	50**	(2.0)	50**	(2.5)
Olfactory Epithelium, Respiratory Metaplasia	14	(1.0)	15	(1.7)	44**	(2.3)	50**	(3.0)
Olfactory Epithelium, Necrosis	0		2	(1.5)	0		0	
Olfactory Epithelium, Vacuolization Cytoplasmic	0		5*	(1.0)	3	(2.0)	0	
Respiratory Epithelium, Accumulation, Hyaline Droplet	11	(1.0)	6	(1.3)	19	(1.5)	30**	(1.1)
Respiratory Epithelium, Metaplasia, Squamous	4	(1.0)	7	(1.0)	16**	(1.0)	34**	(1.4)
Respiratory Epithelium, Necrosis	2	(1.5)	3	(1.3)	3	(1.3)	8	(1.4)
Respiratory Epithelium, Ulcer	1	(1.0)	1	(2.0)	2	(1.0)	4	(1.3)
Respiratory Epithelium, Vacuolization Cytoplasmic	0		1	(1.0)	3	(2.0)	0	
Turbinate, Hyperostosis	5	(1.2)	23**	(1.1)	50**	(2.0)	50**	(3.5)
Turbinate, Necrosis	1	(1.0)	0		0		3	(1.0)
Female								
Number Examined Microscopically	50		49		50		50	
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet	16	(1.3)	28**	(1.4)	45**	(1.9)	42**	(1.8)
Glands, Respiratory Epithelium, Inflammation, Chronic Active	8	(1.0)	11	(1.0)	16	(1.1)	22**	(1.1)
Glands, Respiratory Epithelium, Hyperplasia	43	(1.2)	45	(1.2)	47	(1.4)	50*	(2.0)
Inflammation, Suppurative	2	(1.0)	1	(1.0)	3	(1.0)	9*	(1.1)
Olfactory Epithelium, Atrophy	8	(1.0)	29**	(1.4)	49**	(2.1)	50**	(2.6)
Olfactory Epithelium, Respiratory Metaplasia	4	(1.0)	15**	(1.6)	48**	(2.8)	50**	(3.0)
Olfactory Epithelium, Necrosis	0		0		2	(1.0)	1	(1.0)
Olfactory Epithelium, Vacuolization Cytoplasmic	0		5*	(1.6)	1	(2.0)	1	(2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	20	(1.7)	33**	(1.3)	47**	(2.2)	29	(1.1)
Respiratory Epithelium, Metaplasia, Squamous	0		0		13**	(1.1)	35**	(1.3)
Respiratory Epithelium, Necrosis	1	(1.0)	0		6	(1.5)	16**	(1.6)
Respiratory Epithelium, Ulcer	0		0		0		2	(1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0		2	(2.0)	2	(2.5)	1	(3.0)
Turbinate, Hyperostosis	4	(1.0)	23**	(1.1)	49**	(1.8)	50**	(2.9)
Turbinate, Necrosis	0		0		0		1	(1.0)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

increases in the incidences (chamber control, 5/50; 16 ppm, 2/50; 31 ppm, 3/50; 62.5 ppm, 1/50) or severities (1.8, 2.0, 2.0, 2.0) of cardiomyopathy were observed in exposed females (Table D3). A subsequent review of the slides by two pathologists with experience in cardiac pathology supported the initial findings. The data suggested that diethylamine may have led to small increases in the incidences of minimal cardiomyopathy in male and female mice. However, the incidence of the lesion in 31 ppm males (16%) is within the variability expected for mice (range 0%-24%, unpublished data), and is typical of a common background lesion and, therefore, unlikely to be treatment-related.

In the pituitary gland, incidences of hyperplasia of the pars distalis were significantly increased in 16 and 62.5 ppm males (0/50, 7/50, 2/49, 6/50; Table C3).

A significantly increased incidence of basophilic focus in the liver occurred in 16 ppm males (5/50, 13/50, 10/50, 8/50), and a significantly increased incidence of mixed cell focus occurred in 62.5 ppm males (0/50, 4/50, 1/50, 5/50; Table C3). However, there were no exposure concentration-related increases in the incidences of either lesion.

The incidence of hepatocellular adenoma or carcinoma (combined) was decreased in 62.5 ppm females (16/50, 23/50, 22/50, 11/50; Tables D1 and D2).

Decreased incidences of mammary gland carcinoma occurred in exposed groups of females (4/50, 1/50, 2/50, 0/50; Tables D1 and D2); the incidence in the concurrent chamber control group was at the upper end of the historical control ranges for inhalation studies and all routes combined [inhalation studies: 10/350 (2.9% ± 2.5%), range 0%-8%; all routes: 26/1,498 (1.7% ± 1.9%), range 0%-8%]. The incidence of fibroadenoma or carcinoma (combined) in the mammary gland was significantly decreased in 62.5 ppm females (5/50, 1/50, 2/50, 0/50); the incidence in the concurrent chamber control group was at the upper end of the historical control ranges for inhalation studies and all routes combined [inhalation studies: 11/350 (3.1% ± 3.2%), range 0%-10%; all routes: 27/1,498 (1.8% ± 2.1%), range 0%-10%]. The biological significance of these decreases is uncertain since the concurrent control incidences were at the upper end of the historical chamber control ranges for both inhalation studies and all study routes.

GENETIC TOXICOLOGY

Diethylamine (doses up to 10,000 µg/plate in the first study and 4,000 µg/plate in the second study) was not mutagenic in either of two independent bacterial mutagenicity assays, with or without exogenous metabolic activation (Table E1; Zeiger *et al.*, 1987). Bacterial strains tested in the first study included *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, with and without 10% or 30% induced rat or hamster liver S9 activation enzymes; in the second study, *S. typhimurium* strains TA98 and TA100 were employed, as well as *Escherichia coli* strain WP2 *uvrA*/pKM101, with and without 10% induced rat liver S9. In addition to the negative results in the two bacterial assays, no significant increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood of male or female mice from the 3-month study (Table E2). The percentage of reticulocytes (polychromatic erythrocytes) in the peripheral blood of male and female mice was unaltered by diethylamine exposure, suggesting a lack of chemical-associated bone marrow toxicity.

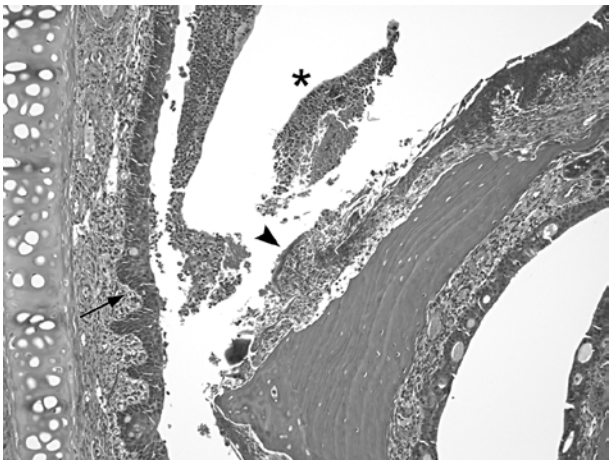


Plate 1
Suppurative exudates in the nasal cavity (asterisk), focal erosion of respiratory epithelium of the lateral wall (arrowhead), and squamous metaplasia of the respiratory epithelium of the nasal septum (arrow), Level I, in a male F344/N rat exposed to 125 ppm diethylamine by inhalation for 2 years. H&E

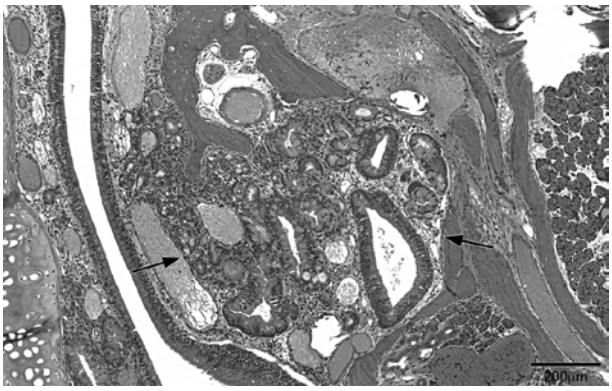


Plate 2
Respiratory glandular hyperplasia with hyaline droplet accumulation (between arrows) in the lateral wall (Level II) of a male F344/N rat exposed to 125 ppm diethylamine by inhalation for 2 years. H&E

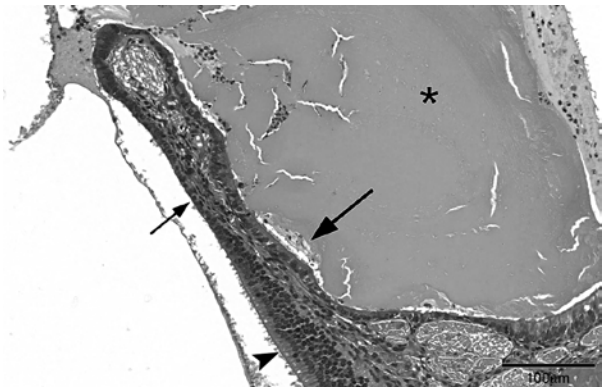


Plate 3
Ethmoid turbinate (Level III), with transition from normal olfactory epithelium (arrowhead) to olfactory atrophy, characterized by thinning of the epithelium (small arrow), to complete replacement of the olfactory epithelium by respiratory metaplasia on the opposite side of the turbinate (large arrow) in a male F344/N rat exposed to 125 ppm diethylamine by inhalation for 2 years. Note the proteinaceous exudates in the nasal cavity (asterisk). H&E

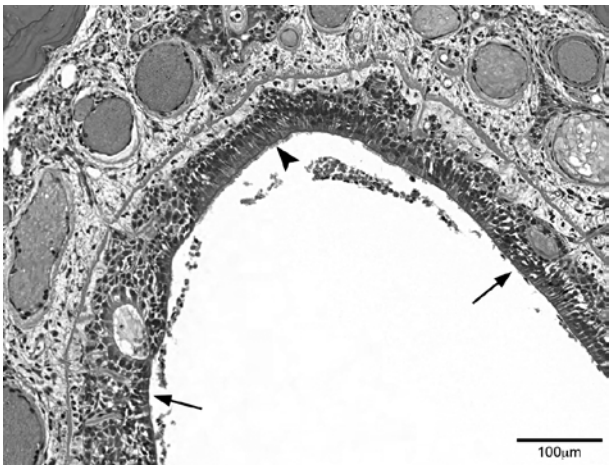


Plate 4
Replacement of olfactory epithelium of the dorsal meatus (Level II) by respiratory metaplastic epithelium (arrowhead), with moderate to marked basal cell hyperplasia on either side (arrows) in a male F344/N rat exposed to 125 ppm diethylamine by inhalation for 2 years. H&E

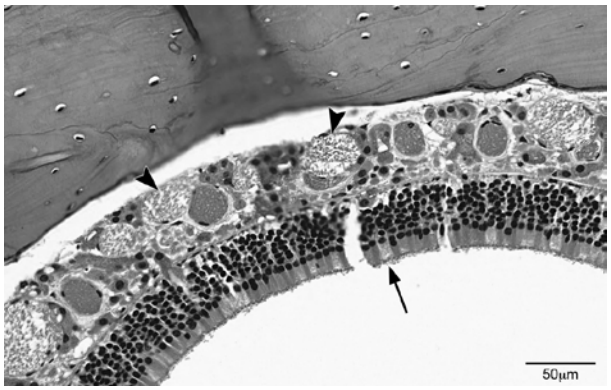


Plate 5
Normal olfactory epithelium (arrow) with underlying nerve bundles (arrowheads) and Bowman's glands in the dorsal meatus (Level II) of a chamber control male B6C3F1 mouse at 2 years in the inhalation study of diethylamine. H&E

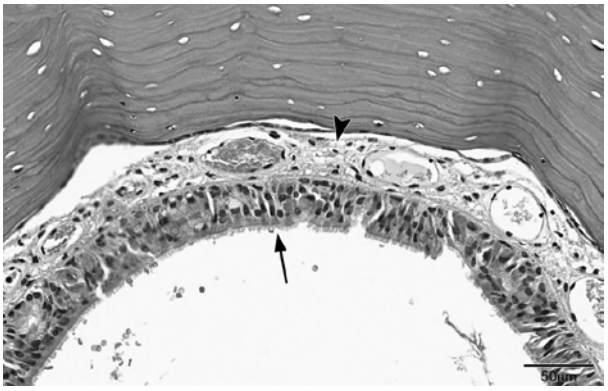


Plate 6
Replacement of olfactory epithelium by respiratory metaplastic epithelium with hyaline droplet accumulation (arrow) in the dorsal meatus (Level II) of a male B6C3F1 mouse exposed to 62.5 ppm diethylamine by inhalation for 2 years. Note the absence of nerve bundles and Bowman's glands (compare to Plate 5) in the underlying lamina propria (arrowhead). H&E

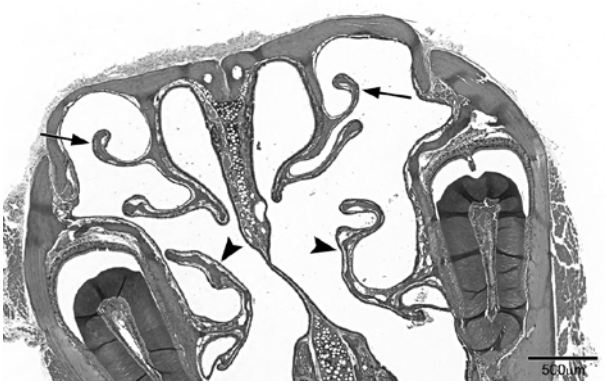


Plate 7
Normal, slender nasoturbinates with lateral hooks (arrows), and maxilloturbinates (arrowheads) at Level I in a chamber control male B6C3F1 mouse at 2 years in the inhalation study of diethylamine. H&E

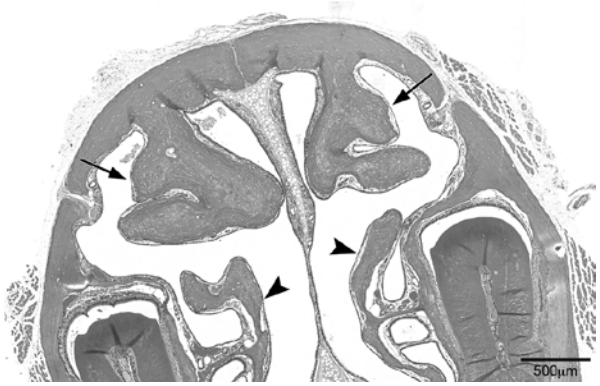


Plate 8
Marked hyperostosis (thickening) of nasoturbinates (arrows) and mild hyperostosis of maxilloturbinates (arrowheads) at Level I in a male B6C3F1 mouse exposed to 62.5 ppm diethylamine by inhalation for 2 years. Compare to Plate 7. H&E

DISCUSSION AND CONCLUSIONS

Diethylamine is a potent contact irritant and the effects of acute exposure to diethylamine vapors in the workplace are well documented (Beard and Noe, 1981; OSHA, 1981). Acute occupational exposures are generally self-limiting because the severe irritation to the eyes, nose, and throat results in worker removal from exposure; however, prolonged exposure of workers may occur at concentrations that do not cause severe irritation. The toxicity and carcinogenicity resulting from chronic inhalation exposure to diethylamine has not been previously investigated. Chronic exposure to diethylamine vapor is a concern because of the repeated injury to the upper respiratory tract, and because under the right conditions diethylamine can be nitrosated to form nitroso-diethylamine, a potent carcinogen (Druckrey *et al.*, 1963).

The toxicity of the aliphatic amines is directly related to their alkalinity, and the most common action is the strong local irritation of the skin, eyes, and mucous membranes. Symptoms in exposed workers include coughing, dyspnea, upper airway obstruction, bronchitis, pneumonitis, and pulmonary edema (Beard and Noe, 1981). In the current studies, rats and mice exposed to diethylamine for 2 weeks exhibited nasal and eye discharge and abnormal breathing, suggesting that the target sites for diethylamine are similar in rodents and humans. Clinical signs indicative of eye irritation were noted in rats and mice exposed to 250 or 500 ppm. Mice were more sensitive than rats to the lethal effects of diethylamine in the 2-week studies. About half of the male and female mice died during the first week of exposure to 500 ppm diethylamine, whereas there was no mortality in rats exposed to diethylamine at concentrations up to 500 ppm. However, body weight gain was significantly decreased in both species exposed to 250 or 500 ppm. Female rats and mice exposed to 500 ppm for 2 weeks weighed about 30% less than the chamber controls, and males of both species weighed about 40% less than the chamber controls. The decreased body weights may have been related to reduced feed intake associated with diethylamine effects on olfaction.

The nasal cavity was a major site of injury in rats and mice exposed to diethylamine vapors. Because diethylamine is highly soluble in water it can be readily absorbed by the mucous lining of the nasal cavity and upper airways. The nasal lesions in rats and mice were similar and included suppurative nasal inflammation, squamous metaplasia of the respiratory epithelium, and atrophy of the olfactory epithelium. Earlier studies on diethyl-amine reported similar nasal lesions in rats (NIOSH, 1984; Lynch *et al.*, 1986) and mice (NIOSH, 1987).

In the 2-week studies, suppurative inflammation was observed at Levels I and II in the nasal cavity of nearly all rats and mice exposed to diethylamine. Inflammation was most prominent at Level I of the nasal cavity and increased in severity with increasing exposure concentration. Squamous metaplasia of the respiratory epithelium was observed in Level I of the nasal cavity of rats at all diethylamine concentrations and in mice exposed to 125 ppm or greater. Squamous metaplasia is a common adaptive response to repeated injury to respiratory epithelium in the nose and results in the replacement of injured respiratory epithelium with the more resistant squamous epithelium. Atrophy of the olfactory epithelium at Level II was observed in all rats exposed to 250 or 500 ppm diethylamine in the 2-week studies, and in most mice exposed to 125 ppm or greater. At level III the epithelium lining the naso-pharyngeal duct was ulcerated in rats of both sexes exposed to 500 ppm. The non-specific distribution of these lesions in nasal respiratory and olfactory epithelium with an anterior to posterior gradient in severity of damage is typical of direct acting irritants (Gaskell, 1990).

In the 2-week studies, turbinate necrosis occurred in almost all exposed mice and almost all rats exposed to 125 ppm or greater. The necrosis was characterized by partial to complete loss of maxillo- and/or nasoturbinates in Level I, with necrosis of respiratory epithelium and underlying bone. The nasal septum was also necrotic in some mice. In the lung, minimal chronic active inflammation of mainstem bronchi at their bifurcation was noted in four male and two female mice in the 500 ppm groups. Respiratory tract lesions in rats in the 2-week study were confined to the nasal cavity.

Because of deaths of mice exposed to 500 ppm and excessive reductions in body weight gain in rats and

mice exposed to 250 or 500 ppm in the 2-week studies, diethylamine concentrations of 0, 8, 16, 32, 62, or 125 ppm were selected for the 3-month studies in rats and mice. The exposure of rats to these diethylamine concentrations for 3 months did not result in mortality or significant changes in body weight gain. As observed in the 2-week studies, mice in the 3-month study were more susceptible than rats to diethylamine exposure, and although there were no deaths, body weight gain was significantly reduced in male (–22%) and female (–16%) mice exposed to 125 ppm compared to the chamber controls. There was no evidence of systemic toxicity associated with diethylamine exposure for 3 months. There were no exposure-related changes in hematology, serum chemistry indices, or organ weights of exposed rats or mice. However, in the 3-month studies, exposure to 32 to 125 ppm diethylamine significantly reduced the sperm motility of both rats and mice, indicating that diethylamine could produce adverse effects in a study of fertility and reproductive performance involving these species and exposure levels.

The nose was a primary site of injury for rats and mice in the 3-month studies. Nasal lesions were generally minimal to mild in severity and consisted of suppurative inflammation and squamous metaplasia of the respiratory epithelium in most rats and mice exposed to 125 ppm. Mild to moderate olfactory epithelial atrophy was present in all 125 ppm rats and mice, in most rats and mice exposed to 62 ppm, and in a few animals exposed to 32 ppm diethylamine. In both species, atrophy of the olfactory epithelium was noted primarily in the dorsal meatus of Level II and sometimes in Level III. The severity of this lesion increased with increasing exposure concentration. Respiratory epithelial hyperplasia was present in most rats in the 62.5 and 125 ppm groups, in a few 16 and 32 ppm male rats, and in one 16 ppm female rat. Respiratory epithelial hyperplasia was not observed in mice exposed to diethylamine.

In the 2-year study, rats were exposed to 0, 31, 62.5, or 125 ppm and mice were exposed to 0, 16, 31, or 62.5 ppm diethylamine. There was no evidence of carcinogenicity in exposed rats or mice.

Although the respiratory tract was a major target site for inhaled diethylamine, no treatment-related neoplasms were observed in the nose, larynx, or lung of rats or mice exposed for 2 years. The spectrum of nasal lesions caused by diethylamine in the 2-week, 3-month, and 2-year studies was typical of those caused by other inhaled irritants. Certain lesions, such as suppurative inflammation, squamous metaplasia of the respiratory epithelium, and olfactory epithelial atrophy, were present in many of the high dose male and female rats in each of the 2-week, 3-month, and 2-year studies. The same was true for the mice, except that suppurative inflammation was much less common in high dose mice (62.5 ppm) after 2 years, probably because the high dose in mice was half that of the 2-year study high dose in rats (125 ppm). Respiratory epithelial hyperplasia was first noted in the 3-month rat study and was common in all exposed groups in the 2-year rat study, but was not seen in mice at either 3 months or 2 years. Respiratory metaplasia of olfactory epithelium was only recorded in the 2-year rat and mouse studies; basal cell hyperplasia of olfactory epithelium was only seen in the 2-year rat study.

Exposure to diethylamine for 2 years also caused hyperostosis (osteopetrosis) of the nasal turbinates, an unusual nonneoplastic thickening of the naso- and maxillo-turbinate bones. Hyperostosis of the nasal turbinates occurred in all mice exposed to 62.5 ppm and in all but one mouse exposed to 31 ppm. In contrast, this lesion was present in only five rats (three males, two females) exposed to 125 ppm. In NTP studies, treatment-related hyperostosis of the nasal turbinates has been previously reported only twice, once in a 2-year inhalation study of rats exposed to 1,2-epoxybutane (NTP, 1988) and once in a 2-year feed study of rats exposed to C.I. Pigment Red 3 (NTP, 1992); however, only small numbers of animals were affected in each study.

Mechanisms of hyperostosis may be divided into proliferative and nonproliferative categories, based upon evidence of either increased bone cell proliferation or decreased bone resorption, respectively (Long *et al.*, 1993). Since the nasal turbinate bone of diethylamine exposed mice in the 2-year study was histologically quiescent (without osteoblastic or osteoclastic activity), the pathogenesis of the bone thickening was uncertain. However, both proliferative and nonproliferative mechanisms could be involved. In the 3-month mouse study, slight thickening of nasal turbinates was noted and this was accompanied by activation of mesenchymal osteoprogenitor cells, indicative of a proliferative response (Rosenberg, 2009). In addition, an imbalance of normal bone remodeling activity associated with decreased bone resorption could have resulted from neutralization of the normally acidic osteoclast resorption pit by the marked alkalinity of diethylamine.

Evidence of eye irritation was observed in some rats exposed to 125 ppm diethylamine for 2 years. These lesions consisted of mild to moderate suppurative inflammation and chronic inflammation of the cornea in some exposed male and female rats, but not in chamber

controls. Cataracts and retinal atrophy were observed in exposed and chamber control animals and were not considered treatment related. The incidences of retinal atrophy and cataracts in historical control rats from NTP studies (unpublished data) are higher in inhalation studies than noninhalation studies, although the reason is unclear.

In an early study (Brieger and Hodes, 1951), diethylamine inhalation was reported to cause a slight, questionable increase in cardiac degeneration in rabbits. In the same study, the structurally related triethylamine caused significant cardiac muscle degeneration in exposed rabbits. The only other aliphatic amines known to cause cardiotoxicity are the unsaturated allylamines (Boor, 1983). Studies were conducted by Lynch *et al.* (1986; 1990) to more fully investigate the potential cardiac toxicity of these amines. There was no electrocardiography or histopathologic evidence of cardiomyopathy after exposure of Fischer 344 rats to 25 or 250 ppm diethylamine for up to 6 months (Lynch *et al.*, 1986) or to 25 or 250 ppm of triethylamine for 4 months (Lynch *et al.*, 1990).

In the current studies, exposure of rats and mice to diethylamine for 2 years did not result in significant histopathologic evidence of cardiomyopathy.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of diethylamine in male or female F344/N rats exposed to 31, 62.5, or 125 ppm. There was *no evidence of carcinogenic activity* of diethylamine in male or female B6C3F1 mice exposed to 16, 31, or 62.5 ppm.

Exposure to diethylamine resulted in increased incidences of nonneoplastic lesions of the nose in male and female rats and mice, of the cornea in male rats, and of the pleura and lung in female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF DIETHYLAMINE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	24	20	13
Natural deaths	3	5	5	1
Survivors				
Terminal sacrifice	28	21	25	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(47)	(49)	(50)
Intestine small, duodenum	(49)	(49)	(49)	(50)
Adenoma		1 (2%)		
Leiomyoma	1 (2%)			
Intestine small, ileum	(48)	(47)	(46)	(49)
Adenoma		1 (2%)		
Intestine small, jejunum	(48)	(47)	(48)	(50)
Liver	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery			1 (2%)	
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hepatocellular adenoma			1 (2%)	
Hepatocellular adenoma, multiple			1 (2%)	
Mesentery	(10)	(13)	(16)	(10)
Fibrosarcoma			1 (6%)	
Pancreas	(49)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(0)	(0)	(1)	(1)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	6 (12%)	3 (6%)	3 (6%)
Adrenal medulla	(50)	(50)	(50)	(49)
Ganglioneuroma				1 (2%)
Pheochromocytoma benign	7 (14%)	3 (6%)	7 (14%)	8 (16%)
Pheochromocytoma benign, multiple				1 (2%)
Pheochromocytoma complex	2 (4%)			
Pheochromocytoma malignant	1 (2%)		2 (4%)	1 (2%)
Bilateral, pheochromocytoma benign		3 (6%)	2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	8 (16%)	7 (14%)	2 (4%)
Carcinoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Pituitary gland	(50)	(48)	(50)	(50)
Ganglioneuroma	1 (2%)			
Pars distalis, adenoma	31 (62%)	32 (67%)	31 (62%)	26 (52%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	7 (14%)	5 (10%)	3 (6%)	9 (18%)
C-cell, carcinoma	1 (2%)	3 (6%)	1 (2%)	
Follicular cell, adenoma		1 (2%)	1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
General Body System				
Peritoneum	(0)	(1)	(1)	(1)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	
Carcinoma	1 (2%)	1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	23 (46%)	23 (46%)	33 (66%)	25 (50%)
Interstitial cell, adenoma	13 (26%)	14 (28%)	6 (12%)	18 (36%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(10)	(5)	(10)	(10)
Lymph node, bronchial	(4)	(4)	(3)	(5)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (25%)			
Basal cell carcinoma, metastatic, skin	1 (25%)			
Lymph node, mandibular	(0)	(0)	(1)	(1)
Lymph node, mediastinal	(30)	(24)	(35)	(29)
Basal cell carcinoma, metastatic, skin	1 (3%)			
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Spleen	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery			1 (2%)	
Thymus	(44)	(43)	(47)	(45)
Thymoma malignant				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		
Fibroadenoma	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Fibroadenoma, multiple		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	1 (2%)		
Keratoacanthoma		2 (4%)	2 (4%)	1 (2%)
Squamous cell carcinoma			1 (2%)	
Sebaceous gland, adenoma	1 (2%)	1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)	4 (8%)	2 (4%)	
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)		
Subcutaneous tissue, lipoma		1 (2%)		1 (2%)
Subcutaneous tissue, osteosarcoma		1 (2%)		
Subcutaneous tissue, sarcoma		2 (4%)		
Subcutaneous tissue, schwannoma benign				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma				1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Cranium, squamous cell carcinoma, metastatic, skin			1 (2%)	
Tibia, osteosarcoma				1 (2%)
Skeletal muscle	(0)	(1)	(1)	(1)
Fibrous histiocytoma, metastatic, skin		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		
Alveolar/bronchiolar carcinoma	1 (2%)		1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Basal cell carcinoma, metastatic, skin	1 (2%)			
Carcinoma, metastatic, mammary gland		1 (2%)		
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Chordoma, metastatic, bone				1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla				1 (2%)
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Nose	(49)	(50)	(50)	(50)
Pleura	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(43)	(39)	(41)	(40)
Adenoma	2 (5%)	1 (3%)		
Carcinoma		3 (8%)		2 (5%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma		1 (2%)		
Urinary bladder	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Transitional epithelium, papilloma	1 (2%)	1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				2 (4%)
Leukemia mononuclear	25 (50%)	18 (36%)	33 (66%)	24 (48%)
Mesothelioma malignant		3 (6%)	1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	50	49
Total primary neoplasms	132	152	146	132
Total animals with benign neoplasms	44	48	49	48
Total benign neoplasms	97	113	104	97
Total animals with malignant neoplasms	31	31	37	32
Total malignant neoplasms	35	39	42	35
Total animals with metastatic neoplasms	2	2	2	3
Total metastatic neoplasms	4	5	4	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	6/50 (12%)	3/50 (6%)	3/50 (6%)
Adjusted rate[b]	2.3%	14.2%	6.8%	6.6%
Terminal rate[c]	1/28 (4%)	3/21 (14%)	2/25 (8%)	3/36 (8%)
First incidence (days)	729 (T)	600	687	729 (T)
Poly-3 test[d]	P=0.525	P=0.053	P=0.317	P=0.326
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	7/50 (14%)	6/50 (12%)	9/50 (18%)	9/49 (18%)
Adjusted rate	16.3%	14.3%	20.3%	19.9%
Terminal rate	6/28 (21%)	3/21 (14%)	7/25 (28%)	7/36 (19%)
First incidence (days)	651	674	656	703
Poly-3 test	P=0.316	P=0.517N	P=0.418	P=0.436
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	10/50 (20%)	6/50 (12%)	11/50 (22%)	10/49 (20%)
Adjusted rate	23.0%	14.3%	24.8%	22.1%
Terminal rate	8/28 (29%)	3/21 (14%)	9/25 (36%)	8/36 (22%)
First incidence (days)	557	674	656	703
Poly-3 test	P=0.447	P=0.223N	P=0.519	P=0.561N
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.7%	4.8%	6.8%	2.2%
Terminal rate	2/28 (7%)	1/21 (5%)	3/25 (12%)	1/36 (3%)
First incidence (days)	729 (T)	710	729 (T)	729 (T)
Poly-3 test	P=0.372N	P=0.686	P=0.513	P=0.480N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.7%	7.2%	6.8%	2.2%
Terminal rate	2/28 (7%)	1/21 (5%)	3/25 (12%)	1/36 (3%)
First incidence (days)	729 (T)	705	729 (T)	729 (T)
Poly-3 test	P=0.312N	P=0.490	P=0.513	P=0.480N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.7%	9.6%	6.8%	2.2%
Terminal rate	2/28 (7%)	2/21 (10%)	3/25 (12%)	1/36 (3%)
First incidence (days)	729 (T)	705	729 (T)	729 (T)
Poly-3 test	P=0.261N	P=0.326	P=0.513	P=0.480N
Pancreatic Islets: Adenoma				
Overall rate	6/50 (12%)	8/50 (16%)	7/50 (14%)	2/50 (4%)
Adjusted rate	14.1%	18.7%	15.9%	4.4%
Terminal rate	6/28 (21%)	2/21 (10%)	5/25 (20%)	2/36 (6%)
First incidence (days)	729 (T)	646	714	729 (T)
Poly-3 test	P=0.061N	P=0.388	P=0.524	P=0.114N
Pancreatic Islets: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.3%	7.2%	4.6%	2.2%
Terminal rate	1/28 (4%)	1/21 (5%)	1/25 (4%)	1/36 (3%)
First incidence (days)	729 (T)	677	724	729 (T)
Poly-3 test	P=0.445N	P=0.298	P=0.510	P=0.748N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	11/50 (22%)	9/50 (18%)	3/50 (6%)
Adjusted rate	16.4%	25.5%	20.4%	6.6%
Terminal rate	7/28 (25%)	3/21 (14%)	6/25 (24%)	3/36 (8%)
First incidence (days)	729 (T)	646	714	729 (T)
Poly-3 test	P=0.055N	P=0.219	P=0.418	P=0.134N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	31/50 (62%)	32/48 (67%)	31/50 (62%)	26/50 (52%)
Adjusted rate	67.2%	71.7%	66.6%	55.6%
Terminal rate	17/28 (61%)	14/20 (70%)	17/25 (68%)	20/36 (56%)
First incidence (days)	557	455	453	562
Poly-3 test	P=0.083N	P=0.404	P=0.562N	P=0.168N
Skin: Keratoacanthoma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	4.8%	6.8%	2.2%
Terminal rate	0/28 (0%)	2/21 (10%)	2/25 (8%)	1/36 (3%)
First incidence (days)	—[e]	729 (T)	569	729 (T)
Poly-3 test	P=0.484	P=0.231	P=0.126	P=0.511
Skin: Keratoacanthoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.3%	7.1%	6.8%	2.2%
Terminal rate	0/28 (0%)	2/21 (10%)	2/25 (8%)	1/36 (3%)
First incidence (days)	660	600	569	729 (T)
Poly-3 test	P=0.469N	P=0.297	P=0.317	P=0.749N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	2.3%	9.5%	4.6%	0.0%
Terminal rate	1/28 (4%)	2/21 (10%)	1/25 (4%)	0/36 (0%)
First incidence (days)	729 (T)	600	712	—
Poly-3 test	P=0.176N	P=0.175	P=0.510	P=0.489N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.3%	9.5%	0.0%	0.0%
Terminal rate	1/28 (4%)	1/21 (5%)	0/25 (0%)	0/36 (0%)
First incidence (days)	729 (T)	635	—	—
Poly-3 test	P=0.112N	P=0.173	P=0.494N	P=0.489N
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	8/50 (16%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.7%	18.7%	4.6%	0.0%
Terminal rate	2/28 (7%)	3/21 (14%)	1/25 (4%)	0/36 (0%)
First incidence (days)	729 (T)	600	712	—
Poly-3 test	P=0.039N	P=0.044	P=0.683N	P=0.225N
Testes: Adenoma				
Overall rate	36/50 (72%)	37/50 (74%)	39/50 (78%)	43/50 (86%)
Adjusted rate	78.6%	80.6%	81.7%	90.7%
Terminal rate	27/28 (96%)	17/21 (81%)	21/25 (84%)	34/36 (94%)
First incidence (days)	549	478	569	611
Poly-3 test	P=0.051	P=0.510	P=0.447	P=0.067

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rate	7/50 (14%)	5/50 (10%)	3/50 (6%)	9/50 (18%)
Adjusted rate	16.0%	11.8%	6.8%	19.7%
Terminal rate	3/28 (11%)	2/21 (10%)	1/25 (4%)	6/36 (17%)
First incidence (days)	621	512	596	634
Poly-3 test	P=0.309	P=0.398N	P=0.149N	P=0.432
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.3%	7.2%	2.3%	0.0%
Terminal rate	0/28 (0%)	2/21 (10%)	1/25 (4%)	0/36 (0%)
First incidence (days)	675	705	729 (T)	—
Poly-3 test	P=0.185N	P=0.294	P=0.756N	P=0.490N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	8/50 (16%)	4/50 (8%)	9/50 (18%)
Adjusted rate	18.2%	18.8%	9.0%	19.7%
Terminal rate	3/28 (11%)	4/21 (19%)	2/25 (8%)	6/36 (17%)
First incidence (days)	621	512	596	634
Poly-3 test	P=0.529	P=0.583	P=0.169N	P=0.539
Zymbal's Gland: Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	0.0%	7.0%	0.0%	4.4%
Terminal rate	0/28 (0%)	0/21 (0%)	0/25 (0%)	1/36 (3%)
First incidence (days)	—	583	—	702
Poly-3 test	P=0.374	P=0.119	—[f]	P=0.250
Zymbal's Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rate	4.7%	9.3%	0.0%	4.4%
Terminal rate	2/28 (7%)	1/21 (5%)	0/25 (0%)	1/36 (3%)
First incidence (days)	729 (T)	583	—	702
Poly-3 test	P=0.383N	P=0.338	P=0.231N	P=0.673N
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.1%	2.3%	0.0%
Terminal rate	0/28 (0%)	1/21 (5%)	0/25 (0%)	0/36 (0%)
First incidence (days)	—	635	715	—
Poly-3 test	P=0.343N	P=0.115	P=0.506	—
All Organs: Mononuclear Cell Leukemia				
Overall rate	25/50 (50%)	18/50 (36%)	33/50 (66%)	24/50 (48%)
Adjusted rate	52.6%	41.2%	69.2%	50.3%
Terminal rate	12/28 (43%)	9/21 (43%)	16/25 (64%)	16/36 (44%)
First incidence (days)	505	473	475	527
Poly-3 test	P=0.421	P=0.185N	P=0.068	P=0.494N
All Organs: Benign Neoplasms				
Overall rate	44/50 (88%)	48/50 (96%)	49/50 (98%)	48/50 (96%)
Adjusted rate	93.0%	98.5%	99.4%	99.1%
Terminal rate	27/28 (96%)	21/21 (100%)	25/25 (100%)	36/36 (100%)
First incidence (days)	549	455	453	562
Poly-3 test	P=0.035	P=0.147	P=0.073	P=0.098

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	31/50 (62%)	37/50 (74%)	32/50 (64%)
Adjusted rate	63.9%	66.3%	76.3%	66.5%
Terminal rate	15/28 (54%)	13/21 (62%)	17/25 (68%)	22/36 (61%)
First incidence (days)	505	473	475	527
Poly-3 test	P=0.395	P=0.487	P=0.128	P=0.478
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	99.1%	100.0%	100.0%	99.9%
Terminal rate	28/28 (100%)	21/21 (100%)	25/25 (100%)	36/36 (100%)
First incidence (days)	505	455	453	527
Poly-3 test	P=0.689	P=0.879	P=0.879	P=0.904

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, pancreatic islets, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	24	20	13
Natural deaths	3	5	5	1
Survivors				
Terminal sacrifice	28	21	25	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(47)	(49)	(50)
Necrosis	1 (2%)		1 (2%)	
Serosa, inflammation, suppurative		1 (2%)		
Intestine small, duodenum	(49)	(49)	(49)	(50)
Intestine small, ileum	(48)	(47)	(46)	(49)
Necrosis			1 (2%)	
Intestine small, jejunum	(48)	(47)	(48)	(50)
Inflammation, suppurative		1 (2%)		
Ulcer		1 (2%)		
Artery, inflammation, chronic				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	2 (4%)	2 (4%)	3 (6%)	5 (10%)
Basophilic focus, multiple	6 (12%)	1 (2%)	1 (2%)	
Clear cell focus	3 (6%)	8 (16%)	2 (4%)	4 (8%)
Clear cell focus, multiple	9 (18%)	1 (2%)	2 (4%)	6 (12%)
Degeneration, cystic	1 (2%)			
Hepatodiaphragmatic nodule	4 (8%)	2 (4%)	4 (8%)	
Necrosis	1 (2%)	2 (4%)	1 (2%)	
Vacuolization cytoplasmic	5 (10%)	4 (8%)	5 (10%)	2 (4%)
Bile duct, cyst		1 (2%)		
Bile duct, dilatation				1 (2%)
Bile duct, hyperplasia			1 (2%)	1 (2%)
Kupffer cell, pigmentation	1 (2%)			
Periportal, inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Periportal, pigmentation				1 (2%)
Mesentery	(10)	(13)	(16)	(10)
Inflammation, chronic			1 (6%)	
Necrosis	9 (90%)	12 (92%)	13 (81%)	10 (100%)
Fat, hemorrhage	1 (10%)	1 (8%)	1 (6%)	
Pancreas	(49)	(50)	(50)	(50)
Acinus, atrophy	24 (49%)	22 (44%)	29 (58%)	31 (62%)
Acinus, hyperplasia				1 (2%)
Duct, cyst	1 (2%)			1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Duct, cyst	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			
Hyperplasia, squamous	2 (4%)			
Inflammation, suppurative			1 (2%)	
Ulcer	1 (2%)	4 (8%)	2 (4%)	2 (4%)
Muscularis, degeneration				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion			3 (6%)	
Inflammation, chronic active				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Alimentary System (continued)				
Tongue	(0)	(0)	(1)	(0)
Epithelium, hyperplasia			1 (100%)	
Cardiovascular System				
Blood vessel	(0)	(0)	(1)	(1)
Adventitia, inflammation, chronic				1 (100%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	23 (46%)	24 (48%)	21 (42%)	25 (50%)
Atrium, thrombosis	1 (2%)	4 (8%)	3 (6%)	
Atrium, ventricle, thrombosis			1 (2%)	
Myocardium, mineralization				1 (2%)
Ventricle, thrombosis			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia	15 (30%)	17 (34%)	10 (20%)	12 (24%)
Hyperplasia, focal		1 (2%)	1 (2%)	
Necrosis		1 (2%)		
Vacuolization cytoplasmic	9 (18%)	7 (14%)	16 (32%)	6 (12%)
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia	15 (30%)	18 (36%)	25 (50%)	17 (35%)
Bilateral, hyperplasia	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Pituitary gland	(50)	(48)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	
Cyst	2 (4%)			1 (2%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	
Pars distalis, hyperplasia	7 (14%)	9 (19%)	9 (18%)	7 (14%)
Thyroid gland	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Ultimobranchial cyst				1 (2%)
C-cell, hyperplasia	17 (34%)	13 (26%)	7 (14%)	11 (22%)
Follicular cell, hyperplasia	1 (2%)		1 (2%)	1 (2%)
General Body System				
Peritoneum	(0)	(1)	(1)	(1)
Mesothelium, tunica vaginalis, hyperplasia				1 (100%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Necrosis, fatty	1 (2%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)			2 (4%)
Hyperplasia		3 (6%)	1 (2%)	1 (2%)
Inflammation, suppurative		1 (2%)		1 (2%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)		1 (2%)	4 (8%)
Inflammation, suppurative	29 (58%)	28 (56%)	29 (58%)	26 (52%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Genital System (continued)				
Seminal vesicle	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Inflammation, suppurative			1 (2%)	2 (4%)
Testes	(50)	(50)	(50)	(50)
Mineralization		2 (4%)	1 (2%)	
Artery, inflammation, chronic active	1 (2%)			1 (2%)
Germinal epithelium, atrophy	9 (18%)	11 (22%)	7 (14%)	15 (30%)
Germinal epithelium, mineralization	1 (2%)			
Interstitial cell, hyperplasia	24 (48%)	18 (36%)	14 (28%)	17 (34%)
Tunic, hyperplasia				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia, reticulum cell	2 (4%)		1 (2%)	
Lymph node	(10)	(5)	(10)	(10)
Ectasia		1 (20%)		
Deep cervical, hemorrhage				1 (10%)
Deep cervical, pigmentation				1 (10%)
Pancreatic, infiltration cellular, histiocyte	1 (10%)			
Pancreatic, pigmentation	1 (10%)			
Lymph node, bronchial	(4)	(4)	(3)	(5)
Ectasia				1 (20%)
Hyperplasia, lymphoid		1 (25%)		
Pigmentation				1 (20%)
Lymph node, mandibular	(0)	(0)	(1)	(1)
Ectasia				1 (100%)
Lymph node, mediastinal	(30)	(24)	(35)	(29)
Infiltration cellular, histiocyte	1 (3%)			1 (3%)
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Ectasia		1 (2%)		
Hyperplasia, lymphoid				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Hemorrhage	2 (4%)	2 (4%)	2 (4%)	
Hyperplasia, lymphoid	1 (2%)			
Necrosis	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Thrombosis	1 (2%)			
Capsule, fibrosis	1 (2%)			
Thymus	(44)	(43)	(47)	(45)
Hyperplasia, tubular	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Galactocele	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		5 (10%)	3 (6%)	1 (2%)
Hyperkeratosis			1 (2%)	
Inflammation, suppurative		1 (2%)		
Ulcer		2 (4%)	1 (2%)	1 (2%)
Sebaceous gland, hyperplasia	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, fracture				1 (2%)
Skeletal muscle	(0)	(1)	(1)	(1)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	11 (22%)	6 (12%)	4 (8%)
Gliosis	2 (4%)			
Hemorrhage	2 (4%)	8 (16%)	5 (10%)	
Hydrocephalus	1 (2%)	1 (2%)	1 (2%)	
Inflammation, suppurative				1 (2%)
Necrosis				1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Inflammation, chronic	1 (2%)			
Epiglottis, hyperplasia			1 (2%)	
Epiglottis, metaplasia, squamous		1 (2%)		
Respiratory epithelium, metaplasia, squamous				1 (2%)
Lung	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Hemorrhage	5 (10%)	3 (6%)	4 (8%)	5 (10%)
Inflammation, suppurative		4 (8%)		2 (4%)
Inflammation, chronic	8 (16%)	8 (16%)	8 (16%)	10 (20%)
Metaplasia, osseous		1 (2%)		2 (4%)
Alveolar epithelium, hyperplasia	6 (12%)	7 (14%)	6 (12%)	5 (10%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Alveolus, infiltration cellular, histiocyte	15 (30%)	12 (24%)	19 (38%)	20 (40%)
Alveolus, metaplasia, osseous				1 (2%)
Alveolus, mineralization				1 (2%)
Alveolus, proteinosis	1 (2%)		2 (4%)	1 (2%)
Artery, thrombosis		2 (4%)		
Bronchiole, hyperplasia				2 (4%)
Interstitium, fibrosis	1 (2%)			3 (6%)
Nose	(49)	(50)	(50)	(50)
Foreign body	6 (12%)	3 (6%)	4 (8%)	2 (4%)
Hemorrhage		1 (2%)		
Inflammation, suppurative	5 (10%)	5 (10%)	10 (20%)	29 (58%)
Inflammation, chronic	1 (2%)			
Thrombosis				1 (2%)
Glands, olfactory epithelium, accumulation, hyaline droplet				1 (2%)
Glands, respiratory epithelium, accumulation, hyaline droplet	6 (12%)	45 (90%)	42 (84%)	45 (90%)
Glands, respiratory epithelium, hyperplasia	44 (90%)	46 (92%)	46 (92%)	48 (96%)
Goblet cell, hyperplasia			2 (4%)	13 (26%)
Nasolacrimal duct, inflammation, suppurative			1 (2%)	

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Respiratory System (continued)				
Nose (continued)	(49)	(50)	(50)	(50)
Olfactory epithelium, accumulation, hyaline droplet	8 (16%)	49 (98%)	49 (98%)	42 (84%)
Olfactory epithelium, atrophy	2 (4%)	49 (98%)	50 (100%)	50 (100%)
Olfactory epithelium, degeneration, hyaline		1 (2%)		1 (2%)
Olfactory epithelium, hyperplasia, basal cell			22 (44%)	50 (100%)
Olfactory epithelium, metaplasia, squamous		2 (4%)		1 (2%)
Olfactory epithelium, necrosis		1 (2%)		2 (4%)
Olfactory epithelium, respiratory metaplasia	2 (4%)	2 (4%)	2 (4%)	37 (74%)
Olfactory epithelium, vacuolization cytoplasmic		2 (4%)	8 (16%)	1 (2%)
Respiratory epithelium, accumulation, hyaline droplet		29 (58%)	42 (84%)	11 (22%)
Respiratory epithelium, hyperplasia	5 (10%)	34 (68%)	35 (70%)	47 (94%)
Respiratory epithelium, inflammation, chronic				1 (2%)
Respiratory epithelium, metaplasia, squamous		2 (4%)	6 (12%)	26 (52%)
Respiratory epithelium, necrosis			1 (2%)	4 (8%)
Respiratory epithelium, ulcer			2 (4%)	22 (44%)
Respiratory epithelium, vacuolization cytoplasmic		5 (10%)	8 (16%)	3 (6%)
Turbinate, hyperostosis				3 (6%)
Turbinate, necrosis			1 (2%)	19 (38%)
Pleura	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hyperplasia		1 (2%)		
Inflammation, chronic	3 (6%)	4 (8%)	3 (6%)	9 (18%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Anterior chamber, inflammation, suppurative			1 (2%)	2 (4%)
Cornea, fibrosis	1 (2%)			
Cornea, hyperplasia				1 (2%)
Cornea, inflammation, suppurative			1 (2%)	5 (10%)
Cornea, inflammation, chronic				2 (4%)
Cornea, inflammation, chronic active				1 (2%)
Cornea, mineralization			1 (2%)	1 (2%)
Cornea, vacuolization cytoplasmic				3 (6%)
Lens, cataract	1 (2%)	3 (6%)	1 (2%)	5 (10%)
Retina, atrophy	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Retina, dysplasia				1 (2%)
Sclera, metaplasia, osseous	11 (22%)	9 (18%)	13 (26%)	6 (12%)
Sclera, mineralization	1 (2%)	1 (2%)	1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)		2 (4%)
Zymbal's gland	(43)	(39)	(41)	(40)
Duct, hyperplasia	1 (2%)			1 (3%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Nephropathy, chronic	44 (88%)	39 (78%)	40 (80%)	39 (78%)
Cortex, cyst	1 (2%)			
Cortex, infarct		2 (4%)	2 (4%)	2 (4%)
Cortex, renal tubule, accumulation, hyaline droplet	1 (2%)			
Cortex, renal tubule, casts granular, focal	1 (2%)			
Cortex, renal tubule, hyperplasia, atypical				1 (2%)
Cortex, renal tubule, mineralization		1 (2%)		
Cortex, renal tubule, necrosis		1 (2%)		
Papilla, mineralization	2 (4%)	3 (6%)		
Pelvis, dilatation		1 (2%)		
Pelvis, inflammation, suppurative				1 (2%)
Pelvis, transitional epithelium, hyperplasia			2 (4%)	4 (8%)
Pelvis, transitional epithelium, mineralization		1 (2%)	1 (2%)	
Urinary bladder	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)	1 (2%)		
Infiltration cellular, histiocyte	1 (2%)			
Muscularis, pigmentation	1 (2%)			
Transitional epithelium, hemorrhage			1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)			2 (4%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DIETHYLAMINE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	15	18	15
Natural deaths	2	4	2	
Survivors				
Died last week of study			1	1
Terminal sacrifice	31	31	29	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(49)	(47)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)			
Mesentery	(16)	(15)	(18)	(10)
Carcinoma, metastatic, uncertain primary site	1 (6%)			
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(2)	(0)	(1)
Squamous cell papilloma		1 (50%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	5 (10%)	2 (4%)	6 (12%)
Bilateral, adenoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)	2 (4%)	1 (2%)
Pheochromocytoma malignant		1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Carcinoma			2 (4%)	
Parathyroid gland	(43)	(42)	(48)	(48)
Adenoma	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, adenoma	29 (58%)	33 (66%)	36 (72%)	23 (46%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	4 (8%)	6 (12%)	3 (6%)	6 (12%)
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma		1 (2%)		
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Mediastinum, carcinoma, metastatic, uncertain primary site	1 (100%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Carcinoma			1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor malignant			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Polyp stromal	8 (16%)	9 (18%)	7 (14%)	9 (18%)
Polyp stromal, multiple		2 (4%)		1 (2%)
Sarcoma stromal		1 (2%)		
Cervix, polyp stromal			1 (2%)	
Endometrium, deciduoma benign		1 (2%)		
Vagina	(1)	(0)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(0)	(1)	(1)
Lymph node, bronchial	(4)	(1)	(5)	(7)
Lymph node, mandibular	(2)	(0)	(1)	(2)
Lymph node, mediastinal	(33)	(27)	(25)	(29)
Carcinoma, metastatic, thyroid gland		1 (4%)		
Fibrosarcoma, metastatic, skin			1 (4%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(44)	(40)	(47)
Thymoma NOS	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma	7 (14%)	3 (6%)	1 (2%)	2 (4%)
Carcinoma, multiple	1 (2%)		1 (2%)	
Fibroadenoma	18 (36%)	16 (32%)	17 (34%)	19 (38%)
Fibroadenoma, multiple	9 (18%)	9 (18%)	8 (16%)	5 (10%)
Fibrosarcoma, metastatic, skin		1 (2%)		
Duct, carcinoma	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Basal cell carcinoma		1 (2%)		
Fibrosarcoma		1 (2%)		
Keratoacanthoma	1 (2%)			
Neural crest tumor				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)		1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, osteosarcoma			1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Granular cell tumor malignant		1 (2%)		
Spinal cord	(0)	(0)	(1)	(0)
Osteosarcoma, metastatic, bone			1 (100%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma				1 (2%)
Carcinoma, metastatic, clitoral gland			1 (2%)	
Carcinoma, metastatic, thyroid gland		1 (2%)		1 (2%)
Carcinoma, metastatic, Zymbal's gland		1 (2%)		
Nose	(50)	(49)	(50)	(50)
Pleura	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(40)	(38)	(42)	(33)
Carcinoma		2 (5%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	15 (30%)	10 (20%)	15 (30%)	11 (22%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	48	48	46
Total primary neoplasms	108	110	107	95
Total animals with benign neoplasms	43	45	46	40
Total benign neoplasms	81	88	82	76
Total animals with malignant neoplasms	24	18	22	16
Total malignant neoplasms	26	22	25	18
Total animals with metastatic neoplasms	3	4	4	2
Total metastatic neoplasms	4	6	4	2
Total animals with malignant neoplasms of uncertain primary site	1			
Total animals with uncertain neoplasms-benign or malignant	1			1
Total uncertain neoplasms	1			1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

[b] Number of animals with any tissue examined microscopically

[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	4/50 (8%)	6/50 (12%)	2/50 (4%)	6/50 (12%)
Adjusted rate[b]	9.2%	13.3%	4.6%	13.4%
Terminal rate[c]	2/31 (7%)	5/31 (16%)	1/30 (3%)	3/34 (9%)
First incidence (days)	688	706	682	647
Poly-3 test[d]	P=0.414	P=0.395	P=0.334N	P=0.389
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	4.4%	6.8%	4.5%
Terminal rate	0/31 (0%)	1/31 (3%)	1/30 (3%)	1/34 (3%)
First incidence (days)	—[e]	645	639	592
Poly-3 test	P=0.248	P=0.249	P=0.121	P=0.244
Clitoral Gland: Adenoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.3%	4.4%	6.9%	4.5%
Terminal rate	1/31 (3%)	1/31 (3%)	3/30 (10%)	1/34 (3%)
First incidence (days)	730 (T)	646	730 (T)	675
Poly-3 test	P=0.411	P=0.518	P=0.308	P=0.509
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.3%	4.4%	9.2%	4.5%
Terminal rate	1/31 (3%)	1/31 (3%)	3/30 (10%)	1/34 (3%)
First incidence (days)	730 (T)	646	725	675
Poly-3 test	P=0.394	P=0.518	P=0.181	P=0.509
Mammary Gland: Fibroadenoma				
Overall rate	27/50 (54%)[f]	25/50 (50%)	25/50 (50%)	24/50 (48%)
Adjusted rate	58.6%	52.7%	55.4%	52.0%
Terminal rate	17/31 (55%)	16/31 (52%)	18/30 (60%)	17/34 (50%)
First incidence (days)	486	575	481	592
Poly-3 test	P=0.336N	P=0.355N	P=0.460N	P=0.330N
Mammary Gland: Carcinoma				
Overall rate	9/50 (18%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	20.5%	6.6%	4.6%	4.5%
Terminal rate	6/31 (19%)	1/31 (3%)	1/30 (3%)	1/34 (3%)
First incidence (days)	638	631	724	712
Poly-3 test	P=0.017N	P=0.049N	P=0.025N	P=0.023N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	22.6%	6.6%	4.6%	4.5%
Terminal rate	6/31 (19%)	1/31 (3%)	1/30 (3%)	1/34 (3%)
First incidence (days)	625	631	724	712
Poly-3 test	P=0.009N	P=0.029N	P=0.014N	P=0.013N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	30/50 (60%)	26/50 (52%)	26/50 (52%)	24/50 (48%)
Adjusted rate	64.3%	54.5%	57.6%	52.0%
Terminal rate	18/31 (58%)	16/31 (52%)	18/30 (60%)	17/34 (50%)
First incidence (days)	486	575	481	592
Poly-3 test	P=0.174N	P=0.218N	P=0.323N	P=0.155N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.6%	2.2%	9.1%	2.3%
Terminal rate	1/31 (3%)	1/31 (3%)	2/30 (7%)	1/34 (3%)
First incidence (days)	665	730 (T)	618	730 (T)
Poly-3 test	P=0.501N	P=0.487N	P=0.343	P=0.495N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	29/50 (58%)	33/50 (66%)	36/50 (72%)	23/50 (46%)
Adjusted rate	62.7%	67.9%	76.3%	50.3%
Terminal rate	18/31 (58%)	20/31 (65%)	22/30 (73%)	15/34 (44%)
First incidence (days)	536	574	544	618
Poly-3 test	P=0.101N	P=0.373	P=0.108	P=0.156N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	31/50 (62%)	34/50 (68%)	37/50 (74%)	24/50 (48%)
Adjusted rate	66.6%	69.3%	77.8%	51.6%
Terminal rate	19/31 (61%)	20/31 (65%)	22/30 (73%)	15/34 (44%)
First incidence (days)	536	574	544	429
Poly-3 test	P=0.062N	P=0.474	P=0.155	P=0.098N
Thyroid Gland (C-cell): Adenoma				
Overall rate	4/50 (8%)	6/50 (12%)	3/50 (6%)	6/50 (12%)
Adjusted rate	9.3%	13.2%	6.8%	13.3%
Terminal rate	4/31 (13%)	5/31 (16%)	2/30 (7%)	2/34 (6%)
First incidence (days)	730 (T)	645	553	647
Poly-3 test	P=0.409	P=0.402	P=0.488N	P=0.397
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	3/50 (6%)	7/50 (14%)
Adjusted rate	9.3%	15.3%	6.8%	15.5%
Terminal rate	4/31 (13%)	5/31 (16%)	2/30 (7%)	2/34 (6%)
First incidence (days)	730 (T)	640	553	647
Poly-3 test	P=0.327	P=0.294	P=0.488N	P=0.287
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	11/50 (22%)	8/50 (16%)	10/50 (20%)
Adjusted rate	18.3%	23.9%	18.0%	22.5%
Terminal rate	5/31 (16%)	7/31 (23%)	6/30 (20%)	8/34 (24%)
First incidence (days)	646	607	481	592
Poly-3 test	P=0.443	P=0.346	P=0.597N	P=0.411
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	8/50 (16%)	12/50 (24%)	8/50 (16%)	10/50 (20%)
Adjusted rate	18.3%	26.1%	18.0%	22.5%
Terminal rate	5/31 (16%)	8/31 (26%)	6/30 (20%)	8/34 (24%)
First incidence (days)	646	607	481	592
Poly-3 test	P=0.482	P=0.262	P=0.597N	P=0.411
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	10/50 (20%)	15/50 (30%)	11/50 (22%)
Adjusted rate	33.4%	21.8%	33.3%	24.1%
Terminal rate	9/31 (29%)	6/31 (19%)	9/30 (30%)	7/34 (21%)
First incidence (days)	361	646	553	449
Poly-3 test	P=0.309N	P=0.156N	P=0.584N	P=0.227N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	45/50 (90%)	46/50 (92%)	40/50 (80%)
Adjusted rate	89.1%	91.7%	94.4%	85.2%
Terminal rate	28/31 (90%)	29/31 (94%)	28/30 (93%)	29/34 (85%)
First incidence (days)	486	574	481	592
Poly-3 test	P=0.283N	P=0.461	P=0.270	P=0.392N
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	18/50 (36%)	22/50 (44%)	16/50 (32%)
Adjusted rate	52.7%	37.9%	48.3%	33.5%
Terminal rate	15/31 (48%)	9/31 (29%)	12/30 (40%)	8/34 (24%)
First incidence (days)	361	578	553	429
Poly-3 test	P=0.072N	P=0.107N	P=0.417N	P=0.045N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	48/50 (96%)	48/50 (96%)	46/50 (92%)
Adjusted rate	96.0%	96.0%	97.8%	93.0%
Terminal rate	29/31 (94%)	29/31 (94%)	29/30 (97%)	31/34 (91%)
First incidence (days)	361	574	481	429
Poly-3 test	P=0.303N	P=0.693	P=0.524	P=0.411N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pancreatic islets, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] One adenoma occurred in an animal that also had multiple fibroadenomas.

[g] Value of statistic cannot be computed.

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	15	18	15
Natural deaths	2	4	2	
Survivors				
Died last week of study			1	1
Terminal sacrifice	31	31	29	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(49)	(47)	(50)	(50)
Epithelium, hyperplasia, focal	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Basophilic focus	3 (6%)	1 (2%)	4 (8%)	7 (14%)
Basophilic focus, multiple	17 (34%)	13 (26%)	19 (38%)	15 (30%)
Clear cell focus	8 (16%)	8 (16%)	6 (12%)	4 (8%)
Clear cell focus, multiple	8 (16%)	1 (2%)	2 (4%)	3 (6%)
Degeneration, cystic		1 (2%)		
Hemorrhage	1 (2%)		2 (4%)	
Hepatodiaphragmatic nodule	9 (18%)	6 (12%)	2 (4%)	9 (18%)
Necrosis		1 (2%)	1 (2%)	
Thrombosis	1 (2%)			
Vacuolization cytoplasmic	3 (6%)	8 (16%)	6 (12%)	2 (4%)
Artery, inflammation			1 (2%)	
Bile duct, hyperplasia			1 (2%)	
Hepatocyte, regeneration			1 (2%)	
Periportal, inflammation, chronic		1 (2%)		
Periportal, pigmentation			1 (2%)	
Mesentery	(16)	(15)	(18)	(10)
Necrosis	15 (94%)	14 (93%)	17 (94%)	10 (100%)
Artery, inflammation			1 (6%)	
Pancreas	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Acinus, atrophy	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Artery, inflammation		1 (2%)	1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion		1 (2%)		
Hyperplasia, squamous	1 (2%)			
Inflammation, suppurative	2 (4%)			
Ulcer	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	1 (2%)		
Tongue	(0)	(2)	(0)	(1)
Epithelium, hyperplasia		1 (50%)		1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	16 (32%)	17 (34%)	23 (46%)	16 (32%)
Atrium, ventricle, thrombosis	1 (2%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hemorrhage	1 (2%)			1 (2%)
Hyperplasia	12 (24%)	6 (12%)	15 (30%)	18 (36%)
Hyperplasia, focal	1 (2%)	1 (2%)	3 (6%)	
Necrosis	1 (2%)	1 (2%)		
Vacuolization cytoplasmic	14 (28%)	13 (26%)	13 (26%)	11 (22%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	1 (2%)	2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Parathyroid gland	(43)	(42)	(48)	(48)
Pituitary gland	(50)	(50)	(50)	(50)
Cyst		1 (2%)	1 (2%)	1 (2%)
Hemorrhage	2 (4%)	2 (4%)		2 (4%)
Pars distalis, hyperplasia	9 (18%)	10 (20%)	7 (14%)	18 (36%)
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)			
C-cell, hyperplasia	25 (50%)	18 (36%)	25 (50%)	21 (42%)
Follicular cell, hyperplasia	1 (2%)			
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Hyperplasia	2 (4%)	5 (10%)	4 (8%)	6 (12%)
Inflammation, chronic	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Cyst	7 (14%)	5 (10%)	4 (8%)	4 (8%)
Uterus	(50)	(50)	(50)	(50)
Hemorrhage			2 (4%)	1 (2%)
Thrombosis			1 (2%)	2 (4%)
Endometrium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Vagina	(1)	(0)	(0)	(0)
Cyst	1 (100%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia, reticulum cell	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Myelofibrosis	1 (2%)			
Lymph node	(3)	(0)	(1)	(1)
Lymph node, bronchial	(4)	(1)	(5)	(7)
Hemorrhage		1 (100%)		
Hyperplasia, histiocytic	1 (25%)			
Infiltration cellular, histiocyte				1 (14%)
Lymph node, mandibular	(2)	(0)	(1)	(2)
Lymph node, mediastinal	(33)	(27)	(25)	(29)
Hyperplasia, lymphoid				1 (3%)
Infiltration cellular, histiocyte				1 (3%)
Pigmentation				2 (7%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Fibrosis		1 (2%)	1 (2%)	
Hematopoietic cell proliferation	7 (14%)	3 (6%)	4 (8%)	3 (6%)
Hemorrhage	1 (2%)			
Hyperplasia, histiocytic		1 (2%)		1 (2%)
Necrosis	1 (2%)		1 (2%)	
Pigmentation	1 (2%)	1 (2%)		
Stromal hyperplasia	1 (2%)			
Thymus	(48)	(44)	(40)	(47)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	4 (8%)	3 (6%)	5 (10%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	6 (12%)	2 (4%)	1 (2%)	3 (6%)
Subcutaneous tissue, cyst		1 (2%)		
Subcutaneous tissue, fibrosis			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, inflammation, suppurative				1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	12 (24%)	11 (22%)	14 (28%)	9 (18%)
Hemorrhage	4 (8%)	1 (2%)	2 (4%)	5 (10%)
Meninges, hemorrhage	1 (2%)			
Meninges, inflammation, chronic			1 (2%)	
Ventricle, hemorrhage	1 (2%)			
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	6 (12%)	4 (8%)	1 (2%)
Inflammation, suppurative	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Epiglottis, metaplasia, squamous			2 (4%)	
Respiratory epithelium, metaplasia, squamous		2 (4%)		
Lung	(50)	(50)	(50)	(50)
Hemorrhage	6 (12%)	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic	4 (8%)	11 (22%)	7 (14%)	24 (48%)
Metaplasia, osseous			1 (2%)	
Alveolar epithelium, hyperplasia	3 (6%)	6 (12%)	4 (8%)	3 (6%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Alveolus, infiltration cellular, histiocyte	13 (26%)	24 (48%)	27 (54%)	35 (70%)
Alveolus, proteinosis			1 (2%)	2 (4%)
Bronchiole, hyperplasia	1 (2%)	1 (2%)		
Interstitium, fibrosis	1 (2%)	2 (4%)		2 (4%)
Perivascular, infiltration cellular, lymphocyte			1 (2%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Respiratory System (continued)				
Nose	(50)	(49)	(50)	(50)
Foreign body	6 (12%)	1 (2%)	1 (2%)	2 (4%)
Inflammation, suppurative	6 (12%)	4 (8%)	15 (30%)	34 (68%)
Glands, respiratory epithelium, accumulation, hyaline droplet	9 (18%)	46 (94%)	45 (90%)	44 (88%)
Glands, respiratory epithelium, hyperplasia	45 (90%)	49 (100%)	48 (96%)	49 (98%)
Goblet cell, hyperplasia	1 (2%)		4 (8%)	20 (40%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	2 (4%)		
Olfactory epithelium, accumulation, hyaline droplet	11 (22%)	49 (100%)	50 (100%)	48 (96%)
Olfactory epithelium, atrophy	1 (2%)	47 (96%)	48 (96%)	50 (100%)
Olfactory epithelium, hyperplasia				1 (2%)
Olfactory epithelium, hyperplasia, basal cell		3 (6%)	29 (58%)	48 (96%)
Olfactory epithelium, metaplasia, squamous			1 (2%)	
Olfactory epithelium, mineralization				1 (2%)
Olfactory epithelium, necrosis		2 (4%)	3 (6%)	
Olfactory epithelium, respiratory metaplasia	3 (6%)	1 (2%)	2 (4%)	19 (38%)
Olfactory epithelium, vacuolization cytoplasmic		1 (2%)	4 (8%)	3 (6%)
Respiratory epithelium, accumulation, hyaline droplet	4 (8%)	48 (98%)	46 (92%)	39 (78%)
Respiratory epithelium, hyperplasia	7 (14%)	31 (63%)	41 (82%)	50 (100%)
Respiratory epithelium, inflammation, chronic		1 (2%)		
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)	5 (10%)	39 (78%)
Respiratory epithelium, necrosis			1 (2%)	4 (8%)
Respiratory epithelium, ulcer				34 (68%)
Respiratory epithelium, vacuolization cytoplasmic		1 (2%)	4 (8%)	3 (6%)
Turbinate, hyperostosis				2 (4%)
Turbinate, necrosis				32 (64%)
Pleura	(50)	(50)	(50)	(50)
Inflammation, chronic	6 (12%)	14 (28%)	12 (24%)	21 (42%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Anterior chamber, inflammation, suppurative				1 (2%)
Bilateral, lens, cataract			1 (2%)	
Cornea, inflammation, suppurative		2 (4%)	2 (4%)	1 (2%)
Cornea, mineralization			1 (2%)	
Cornea, vacuolization cytoplasmic			1 (2%)	
Lens, cataract	3 (6%)	2 (4%)	5 (10%)	4 (8%)
Retina, atrophy	4 (8%)	2 (4%)	8 (16%)	6 (12%)
Sclera, metaplasia, osseous	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Zymbal's gland	(40)	(38)	(42)	(33)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	31 ppm	62.5 ppm	125 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Nephropathy, chronic	26 (52%)	31 (62%)	30 (60%)	24 (48%)
Cortex, infarct			1 (2%)	1 (2%)
Cortex, renal tubule, accumulation, hyaline droplet				1 (2%)
Cortex, renal tubule, necrosis			1 (2%)	
Papilla, mineralization	12 (24%)	13 (26%)	9 (18%)	4 (8%)
Pelvis, transitional epithelium, hyperplasia		2 (4%)	2 (4%)	
Pelvis, transitional epithelium, mineralization	2 (4%)			1 (2%)
Renal tubule, vacuolization cytoplasmic		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	1 (2%)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF DIETHYLAMINE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	10	12	11
Natural deaths	4	2	6	2
Survivors				
Terminal sacrifice	31	38	32	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(36)	(42)	(41)	(38)
Adenoma			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Serosa, carcinoma, metastatic, pancreas		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Serosa, carcinoma, metastatic, pancreas		1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Hemangiosarcoma			1 (2%)	
Polyp adenomatous				1 (2%)
Serosa, carcinoma, metastatic, pancreas		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)			1 (2%)
Serosa, sarcoma, metastatic, uncertain primary site	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Hemangiosarcoma	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Hepatoblastoma	1 (2%)	1 (2%)		
Hepatocellular adenoma	14 (28%)	21 (42%)	15 (30%)	20 (40%)
Hepatocellular adenoma, multiple	14 (28%)	8 (16%)	13 (26%)	11 (22%)
Hepatocellular carcinoma	14 (28%)	14 (28%)	11 (22%)	16 (32%)
Hepatocellular carcinoma, multiple	6 (12%)	3 (6%)	3 (6%)	4 (8%)
Mesentery	(10)	(6)	(4)	(8)
Carcinoma, metastatic, pancreas		1 (17%)		
Carcinoma, metastatic, uncertain primary site			1 (25%)	
Hemangiosarcoma	1 (10%)			3 (38%)
Hepatoblastoma, metastatic, liver	1 (10%)			
Sarcoma, metastatic, uncertain primary site	1 (10%)			
Pancreas	(50)	(50)	(49)	(50)
Carcinoma		1 (2%)		
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Sarcoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	2 (4%)			
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Tooth	(6)	(6)	(8)	(5)
Odontoma	2 (33%)	3 (50%)	3 (38%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(1)
Aorta, carcinoma, metastatic, pancreas		1 (100%)		
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Hepatoblastoma, metastatic, liver	1 (2%)			
Hepatocellular carcinoma, metastatic, liver			2 (4%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)	
Pericardium, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Carcinoma, metastatic, pancreas		1 (2%)		
Capsule, sarcoma, metastatic, uncertain primary site	1 (2%)			
Subcapsular, adenoma	5 (10%)	2 (4%)	4 (8%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	2 (4%)	1 (2%)		1 (2%)
Carcinoma				1 (2%)
Carcinoma, metastatic, pancreas		1 (2%)		
Parathyroid gland	(28)	(32)	(25)	(21)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, oligodendroglioma malignant, metastatic, brain			1 (2%)	
Thyroid gland	(50)	(50)	(48)	(49)
Sarcoma, metastatic, skeletal muscle				1 (2%)
Follicular cell, adenoma		2 (4%)		
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Carcinoma, metastatic, pancreas		1 (100%)		
Epididymis	(49)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hemangiosarcoma		1 (2%)		
Penis	(0)	(0)	(0)	(2)
Preputial gland	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Prostate	(50)	(50)	(50)	(49)
Carcinoma, metastatic, pancreas		1 (2%)		
Fibrous histiocytoma, metastatic, skin				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas		1 (2%)		
Fibrous histiocytoma, metastatic, skin				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hemangiosarcoma	2 (4%)	2 (4%)		
Oligodendroglioma malignant, metastatic, brain			1 (2%)	
Lymph node	(0)	(5)	(5)	(0)
Carcinoma, metastatic, pancreas		1 (20%)		
Iliac, sarcoma, metastatic, uncertain primary site			1 (20%)	
Renal, hemangiosarcoma		1 (20%)		
Lymph node, bronchial	(20)	(29)	(25)	(25)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (5%)			
Carcinoma, metastatic, pancreas		1 (3%)		
Hepatoblastoma, metastatic, liver	1 (5%)			
Hepatocellular carcinoma, metastatic, liver	1 (5%)		1 (4%)	1 (4%)
Lymph node, mandibular	(16)	(20)	(18)	(20)
Hemangiosarcoma		1 (5%)		
Lymph node, mediastinal	(33)	(35)	(36)	(38)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)			
Carcinoma, metastatic, pancreas		1 (3%)		
Hepatoblastoma, metastatic, liver	1 (3%)			
Hepatocellular carcinoma, metastatic, liver	1 (3%)		1 (3%)	
Sarcoma, metastatic, uncertain primary site	1 (3%)			
Lymph node, mesenteric	(49)	(47)	(48)	(50)
Carcinoma, metastatic, intestine large, cecum		1 (2%)		
Carcinoma, metastatic, pancreas		1 (2%)		
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Hemangiosarcoma				1 (2%)
Spleen	(50)	(50)	(49)	(50)
Hemangiosarcoma	3 (6%)	4 (8%)	4 (8%)	1 (2%)
Thymus	(38)	(36)	(36)	(37)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)			1 (3%)
Hepatoblastoma, metastatic, liver	1 (3%)			
Hepatocellular carcinoma, metastatic, liver			1 (3%)	
Sarcoma, metastatic, uncertain primary site	1 (3%)		1 (3%)	
Integumentary System				
Skin	(50)	(50)	(49)	(50)
Subcutaneous tissue, fibroma			1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		2 (4%)		1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Musculoskeletal System				
Bone	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		
Maxilla, carcinoma, metastatic, Harderian gland				1 (2%)
Skeletal muscle	(2)	(3)	(3)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)			
Carcinoma, metastatic, pancreas		1 (33%)		
Hemangiosarcoma		1 (33%)	2 (67%)	
Sarcoma				1 (100%)
Sarcoma, metastatic, uncertain primary site	1 (50%)		1 (33%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland				2 (4%)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Meningioma benign	1 (2%)			
Oligodendroglioma malignant			1 (2%)	
Peripheral nerve	(0)	(1)	(1)	(0)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	4 (8%)	5 (10%)	3 (6%)
Alveolar/bronchiolar carcinoma	12 (24%)	10 (20%)	9 (18%)	9 (18%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Carcinoma, metastatic, Harderian gland		1 (2%)		
Carcinoma, metastatic, intestine small, jejunum	1 (2%)			
Carcinoma, metastatic, pancreas		1 (2%)		
Hemangiosarcoma			1 (2%)	
Hepatoblastoma, metastatic, liver	1 (2%)	1 (2%)		
Hepatocellular carcinoma, metastatic, liver	8 (16%)	6 (12%)	6 (12%)	9 (18%)
Sarcoma, metastatic, uncertain primary site	1 (2%)		1 (2%)	
Nose	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Oligodendroglioma malignant, metastatic, brain			1 (2%)	
Pleura	(1)	(0)	(0)	(0)
Trachea	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skeletal muscle				1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	6 (12%)	2 (4%)	1 (2%)
Carcinoma	4 (8%)	7 (14%)	1 (2%)	3 (6%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	1 (2%)		
Carcinoma, metastatic, pancreas		1 (2%)		
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Sarcoma, metastatic, uncertain primary site	1 (2%)		1 (2%)	
Renal tubule, adenoma			1 (2%)	
Renal tubule, carcinoma			1 (2%)	
Urethra	(0)	(0)	(1)	(0)
Bulbourethral gland, carcinoma			1 (100%)	
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant	2 (4%)	3 (6%)	3 (6%)	
Mesothelioma malignant	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	46	47	44
Total primary neoplasms	104	109	94	85
Total animals with benign neoplasms	33	34	34	34
Total benign neoplasms	47	50	47	41
Total animals with malignant neoplasms	36	34	31	32
Total malignant neoplasms	57	59	47	44
Total animals with metastatic neoplasms	12	10	8	14
Total metastatic neoplasms	42	32	23	24
Total animals with malignant neoplasms of uncertain primary site	1		1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	6/50 (12%)	4/50 (8%)	6/50 (12%)	2/50 (4%)
Adjusted rate[b]	13.9%	8.7%	13.8%	4.4%
Terminal rate[c]	5/31 (16%)	3/38 (8%)	5/32 (16%)	2/37 (5%)
First incidence (days)	606	610	610	729
Poly-3 test[d]	P=0.127N	P=0.329N	P=0.617N	P=0.118N
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	6/50 (12%)	2/50 (4%)	1/50 (2%)
Adjusted rate	9.3%	13.0%	4.6%	2.2%
Terminal rate	4/31 (13%)	5/38 (13%)	1/32 (3%)	0/37 (0%)
First incidence (days)	729 (T)	541	705	715
Poly-3 test	P=0.059N	P=0.419	P=0.332N	P=0.161N
Harderian Gland: Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	1/50 (2%)	3/50 (6%)
Adjusted rate	9.3%	15.3%	2.3%	6.6%
Terminal rate	4/31 (13%)	7/38 (18%)	1/32 (3%)	3/37 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.206N	P=0.299	P=0.176N	P=0.470N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	13/50 (26%)	3/50 (6%)	4/50 (8%)
Adjusted rate	18.7%	28.1%	6.9%	8.8%
Terminal rate	8/31 (26%)	12/38 (32%)	2/32 (6%)	3/37 (8%)
First incidence (days)	729 (T)	541	705	715
Poly-3 test	P=0.027N	P=0.212	P=0.093N	P=0.149N
Liver: Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.7%	2.2%	9.1%	2.2%
Terminal rate	2/31 (7%)	0/38 (0%)	1/32 (3%)	1/37 (3%)
First incidence (days)	729 (T)	684	590	729 (T)
Poly-3 test	P=0.480N	P=0.476N	P=0.349	P=0.481N
Liver: Hepatocellular Adenoma				
Overall rate	28/50 (56%)	29/50 (58%)	28/50 (56%)	31/50 (62%)
Adjusted rate	60.8%	60.8%	63.2%	63.6%
Terminal rate	21/31 (68%)	23/38 (61%)	25/32 (78%)	23/37 (62%)
First incidence (days)	491	484	551	442
Poly-3 test	P=0.408	P=0.585	P=0.490	P=0.471
Liver: Hepatocellular Carcinoma				
Overall rate	20/50 (40%)[e]	17/50 (34%)[e]	14/50 (28%)	20/50 (40%)
Adjusted rate	42.9%	35.5%	30.8%	42.0%
Terminal rate	10/31 (32%)	11/38 (29%)	8/32 (25%)	12/37 (32%)
First incidence (days)	491	536	484	563
Poly-3 test	P=0.522	P=0.297N	P=0.159N	P=0.545N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	40/50 (80%)[e]	37/50 (74%)[e]	36/50 (72%)	38/50 (76%)
Adjusted rate	83.1%	75.5%	77.4%	76.3%
Terminal rate	26/31 (84%)	27/38 (71%)	27/32 (84%)	26/37 (70%)
First incidence (days)	491	484	484	442
Poly-3 test	P=0.302N	P=0.242N	P=0.322N	P=0.274N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	5/50 (10%)	3/50 (6%)
Adjusted rate	9.3%	8.6%	11.2%	6.5%
Terminal rate	4/31 (13%)	2/38 (5%)	2/32 (6%)	1/37 (3%)
First incidence (days)	729 (T)	484	442	554
Poly-3 test	P=0.400N	P=0.598N	P=0.527	P=0.462N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	12/50 (24%)	10/50 (20%)	10/50 (20%)	9/50 (18%)
Adjusted rate	26.5%	21.5%	22.6%	19.6%
Terminal rate	6/31 (19%)	8/38 (21%)	7/32 (22%)	7/37 (19%)
First incidence (days)	503	561	506	555
Poly-3 test	P=0.283N	P=0.375N	P=0.429N	P=0.296N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	15/50 (30%)	14/50 (28%)	14/50 (28%)	12/50 (24%)
Adjusted rate	33.1%	29.6%	30.6%	25.8%
Terminal rate	9/31 (29%)	10/38 (26%)	8/32 (25%)	8/37 (22%)
First incidence (days)	503	484	442	554
Poly-3 test	P=0.269N	P=0.443N	P=0.486N	P=0.293N
Mesentery: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	0.0%	0.0%	6.6%
Terminal rate	1/31 (3%)	0/38 (0%)	0/32 (0%)	3/37 (8%)
First incidence (days)	729 (T)	—[f]	—	729 (T)
Poly-3 test	P=0.084	P=0.487N	P=0.498N	P=0.325
Spleen: Hemangiosarcoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/49 (8%)	1/50 (2%)
Adjusted rate	6.9%	8.7%	9.2%	2.2%
Terminal rate	1/31 (3%)	1/38 (3%)	2/32 (6%)	1/37 (3%)
First incidence (days)	632	684	590	729 (T)
Poly-3 test	P=0.197N	P=0.533	P=0.500	P=0.289N
Tooth: Odontoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.6%	6.6%	6.9%	0.0%
Terminal rate	1/31 (3%)	2/38 (5%)	2/32 (6%)	0/37 (0%)
First incidence (days)	620	710	723	—
Poly-3 test	P=0.153N	P=0.526	P=0.500	P=0.227N
All Organs: Hemangiosarcoma				
Overall rate	7/50 (14%)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted rate	16.1%	8.7%	13.5%	8.8%
Terminal rate	4/31 (13%)	1/38 (3%)	2/32 (6%)	4/37 (11%)
First incidence (days)	632	684	590	729 (T)
Poly-3 test	P=0.267N	P=0.229N	P=0.485N	P=0.237N
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.6%	6.5%	6.8%	0.0%
Terminal rate	1/31 (3%)	2/38 (5%)	2/32 (6%)	0/37 (0%)
First incidence (days)	589	684	508	—
Poly-3 test	P=0.153N	P=0.526	P=0.505	P=0.227N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	34/50 (68%)	34/50 (68%)	34/50 (68%)
Adjusted rate	70.6%	70.7%	74.3%	69.7%
Terminal rate	24/31 (77%)	27/38 (71%)	27/32 (84%)	25/37 (68%)
First incidence (days)	491	484	442	442
Poly-3 test	P=0.513N	P=0.590	P=0.430	P=0.549N
All Organs: Malignant Neoplasms				
Overall rate	36/50 (72%)	34/50 (68%)	32/50 (64%)	32/50 (64%)
Adjusted rate	73.7%	68.0%	65.0%	65.3%
Terminal rate	20/31 (65%)	22/38 (58%)	17/32 (53%)	22/37 (60%)
First incidence (days)	491	484	442	442
Poly-3 test	P=0.230N	P=0.344N	P=0.237N	P=0.247N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	46/50 (92%)	47/50 (94%)	44/50 (88%)
Adjusted rate	96.0%	92.0%	94.4%	88.3%
Terminal rate	29/31 (94%)	34/38 (90%)	30/32 (94%)	32/37 (87%)
First incidence (days)	491	484	442	442
Poly-3 test	P=0.121N	P=0.338N	P=0.535N	P=0.144N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] One hepatoblastoma occurred in an animal that also had hepatocellular carcinoma.

[f] Not applicable; no neoplasms in animal group

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	10	12	11
Natural deaths	4	2	6	2
Survivors				
Terminal sacrifice	31	38	32	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(36)	(42)	(41)	(38)
Degeneration, hyaline		1 (2%)	1 (2%)	
Inflammation, suppurative		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, chronic active	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation, chronic active				1 (2%)
Serosa, fibrosis	1 (2%)			
Intestine small, duodenum	(49)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	
Peyer's patch, hyperplasia, lymphoid			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Peyer's patch, hyperplasia, lymphoid		1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Basophilic focus	5 (10%)	13 (26%)	10 (20%)	8 (16%)
Clear cell focus	5 (10%)	5 (10%)	7 (14%)	2 (4%)
Congestion	2 (4%)			
Degeneration, fatty	2 (4%)			
Eosinophilic focus	2 (4%)	8 (16%)	6 (12%)	7 (14%)
Fibrosis			1 (2%)	
Hemorrhage	1 (2%)		1 (2%)	
Inflammation, chronic active			1 (2%)	
Mixed cell focus		4 (8%)	1 (2%)	5 (10%)
Necrosis	4 (8%)	1 (2%)	4 (8%)	3 (6%)
Tension lipidosis	1 (2%)	6 (12%)	2 (4%)	3 (6%)
Thrombosis	1 (2%)		1 (2%)	
Mesentery	(10)	(6)	(4)	(8)
Inflammation, chronic active	2 (20%)	1 (17%)	1 (25%)	1 (13%)
Fat, fibrosis			1 (25%)	
Fat, necrosis	5 (50%)	4 (67%)	1 (25%)	4 (50%)
Pancreas	(50)	(50)	(49)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Cyst	1 (2%)	1 (2%)		
Cytoplasmic alteration				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Artery, inflammation, chronic				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	3 (6%)	4 (8%)	6 (12%)	4 (8%)
Inflammation	2 (4%)	2 (4%)		
Ulcer			1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Hyperplasia			3 (6%)	
Inflammation			1 (2%)	
Mineralization			1 (2%)	
Necrosis		1 (2%)		
Glands, ectasia	1 (2%)			
Tooth	(6)	(6)	(8)	(5)
Dentine, malformation	4 (67%)	2 (33%)	5 (63%)	5 (100%)
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(1)
Media, hypertrophy				1 (100%)
Heart	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Cardiomyopathy		1 (2%)	8 (16%)	1 (2%)
Artery, inflammation				4 (8%)
Atrium, thrombosis		2 (4%)	1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule			3 (6%)	
Hyperplasia	10 (20%)	12 (24%)	4 (8%)	5 (10%)
Hypertrophy	37 (74%)	31 (62%)	26 (52%)	26 (52%)
Mineralization			1 (2%)	
Vacuolization cytoplasmic		1 (2%)	1 (2%)	
Subcapsular, hyperplasia		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Hypertrophy			1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia	2 (4%)	1 (2%)	1 (2%)	
Parathyroid gland	(28)	(32)	(25)	(21)
Cyst		1 (3%)	1 (4%)	
Hypertrophy			1 (4%)	
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, cyst	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Pars distalis, hyperplasia		7 (14%)	2 (4%)	6 (12%)
Thyroid gland	(50)	(50)	(48)	(49)
Cyst		1 (2%)	2 (4%)	1 (2%)
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Epididymis	(49)	(50)	(50)	(50)
Granuloma sperm		1 (2%)		2 (4%)
Penis	(0)	(0)	(0)	(2)
Inflammation, suppurative				2 (100%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Genital System (continued)				
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Ectasia				2 (4%)
Inflammation	2 (4%)	1 (2%)	1 (2%)	
Prostate	(50)	(50)	(50)	(49)
Inflammation				1 (2%)
Arteriole, inflammation, chronic				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	1 (2%)	2 (4%)	1 (2%)	
Inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Testes	(50)	(50)	(50)	(50)
Degeneration		2 (4%)	1 (2%)	
Hyperplasia, atypical	1 (2%)			
Mineralization		1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Lymph node	(0)	(5)	(5)	(0)
Renal, hyperplasia, lymphoid			1 (20%)	
Lymph node, bronchial	(20)	(29)	(25)	(25)
Congestion	1 (5%)			
Lymph node, mandibular	(16)	(20)	(18)	(20)
Lymph node, mediastinal	(33)	(35)	(36)	(38)
Hematopoietic cell proliferation	1 (3%)			1 (3%)
Hyperplasia, lymphoid				1 (3%)
Infiltration cellular, mixed cell	1 (3%)			
Lymph node, mesenteric	(49)	(47)	(48)	(50)
Hematopoietic cell proliferation	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Hyperplasia, plasma cell	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular	2 (4%)	3 (6%)	1 (2%)	
Spleen	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)			
Hematopoietic cell proliferation		2 (4%)	3 (6%)	2 (4%)
Infiltration cellular, histiocyte	1 (2%)			
Necrosis			1 (2%)	
Thymus	(38)	(36)	(36)	(37)
Cyst	1 (3%)		2 (6%)	2 (5%)
Hyperplasia, lymphoid				1 (3%)
Integumentary System				
Skin	(50)	(50)	(49)	(50)
Inflammation		1 (2%)		3 (6%)
Ulcer	1 (2%)	3 (6%)	5 (10%)	3 (6%)
Epidermis, hyperplasia			1 (2%)	
Hair follicle, congestion		1 (2%)		
Sebaceous gland, hyperplasia			1 (2%)	
Subcutaneous tissue, fibrosis			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(49)	(50)
Joint, inflammation, chronic		1 (2%)		
Skeletal muscle	(2)	(3)	(3)	(1)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hydrocephalus			1 (2%)	
Meninges, infiltration cellular	1 (2%)			1 (2%)
Meninges, infiltration cellular, mixed cell				1 (2%)
Peripheral nerve	(0)	(1)	(1)	(0)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Arteriole, infiltration cellular, mixed cell				1 (2%)
Squamous epithelium, hyperplasia				1 (2%)
Lung	(50)	(50)	(50)	(50)
Hemorrhage	3 (6%)		2 (4%)	1 (2%)
Infiltration cellular, histiocyte	12 (24%)	1 (2%)	7 (14%)	3 (6%)
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	3 (6%)	2 (4%)	5 (10%)	2 (4%)
Arteriole, inflammation, chronic active		2 (4%)	1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	6 (12%)	5 (10%)	6 (12%)	14 (28%)
Glands, respiratory epithelium, accumulation, hyaline droplet	5 (10%)	5 (10%)	16 (32%)	33 (66%)
Glands, respiratory epithelium, hyperplasia	42 (84%)	41 (82%)	44 (88%)	50 (100%)
Glands, respiratory epithelium, inflammation, chronic active	6 (12%)	9 (18%)	8 (16%)	11 (22%)
Olfactory epithelium, accumulation, hyaline droplet	7 (14%)	2 (4%)	4 (8%)	6 (12%)
Olfactory epithelium, atrophy	9 (18%)	19 (38%)	50 (100%)	50 (100%)
Olfactory epithelium, necrosis		2 (4%)		
Olfactory epithelium, respiratory metaplasia	14 (28%)	15 (30%)	44 (88%)	50 (100%)
Olfactory epithelium, ulcer		1 (2%)		
Olfactory epithelium, vacuolization cytoplasmic		5 (10%)	3 (6%)	
Respiratory epithelium, accumulation, hyaline droplet	11 (22%)	6 (12%)	19 (38%)	30 (60%)
Respiratory epithelium, inflammation, suppurative	1 (2%)			
Respiratory epithelium, metaplasia, squamous	4 (8%)	7 (14%)	16 (32%)	34 (68%)
Respiratory epithelium, necrosis	2 (4%)	3 (6%)	3 (6%)	8 (16%)
Respiratory epithelium, ulcer	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Respiratory epithelium, vacuolization cytoplasmic		1 (2%)	3 (6%)	
Turbinate, hyperostosis	5 (10%)	23 (46%)	50 (100%)	50 (100%)
Turbinate, necrosis	1 (2%)			3 (6%)
Pleura	(1)	(0)	(0)	(0)
Trachea	(50)	(50)	(50)	(50)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	1 (2%)
Cataract			2 (4%)	
Cornea, hyperplasia, squamous			1 (2%)	1 (2%)
Cornea, inflammation, chronic active		2 (4%)		2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia		1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Casts granular	1 (2%)			
Cyst	4 (8%)	6 (12%)	6 (12%)	5 (10%)
Hydronephrosis	1 (2%)		1 (2%)	
Infarct	1 (2%)	4 (8%)	4 (8%)	2 (4%)
Metaplasia, osseous	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Nephropathy	44 (88%)	47 (94%)	44 (90%)	45 (90%)
Thrombosis			1 (2%)	
Artery, inflammation, chronic active	1 (2%)			1 (2%)
Vein, dilatation	1 (2%)			
Urethra	(0)	(0)	(1)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Inflammation			1 (2%)	1 (2%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF DIETHYLAMINE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	13	11	7	10
Natural deaths	5	4	7	1
Survivors				
Terminal sacrifice	32	35	36	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(38)	(45)	(44)	(42)
Intestine large, cecum	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)			
Polyp adenomatous		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(49)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Sarcoma				1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Polyp adenomatous			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		
Hepatocellular adenoma	8 (16%)	14 (28%)	14 (28%)	10 (20%)
Hepatocellular adenoma, multiple	6 (12%)	5 (10%)	3 (6%)	
Hepatocellular carcinoma	2 (4%)	5 (10%)	6 (12%)	1 (2%)
Hepatocellular carcinoma, multiple	2 (4%)		1 (2%)	1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle				1 (2%)
Mesentery	(12)	(16)	(15)	(10)
Carcinoma, metastatic, urinary bladder				1 (10%)
Hemangiosarcoma	2 (17%)			1 (10%)
Rhabdomyosarcoma, metastatic, skeletal muscle				1 (10%)
Sarcoma, metastatic, intestine small, ileum				1 (10%)
Sarcoma, metastatic, skin	1 (8%)		1 (7%)	
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma, metastatic, urinary bladder				1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle				1 (2%)
Sarcoma, metastatic, intestine small, ileum				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin			1 (2%)	
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma			1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(2)	(0)	(1)	(0)
Heart	(50)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Subcapsular, adenoma		1 (2%)		2 (4%)
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma benign		1 (2%)		1 (2%)
Pheochromocytoma malignant		1 (2%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Parathyroid gland	(23)	(32)	(33)	(33)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	10 (20%)	5 (10%)	6 (12%)	7 (14%)
Pars distalis, carcinoma		1 (2%)		
Pars distalis, schwannoma malignant, metastatic, uncertain primary site			1 (2%)	
Pars intermedia, adenoma	2 (4%)	1 (2%)		1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	
General Body System				
Peritoneum	(0)	(0)	(0)	(1)
Genital System				
Clitoral gland	(46)	(43)	(43)	(46)
Sarcoma, metastatic, skin	1 (2%)	1 (2%)		
Ovary	(49)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland	1 (2%)			
Cystadenocarcinoma		1 (2%)		
Cystadenoma	3 (6%)	2 (4%)		
Hemangiosarcoma	1 (2%)			
Luteoma		1 (2%)	1 (2%)	1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle				1 (2%)
Periovarian tissue, carcinoma, metastatic, urinary bladder				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Cystadenocarcinoma, metastatic, ovary		1 (2%)		
Hemangiosarcoma				1 (2%)
Leiomyoma		1 (2%)		1 (2%)
Polyp stromal	1 (2%)	2 (4%)	2 (4%)	
Sarcoma, metastatic, intestine small, ileum				1 (2%)
Sarcoma stromal		1 (2%)		
Vagina	(0)	(0)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		
Lymph node	(5)	(6)	(11)	(5)
Iliac, hemangiosarcoma		1 (17%)		
Lymph node, bronchial	(29)	(32)	(31)	(38)
Carcinoma, metastatic, mammary gland	1 (3%)			
Hemangiosarcoma	1 (3%)			
Lymph node, mandibular	(23)	(40)	(30)	(33)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (4%)			
Mast cell tumor malignant, metastatic, uncertain primary site				1 (3%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Hematopoietic System (continued)				
Lymph node, mediastinal	(34)	(33)	(44)	(40)
Sarcoma, metastatic, intestine small, ileum				1 (3%)
Sarcoma, metastatic, skin	1 (3%)		1 (2%)	
Lymph node, mesenteric	(50)	(46)	(46)	(49)
Carcinoma, metastatic, urinary bladder				1 (2%)
Hemangiosarcoma	1 (2%)			
Sarcoma, metastatic, intestine small, ileum				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)			
Capsule, carcinoma, metastatic, urinary bladder				1 (2%)
Capsule, rhabdomyosarcoma, metastatic, skeletal muscle				1 (2%)
Thymus	(47)	(47)	(45)	(47)
Sarcoma, metastatic, intestine small, ileum				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Carcinoma	4 (8%)	1 (2%)	2 (4%)	
Fibroadenoma	1 (2%)			
Sarcoma, metastatic, skin		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)	1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, sarcoma	3 (6%)	1 (2%)	5 (10%)	2 (4%)
Subcutaneous tissue, sarcoma, multiple	1 (2%)		1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(49)
Sarcoma, metastatic, skin	1 (2%)			
Cranium, schwannoma malignant, metastatic, uncertain primary site			1 (2%)	
Skeletal muscle	(1)	(2)	(2)	(3)
Carcinoma, metastatic, urinary bladder				1 (33%)
Rhabdomyosarcoma		1 (50%)		1 (33%)
Sarcoma, metastatic, skin	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, carcinoma, metastatic, pituitary gland		1 (2%)		
Meninges, schwannoma malignant, metastatic, uncertain primary site			1 (2%)	
Peripheral nerve	(0)	(2)	(0)	(1)
Spinal cord	(1)	(1)	(0)	(1)
Sarcoma, metastatic, skin	1 (100%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	3 (6%)	1 (2%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Carcinoma, metastatic, mammary gland	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Sarcoma, metastatic, skin			1 (2%)	
Mediastinum, hemangiosarcoma	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)		5 (10%)
Carcinoma	2 (4%)	1 (2%)		4 (8%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(49)	(50)
Transitional epithelium, carcinoma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymphoma malignant	8 (16%)	15 (30%)	15 (30%)	13 (26%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	40	40	41
Total primary neoplasms	75	75	68	62
Total animals with benign neoplasms	25	28	26	26
Total benign neoplasms	38	40	32	34
Total animals with malignant neoplasms	23	28	29	26
Total malignant neoplasms	37	35	36	28
Total animals with metastatic neoplasms	7	5	5	5
Total metastatic neoplasms	13	6	9	19
Total animals with malignant neoplasms of uncertain primary site			1	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

[b] Number of animals with any tissue examined microscopically

[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted rate[b]	6.9%	2.3%	0.0%	10.5%
Terminal rate[c]	3/32 (9%)	1/35 (3%)	0/36 (0%)	3/39 (8%)
First incidence (days)	731 (T)	731 (T)	—[e]	610
Poly-3 test[d]	P=0.180	P=0.307N	P=0.103N	P=0.410
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
Adjusted rate	4.6%	2.3%	0.0%	8.5%
Terminal rate	0/32 (0%)	1/35 (3%)	0/36 (0%)	4/39 (10%)
First incidence (days)	712	731 (T)	—	731 (T)
Poly-3 test	P=0.179	P=0.502N	P=0.218N	P=0.376
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	0/50 (0%)	9/50 (18%)
Adjusted rate	11.5%	4.6%	0.0%	18.8%
Terminal rate	3/32 (9%)	2/35 (6%)	0/36 (0%)	7/39 (18%)
First incidence (days)	712	731 (T)	—	610
Poly-3 test	P=0.062	P=0.218N	P=0.024N	P=0.248
Liver: Hepatocellular Adenoma				
Overall rate	14/50 (28%)	19/50 (38%)	17/50 (34%)	10/50 (20%)
Adjusted rate	31.4%	41.9%	35.5%	20.8%
Terminal rate	11/32 (34%)	14/35 (40%)	14/36 (39%)	7/39 (18%)
First incidence (days)	592	444	659	653
Poly-3 test	P=0.070N	P=0.205	P=0.423	P=0.178N
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	7/50 (14%)	2/50 (4%)
Adjusted rate	9.2%	11.3%	14.7%	4.2%
Terminal rate	4/32 (13%)	3/35 (9%)	6/36 (17%)	1/39 (3%)
First incidence (days)	731 (T)	533	689	719
Poly-3 test	P=0.220N	P=0.513	P=0.317	P=0.298N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	16/50 (32%)	23/50 (46%)	22/50 (44%)	11/50 (22%)
Adjusted rate	35.9%	49.6%	45.8%	22.9%
Terminal rate	13/32 (41%)	16/35 (46%)	18/36 (50%)	8/39 (21%)
First incidence (days)	592	444	659	653
Poly-3 test	P=0.037N	P=0.131	P=0.224	P=0.125N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.6%	6.9%	4.2%	8.4%
Terminal rate	2/32 (6%)	2/35 (6%)	2/36 (6%)	3/39 (8%)
First incidence (days)	731 (T)	649	731 (T)	610
Poly-3 test	P=0.327	P=0.501	P=0.661N	P=0.382
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.9%	2.3%	8.4%	2.1%
Terminal rate	2/32 (6%)	0/35 (0%)	4/36 (11%)	1/39 (3%)
First incidence (days)	695	600	731 (T)	731 (T)
Poly-3 test	P=0.299N	P=0.304N	P=0.547	P=0.277N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	6/50 (12%)	5/50 (10%)
Adjusted rate	11.5%	9.1%	12.6%	10.5%
Terminal rate	4/32 (13%)	2/35 (6%)	6/36 (17%)	4/39 (10%)
First incidence (days)	695	600	731 (T)	610
Poly-3 test	P=0.557N	P=0.494N	P=0.560	P=0.572N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	9.0%	2.3%	4.2%	0.0%
Terminal rate	1/32 (3%)	1/35 (3%)	2/36 (6%)	0/39 (0%)
First incidence (days)	584	731 (T)	731 (T)	—
Poly-3 test	P=0.043N	P=0.186N	P=0.306N	P=0.053N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	5/50 (10%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	11.1%	2.3%	4.2%	0.0%
Terminal rate	1/32 (3%)	1/35 (3%)	2/36 (6%)	0/39 (0%)
First incidence (days)	465	731 (T)	731 (T)	—
Poly-3 test	P=0.020N	P=0.111N	P=0.196N	P=0.027N
Ovary: Cystadenoma				
Overall rate	3/49 (6%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.1%	4.6%	0.0%	0.0%
Terminal rate	3/32 (9%)	2/35 (6%)	0/36 (0%)	0/39 (0%)
First incidence (days)	731 (T)	731 (T)	—	—
Poly-3 test	P=0.034N	P=0.492N	P=0.100N	P=0.100N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	10/50 (20%)	5/50 (10%)	6/50 (12%)	7/49 (14%)
Adjusted rate	22.3%	11.6%	12.5%	15.1%
Terminal rate	7/32 (22%)	5/35 (14%)	4/36 (11%)	7/38 (18%)
First incidence (days)	465	731 (T)	680	731 (T)
Poly-3 test	P=0.301N	P=0.144N	P=0.167N	P=0.270N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	10/50 (20%)	6/50 (12%)	6/50 (12%)	7/49 (14%)
Adjusted rate	22.3%	13.9%	12.5%	15.1%
Terminal rate	7/32 (22%)	6/35 (17%)	4/36 (11%)	7/38 (18%)
First incidence (days)	465	731 (T)	680	731 (T)
Poly-3 test	P=0.269N	P=0.226N	P=0.167N	P=0.270N
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	6/50 (12%)	2/50 (4%)
Adjusted rate	9.0%	2.3%	12.5%	4.2%
Terminal rate	0/32 (0%)	0/35 (0%)	3/36 (8%)	1/39 (3%)
First incidence (days)	599	551	676	653
Poly-3 test	P=0.384N	P=0.183N	P=0.417	P=0.306N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Sarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	6/50 (12%)	2/50 (4%)
Adjusted rate	9.0%	4.5%	12.5%	4.2%
Terminal rate	0/32 (0%)	0/35 (0%)	3/36 (8%)	1/39 (3%)
First incidence (days)	599	551	676	653
Poly-3 test	P=0.337N	P=0.338N	P=0.417	P=0.306N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	2.3%	6.9%	4.2%	0.0%
Terminal rate	1/32 (3%)	3/35 (9%)	1/36 (3%)	0/39 (0%)
First incidence (days)	731 (T)	731 (T)	676	—
Poly-3 test	P=0.212N	P=0.303	P=0.533	P=0.483N
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	11.4%	6.9%	0.0%	4.2%
Terminal rate	4/32 (13%)	2/35 (6%)	0/36 (0%)	2/39 (5%)
First incidence (days)	599	649	—	731 (T)
Poly-3 test	P=0.102N	P=0.360N	P=0.024N	P=0.187N
All Organs: Malignant Lymphoma				
Overall rate	8/50 (16%)	15/50 (30%)	15/50 (30%)	13/50 (26%)
Adjusted rate	17.4%	33.7%	31.1%	27.3%
Terminal rate	1/32 (3%)	12/35 (34%)	9/36 (25%)	11/39 (28%)
First incidence (days)	522	551	680	684
Poly-3 test	P=0.278	P=0.059	P=0.094	P=0.184
All Organs: Benign Neoplasms				
Overall rate	25/50 (50%)	28/50 (56%)	26/50 (52%)	26/50 (52%)
Adjusted rate	55.0%	61.4%	53.7%	53.7%
Terminal rate	20/32 (63%)	22/35 (63%)	20/36 (56%)	22/39 (56%)
First incidence (days)	465	444	659	610
Poly-3 test	P=0.394N	P=0.338	P=0.534N	P=0.535N
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	28/50 (56%)	30/50 (60%)	27/50 (54%)
Adjusted rate	48.9%	58.8%	61.6%	54.9%
Terminal rate	11/32 (34%)	19/35 (54%)	20/36 (56%)	19/39 (49%)
First incidence (days)	522	444	676	626
Poly-3 test	P=0.381	P=0.223	P=0.146	P=0.352
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	40/50 (80%)	41/50 (82%)	42/50 (84%)
Adjusted rate	84.7%	83.1%	83.7%	84.2%
Terminal rate	26/32 (81%)	29/35 (83%)	29/36 (81%)	32/39 (82%)
First incidence (days)	465	444	659	610
Poly-3 test	P=0.548N	P=0.526N	P=0.559N	P=0.581N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Diethylamine[a]

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	13	11	7	10
Natural deaths	5	4	7	1
Survivors				
Terminal sacrifice	32	35	36	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(38)	(45)	(44)	(42)
Cyst				1 (2%)
Hyperplasia		1 (2%)		
Mineralization		1 (2%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Amyloid deposition		1 (2%)		
Hemorrhage		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Polyp, inflammatory	1 (2%)			
Arteriole, inflammation, chronic active			1 (2%)	
Intestine small, duodenum	(50)	(50)	(49)	(50)
Necrosis				1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	
Basophilic focus	1 (2%)	3 (6%)	6 (12%)	1 (2%)
Clear cell focus	1 (2%)		2 (4%)	
Cyst	1 (2%)	2 (4%)		
Degeneration, fatty	1 (2%)	1 (2%)		
Eosinophilic focus	4 (8%)	7 (14%)	8 (16%)	2 (4%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)		1 (2%)
Infiltration cellular, lymphoid	1 (2%)			
Inflammation, chronic active	1 (2%)			1 (2%)
Mixed cell focus			2 (4%)	2 (4%)
Necrosis	1 (2%)	2 (4%)	3 (6%)	3 (6%)
Pigmentation				1 (2%)
Tension lipidosis	5 (10%)	8 (16%)	3 (6%)	9 (18%)
Thrombosis	1 (2%)			
Vacuolization cytoplasmic		1 (2%)		
Bile duct, hyperplasia				1 (2%)
Mesentery	(12)	(16)	(15)	(10)
Inflammation, chronic active		1 (6%)		
Fat, necrosis	10 (83%)	12 (75%)	11 (73%)	6 (60%)
Oral mucosa	(1)	(0)	(0)	(0)
Gingival, inflammation, suppurative	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Cyst	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Cytoplasmic alteration	1 (2%)			
Salivary glands	(49)	(50)	(50)	(50)
Necrosis		1 (2%)		
Arteriole, inflammation, chronic active			1 (2%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous			4 (8%)	1 (2%)
Inflammation	2 (4%)			
Mineralization			1 (2%)	1 (2%)
Ulcer		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	
Mineralization		1 (2%)		
Epithelium, degeneration, hyaline	1 (2%)			
Tooth	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(2)	(0)	(1)	(0)
Aorta, mineralization			1 (100%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	5 (10%)	2 (4%)	3 (6%)	1 (2%)
Congestion			1 (2%)	
Mineralization		1 (2%)		
Artery, inflammation		2 (4%)		1 (2%)
Capillary, hyperplasia	2 (4%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)		3 (6%)	1 (2%)
Angiectasis	1 (2%)			1 (2%)
Atrophy		1 (2%)	1 (2%)	
Hematopoietic cell proliferation		2 (4%)	1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia	5 (10%)	3 (6%)	8 (16%)	7 (14%)
Hypertrophy	10 (20%)	13 (26%)	7 (14%)	8 (16%)
Vacuolization cytoplasmic			3 (6%)	
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia				3 (6%)
Hypertrophy	1 (2%)			1 (2%)
Vacuolization cytoplasmic			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Parathyroid gland	(23)	(32)	(33)	(33)
Cyst		1 (3%)	2 (6%)	
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, angiectasis	1 (2%)		4 (8%)	2 (4%)
Pars distalis, cyst			3 (6%)	2 (4%)
Pars distalis, hyperplasia	8 (16%)	15 (30%)	16 (32%)	10 (20%)
Pars intermedia, hemorrhage	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Cyst		2 (4%)	7 (14%)	3 (6%)
Follicular cell, hyperplasia	2 (4%)		1 (2%)	
General Body System				
Peritoneum	(0)	(0)	(0)	(1)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Genital System				
Clitoral gland	(46)	(43)	(43)	(46)
Ovary	(49)	(50)	(50)	(50)
Cyst	8 (16%)	11 (22%)	7 (14%)	9 (18%)
Hemorrhage	2 (4%)			2 (4%)
Infiltration cellular, histiocyte	1 (2%)			
Mineralization		1 (2%)		
Thrombosis		1 (2%)		1 (2%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Fibrosis	1 (2%)			
Inflammation, chronic active	1 (2%)		2 (4%)	
Thrombosis	2 (4%)			3 (6%)
Arteriole, inflammation, chronic active		1 (2%)		
Endometrium, hyperplasia, cystic	26 (52%)	18 (36%)	21 (42%)	27 (54%)
Vagina	(0)	(0)	(1)	(0)
Arteriole, inflammation, chronic active			1 (100%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Myelofibrosis		1 (2%)		
Lymph node	(5)	(6)	(11)	(5)
Iliac, ectasia	1 (20%)			
Lumbar, ectasia			1 (9%)	
Lumbar, hemorrhage	1 (20%)	1 (17%)		
Lumbar, hyperplasia, lymphoid				1 (20%)
Renal, hemorrhage			1 (9%)	
Lymph node, bronchial	(29)	(32)	(31)	(38)
Lymph node, mandibular	(23)	(40)	(30)	(33)
Ectasia		1 (3%)		1 (3%)
Hyperplasia, lymphoid	2 (9%)			1 (3%)
Lymph node, mediastinal	(34)	(33)	(44)	(40)
Hyperplasia, lymphoid	1 (3%)		1 (2%)	
Lymph node, mesenteric	(50)	(46)	(46)	(49)
Angiectasis				1 (2%)
Ectasia			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	2 (4%)		1 (2%)	
Necrosis			1 (2%)	
Thymus	(47)	(47)	(45)	(47)
Cyst	1 (2%)			
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Inflammation	1 (2%)		3 (6%)	
Ulcer	1 (2%)	2 (4%)		
Sebaceous gland, hyperplasia				1 (2%)
Subcutaneous tissue, metaplasia, osseous				1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Musculoskeletal System				
Bone	(49)	(50)	(50)	(49)
Cranium, hyperostosis		1 (2%)	1 (2%)	
Joint, hyperostosis	1 (2%)			
Skeletal muscle	(1)	(2)	(2)	(3)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Atrophy	2 (4%)	1 (2%)	1 (2%)	
Hydrocephalus			1 (2%)	
Hippocampus, necrosis, acute		1 (2%)		
Meninges, infiltration cellular	1 (2%)	1 (2%)		
Meninges, infiltration cellular, mononuclear cell				1 (2%)
Peripheral nerve	(0)	(2)	(0)	(1)
Infiltration cellular, lymphocyte				1 (100%)
Spinal cord	(1)	(1)	(0)	(1)
Meninges, infiltration cellular, mononuclear cell				1 (100%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Atypia cellular			1 (2%)	
Hyperplasia, squamous	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Lung	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hemorrhage		1 (2%)		1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Infiltration cellular, histiocyte	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Inflammation, suppurative	1 (2%)			
Metaplasia, osseous	1 (2%)			
Thrombosis	1 (2%)	1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia		1 (2%)	3 (6%)	1 (2%)
Arteriole, inflammation, chronic active				1 (2%)
Bronchiole, degeneration, hyaline	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Inflammation, suppurative	2 (4%)	1 (2%)	3 (6%)	9 (18%)
Glands, respiratory epithelium, accumulation, hyaline droplet	16 (32%)	28 (57%)	45 (90%)	42 (84%)
Glands, respiratory epithelium, hyperplasia	43 (86%)	45 (92%)	47 (94%)	50 (100%)
Glands, respiratory epithelium, inflammation, chronic active	8 (16%)	11 (22%)	16 (32%)	22 (44%)
Olfactory epithelium, accumulation, hyaline droplet	11 (22%)	19 (39%)	8 (16%)	17 (34%)
Olfactory epithelium, atrophy	8 (16%)	29 (59%)	49 (98%)	50 (100%)
Olfactory epithelium, necrosis			2 (4%)	1 (2%)
Olfactory epithelium, respiratory metaplasia	4 (8%)	15 (31%)	48 (96%)	50 (100%)
Olfactory epithelium, vacuolization cytoplasmic		5 (10%)	1 (2%)	1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Diethylamine

	Chamber Control	16 ppm	31 ppm	62.5 ppm
Respiratory System (continued)				
Nose (continued)	(50)	(49)	(50)	(50)
Respiratory epithelium, accumulation, hyaline droplet	20 (40%)	33 (67%)	47 (94%)	29 (58%)
Respiratory epithelium, metaplasia, squamous			13 (26%)	35 (70%)
Respiratory epithelium, necrosis	1 (2%)		6 (12%)	16 (32%)
Respiratory epithelium, ulcer				2 (4%)
Respiratory epithelium, vacuolization cytoplasmic		2 (4%)	2 (4%)	1 (2%)
Turbinate, hyperostosis	4 (8%)	23 (47%)	49 (98%)	50 (100%)
Turbinate, necrosis				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Metaplasia, osseous		1 (2%)	1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Cataract			1 (2%)	
Arteriole, thrombosis	1 (2%)			
Cornea, hyperplasia, squamous			1 (2%)	
Cornea, inflammation, chronic active	2 (4%)			1 (2%)
Cornea, mineralization	1 (2%)			
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)	2 (4%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Cyst		1 (2%)	1 (2%)	
Hydronephrosis				1 (2%)
Infarct	2 (4%)	2 (4%)	4 (8%)	6 (12%)
Metaplasia, osseous	8 (16%)	3 (6%)	1 (2%)	4 (8%)
Nephropathy	39 (78%)	40 (80%)	43 (86%)	46 (92%)
Renal tubule, necrosis		1 (2%)		
Renal tubule, pigmentation, bile				1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)
Inflammation		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing procedures used in the first study, conducted at SRI International (Menlo Park, CA), followed protocols reported by Zeiger *et al.* (1987); in the test conducted at SITEK Research Laboratories (Rockville, MD), a slightly modified procedure was used, and that is described below. Diethylamine was tested at both laboratories as a coded sample. The study conducted at SITEK Research Laboratories used the same lot of diethylamine that was used for the 2-week, 3-month, and 2-year studies (lot BE/07/01). In the tests conducted at SRI International, diethylamine was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37º C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days incubation at 37º C.

The slightly modified protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation, and employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with diethylamine and subsequent plating were carried out as described above for the traditional protocol.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of diethylamine. In the first study, doses up to 10,000 µg/plate were tested; toxicity was observed above 3,333 µg/plate. In the second study, 4,000 µg/plate was the highest dose tested. All trials were repeated, and those that were conducted with S9 activation enzymes were repeated using the same concentrations of S9.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice exposed to 8 to 125 ppm diethylamine by inhalation. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs; mature erythrocytes) in each of five animals per exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs; reticulocytes) in a population of 1,000 erythrocytes was scored for each exposure group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

Diethylamine (doses up to 10,000 µg/plate in the first study and 4,000 µg/plate in the second study) was not mutagenic in either of two independent bacterial mutagenicity assays, with or without exogenous metabolic activation (Table E1; Zeiger *et al.*, 1987). Bacterial strains tested in the first study included *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, with and without 10% induced rat or hamster liver S9 activation enzymes; in the second study, *S. typhimurium* strains TA98 and TA100 were employed, as well as *E. coli* strain WP2 *uvrA*/pKM101, with and without 10% induced rat liver S9. In addition to the negative results in the two bacterial assays, no significant increases in the frequencies of micronucleated NCEs were seen in peripheral blood of male or female mice from the 3-month study (Table E2). The percentage of reticulocytes (PCEs) in the peripheral blood of male and female mice was unaltered by diethylamine exposure, suggesting a lack of chemical-associated bone marrow toxicity.

TABLE E1
Mutagenicity of Diethylamine in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]					
		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Study performed at SRI International							
TA100	0	108 ± 3	101 ± 9	134 ± 8	117 ± 10	116 ± 8	95 ± 2
	33		122 ± 9		112 ± 3		106 ± 3
	100	158 ± 15	109 ± 4	160 ± 9	119 ± 5	152 ± 4	111 ± 3
	333	144 ± 7	121 ± 7	142 ± 9	116 ± 14	141 ± 4	119 ± 15
	1,000	153 ± 5	121 ± 10	146 ± 8	123 ± 13	141 ± 3	104 ± 11
	3,333	0 ± 0[c]	111 ± 8	104 ± 52[c]	97 ± 13	116 ± 18	102 ± 4
	10,000	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		467 ± 18	477 ± 6	2,355 ± 34	1,511 ± 50	846 ± 26	820 ± 37
TA1535	0	31 ± 1	37 ± 6	42 ± 4	14 ± 2	41 ± 5	23 ± 4
	33		26 ± 4		12 ± 2		17 ± 1
	100	22 ± 2	25 ± 1	47 ± 1	13 ± 1	40 ± 6	12 ± 2
	333	21 ± 3	25 ± 0	41 ± 8	7 ± 1	36 ± 2	13 ± 2
	1,000	31 ± 3	19 ± 1	55 ± 3	10 ± 2	42 ± 6	17 ± 5
	3,333	0 ± 0[c]	20 ± 4	30 ± 15[c]	7 ± 1	31 ± 3	14 ± 5
	10,000	0 ± 0[c]		Toxic		0 ± 0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		443 ± 29	399 ± 18	645 ± 25	563 ± 28	331 ± 14	266 ± 34
TA1537	0	11 ± 2	7 ± 2	19 ± 5	9 ± 1	14 ± 1	11 ± 1
	33		7 ± 1		6 ± 2		9 ± 0
	100	5 ± 1	7 ± 0	23 ± 3	7 ± 1	10 ± 3	17 ± 1
	333	7 ± 2	9 ± 1	27 ± 1	5 ± 1	13 ± 3	13 ± 3
	1,000	6 ± 0	10 ± 1	21 ± 7	10 ± 2	13 ± 1	15 ± 2
	3,333	0 ± 0[c]	7 ± 1	0 ± 0[c]	5 ± 1c	6 ± 2	12 ± 0
	10,000	0 ± 0[c]		Toxic		0 ± 0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		388 ± 33	205 ± 40	591 ± 17	465 ± 15	266 ± 10	241 ± 16
TA98	0	28 ± 2	21 ± 4	48 ± 6	28 ± 3	37 ± 2	36 ± 3
	33		20 ± 2		23 ± 2		31 ± 5
	100	19 ± 1	15 ± 1	46 ± 7	30 ± 4	43 ± 1	30 ± 2
	333	16 ± 1	17 ± 2	52 ± 1	24 ± 3	34 ± 5	31 ± 5
	1,000	15 ± 1	20 ± 2	57 ± 3	22 ± 3	37 ± 6	35 ± 4
	3,333	1 ± 1[c]	17 ± 1	0 ± 0[c]	23 ± 2	43 ± 4	26 ± 0
	10,000	0 ± 0[c]		Toxic		0 ± 0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		758 ± 14	722 ± 8	1,856 ± 20	1,102 ± 67	436 ± 5	591 ± 44

TABLE E1
Mutagenicity of Diethylamine in Bacterial Tester Strains

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SITEK Research Laboratories (lot BE/07/01 used in the 2-week, 3-month, and 2-year studies)					
TA100	0	61 ± 4	51 ± 6	84 ± 3	56 ± 4
	50	63 ± 7	65 ± 2	90 ± 7	51 ± 6
	100	55 ± 2	63 ± 7	105 ± 10	66 ± 1
	250		64 ± 2		
	500	54 ± 3	48 ± 4	90 ± 4	57 ± 4
	750		55 ± 5		
	1,000	Toxic		76 ± 1	47 ± 6
	2,000	Toxic		83 ± 8	63 ± 9
Trial summary		Negative	Negative	Negative	Negative
Positive control		553 ± 14	609 ± 29	768 ± 36	609 ± 29
TA 98	0	20 ± 1	20 ± 2	30 ± 1	25 ± 3
	50	17 ± 1	17 ± 1	40 ± 2	27 ± 2
	100	19 ± 0	15 ± 1	29 ± 2	22 ± 1
	250		12 ± 4		
	500	24 ± 1	13 ± 2	38 ± 2	29 ± 4
	750		13 ± 1		
	1,000	Toxic		36 ± 4	19 ± 2
	2,000	Toxic		27 ± 1	25 ± 4
Trial summary		Negative	Negative	Negative	Negative
Positive control		417 ± 15	572 ± 8	752 ± 58	1,137 ± 19
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	132 ± 9	122 ± 7	157 ± 2	139 ± 3
	50	186 ± 14	132 ± 9		155 ± 5
	100	152 ± 14	158 ± 13		172 ± 10
	500	136 ± 5	140 ± 3	201 ± 12	151 ± 5
	1,000	105 ± 5	67 ± 0	199 ± 31	161 ± 7
	1,500	150 ± 12			
	2,000	18 ± 9	0 ± 0	182 ± 5	178 ± 14
	3,000			110 ± 9	
	4,000			0 ± 0	
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,101 ± 55	812 ± 14	734 ± 7	711 ± 31

[a] The detailed protocol for the SRI International assay is presented by Zeiger *et al.* (1987); SITEK Research Laboratories used a modified version of this protocol. 0 µg/plate was the solvent control

[b] Revertants are presented as mean ± standard error from three plates

[c] Slight toxicity

[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Diethylamine by Inhalation for 3 Months[a]

Compound	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Chamber control	0	5	2.80 ± 0.30		6.300 ± 0.69
Diethylamine	8	5	4.60 ± 0.60	0.0180	7.220 ± 0.76
	16	5	4.10 ± 0.48	0.0585	6.820 ± 0.61
	32	5	3.30 ± 0.34	0.2607	7.960 ± 0.83
	62	5	4.00 ± 0.52	0.0725	6.920 ± 0.66
	125	5	2.60 ± 0.33	0.6074	5.700 ± 0.39
			P=0.915[d]		
Female					
Chamber control	0	5	2.60 ± 0.29		6.560 ± 0.09
Diethylamine	8	5	2.50 ± 0.61	0.5558	4.160 ± 0.51
	16	5	2.20 ± 0.25	0.7184	6.800 ± 1.00
	32	5	3.50 ± 0.57	0.1242	7.080 ± 0.82
	62	5	3.80 ± 0.60	0.0665	7.560 ± 1.36
	125	5	2.20 ± 0.25	0.7184	6.240 ± 1.11
			P=0.519		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte

[b] Mean ± standard error

[c] Pairwise comparison with the chamber control group; significant at P≤0.005

[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025.

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	46.5 ± 0.6	45.9 ± 0.4	46.2 ± 0.5	46.0 ± 0.4	46.0 ± 0.6	47.4 ± 0.3
Day 23	48.2 ± 0.4	47.6 ± 0.7	47.0 ± 0.3	47.4 ± 0.3	47.0 ± 0.3	48.5 ± 0.3
Week 14	48.7 ± 0.5	48.8 ± 0.3	48.9 ± 0.5	48.7 ± 0.4	48.2 ± 0.3	49.1 ± 0.4
Packed cell volume (mL/dL)						
Day 3	45.7 ± 0.6	44.7 ± 0.4	45.2 ± 0.3	44.8 ± 0.3	44.8 ± 0.6	46.2 ± 0.4
Day 23	46.9 ± 0.5	46.5 ± 0.7	46.1 ± 0.2	45.9 ± 0.2	45.9 ± 0.4	47.0 ± 0.3
Week 14	47.8 ± 0.6	48.1 ± 0.4	48.2 ± 0.3	48.1 ± 0.2	47.7 ± 0.4	48.0 ± 0.3
Hemoglobin (g/dL)						
Day 3	14.1 ± 0.2	14.0 ± 0.1	14.2 ± 0.1	14.0 ± 0.2	14.0 ± 0.2	14.6 ± 0.2
Day 23	15.2 ± 0.2	15.0 ± 0.2	14.8 ± 0.1	14.8 ± 0.1	14.7 ± 0.1	15.2 ± 0.1
Week 14	15.5 ± 0.1	15.6 ± 0.1	15.7 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.7 ± 0.1
Erythrocytes (10^6/μL)						
Day 3	7.39 ± 0.14	7.25 ± 0.08	7.44 ± 0.08	7.36 ± 0.09	7.38 ± 0.14	7.70 ± 0.08
Day 23	8.05 ± 0.13	7.94 ± 0.14	7.88 ± 0.08	7.85 ± 0.06	7.82 ± 0.08	8.03 ± 0.06
Week 14	8.98 ± 0.09	9.05 ± 0.05	9.06 ± 0.06	9.06 ± 0.04	9.01 ± 0.05	9.08 ± 0.07
Reticulocytes (10^6/μL)						
Day 3	0.32 ± 0.02	0.29 ± 0.02	0.28 ± 0.02	0.31 ± 0.02	0.27 ± 0.01	0.30 ± 0.02
Day 23	0.21 ± 0.01	0.20 ± 0.02	0.20 ± 0.01	0.24 ± 0.01*	0.28 ± 0.02**	0.26 ± 0.02*
Week 14	0.15 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.16 ± 0.01	0.19 ± 0.02
Nucleated erythrocytes/100 leukocytes						
Day 3	1.1 ± 0.4	0.5 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.1 ± 0.3	0.4 ± 0.2
Day 23	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.2	0.4 ± 0.2
Week 14	0.2 ± 0.1	0.5 ± 0.2	0.4 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
Mean cell volume (fL)						
Day 3	61.9 ± 0.4	61.7 ± 0.5	60.8 ± 0.4	60.9 ± 0.5	60.7 ± 0.4	60.1 ± 0.4*
Day 23	58.3 ± 0.4	58.5 ± 0.3	58.5 ± 0.5	58.5 ± 0.4	58.6 ± 0.3	58.6 ± 0.4
Week 14	53.3 ± 0.2	53.1 ± 0.2	53.2 ± 0.1	53.1 ± 0.2	53.0 ± 0.2	52.9 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	19.1 ± 0.1	19.3 ± 0.1	19.1 ± 0.1	19.0 ± 0.1	19.0 ± 0.1	18.9 ± 0.1*
Day 23	18.8 ± 0.1	18.9 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.9 ± 0.1
Week 14	17.3 ± 0.1	17.2 ± 0.0	17.3 ± 0.1	17.2 ± 0.1	17.2 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	30.9 ± 0.2	31.3 ± 0.2	31.4 ± 0.2	31.2 ± 0.2	31.3 ± 0.2	31.5 ± 0.2
Day 23	32.3 ± 0.1	32.3 ± 0.1	32.1 ± 0.1	32.2 ± 0.2	32.1 ± 0.2	32.3 ± 0.1
Week 14	32.5 ± 0.2	32.4 ± 0.1	32.4 ± 0.1	32.5 ± 0.1	32.4 ± 0.1	32.6 ± 0.1
Platelets (10^3/μL)						
Day 3	928.4 ± 34.3	902.1 ± 13.3	908.5 ± 20.7	891.1 ± 23.5	903.3 ± 26.8	865.8 ± 17.6
Day 23	744.1 ± 26.6	766.0 ± 18.4	806.2 ± 14.1	819.2 ± 16.6	803.2 ± 17.5	830.0 ± 15.1**
Week 14	672.8 ± 11.5	629.7 ± 28.1	679.9 ± 12.9	642.3 ± 14.7	652.3 ± 7.7	624.1 ± 14.6
Leukocytes (10^3/μL)						
Day 3	11.25 ± 0.62	11.34 ± 0.75	11.13 ± 0.64	10.04 ± 0.51	9.17 ± 0.57*	9.00 ± 0.43**
Day 23	8.37 ± 0.54	8.65 ± 0.53	8.62 ± 0.53	8.63 ± 0.35	9.75 ± 0.66	8.28 ± 0.41
Week 14	8.50 ± 0.58	8.32 ± 0.59	8.78 ± 0.62	8.56 ± 0.47	8.43 ± 0.54	8.73 ± 0.23
Segmented neutrophils (10^3/μL)						
Day 3	1.30 ± 0.05	1.21 ± 0.07	1.24 ± 0.06	1.15 ± 0.07	1.09 ± 0.04*	1.07 ± 0.09*
Day 23	1.34 ± 0.09	1.20 ± 0.05	1.40 ± 0.11	1.51 ± 0.05	1.60 ± 0.08	1.42 ± 0.09
Week 14	1.48 ± 0.07	1.29 ± 0.09	1.47 ± 0.15	1.18 ± 0.08	1.45 ± 0.14	1.48 ± 0.06
Bands (10^3/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Male (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Lymphocytes (10^3/μL)						
Day 3	9.69 ± 0.60	9.90 ± 0.71	9.64 ± 0.57	8.66 ± 0.45	7.87 ± 0.54*	7.75 ± 0.36*
Day 23	6.86 ± 0.46	7.29 ± 0.50	7.06 ± 0.55	6.97 ± 0.36	7.98 ± 0.59	6.72 ± 0.41
Week 14	6.78 ± 0.51	6.81 ± 0.54	7.07 ± 0.50	7.17 ± 0.44	6.75 ± 0.47	6.92 ± 0.24
Monocytes (10^3/μL)						
Day 3	0.09 ± 0.02	0.10 ± 0.03	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.12 ± 0.03
Day 23	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
Week 14	0.13 ± 0.06	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.17 ± 0.07
Basophils (10^3/μL)						
Day 3	0.011 ± 0.003	0.004 ± 0.002	0.011 ± 0.003	0.006 ± 0.002	0.011 ± 0.002	0.004 ± 0.002
Day 23	0.004 ± 0.002	0.012 ± 0.008	0.003 ± 0.002	0.006 ± 0.002	0.004 ± 0.002	0.006 ± 0.002
Week 14	0.011 ± 0.008	0.003 ± 0.002	0.002 ± 0.001	0.001 ± 0.001	0.009 ± 0.008	0.006 ± 0.002
Eosinophils (10^3/μL)						
Day 3	0.15 ± 0.03	0.12 ± 0.03	0.15 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.07 ± 0.01**
Day 23	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.02	0.09 ± 0.02
Week 14	0.09 ± 0.02	0.11 ± 0.02	0.13 ± 0.03	0.11 ± 0.03	0.14 ± 0.03	0.17 ± 0.01*
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	9.8 ± 0.7	8.7 ± 0.2	10.3 ± 0.6	8.2 ± 0.2	9.0 ± 0.4	9.4 ± 0.4
Day 23	10.5 ± 0.5	9.6 ± 0.4	10.3 ± 0.3	9.2 ± 0.4	9.9 ± 0.3	10.1 ± 0.3
Week 14	15.0 ± 0.3	15.0 ± 0.5	15.4 ± 0.4	15.7 ± 0.3	15.7 ± 0.4	17.0 ± 0.5**
Creatinine (mg/dL)						
Day 3	0.25 ± 0.02	0.24 ± 0.02	0.27 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.02
Day 23	0.51 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.56 ± 0.02	0.54 ± 0.02	0.55 ± 0.02
Week 14	0.57 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	0.59 ± 0.02	0.52 ± 0.03	0.54 ± 0.02
Total protein (g/dL)						
Day 3	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.0	6.0 ± 0.1
Day 23	6.5 ± 0.1	6.5 ± 0.1	6.3 ± 0.0	6.3 ± 0.0	6.3 ± 0.1	6.5 ± 0.1
Week 14	7.2 ± 0.1	7.2 ± 0.1	7.3 ± 0.0	7.2 ± 0.0	7.2 ± 0.1	7.0 ± 0.1**
Albumin (g/dL)						
Day 3	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.4 ± 0.1
Day 23	4.6 ± 0.0	4.5 ± 0.0	4.4 ± 0.0**	4.5 ± 0.0	4.4 ± 0.0**	4.5 ± 0.0
Week 14	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.7 ± 0.0	4.6 ± 0.0*
Globulin (g/dL)						
Day 3	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.1	1.6 ± 0.0
Day 23	2.0 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Week 14	2.5 ± 0.0	2.5 ± 0.0	2.6 ± 0.0	2.5 ± 0.0	2.5 ± 0.0	2.4 ± 0.0*
Albumin/globulin ratio						
Day 3	2.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.0	2.7 ± 0.1	2.9 ± 0.1	2.8 ± 0.0
Day 23	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.0	2.4 ± 0.0	2.2 ± 0.0	2.3 ± 0.0
Week 14	1.9 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.0
Alkaline phosphatase (IU/L)						
Day 3	685 ± 19	678 ± 11	657 ± 15	651 ± 23	657 ± 11	620 ± 11**
Day 23	447 ± 14	431 ± 17	437 ± 11	442 ± 15	455 ± 15	465 ± 9
Week 14	246 ± 7	254 ± 4	261 ± 6	252 ± 4	246 ± 5	261 ± 6

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Male (continued)						
n	10	10	10	10	10	10
Clinical Chemistry (continued)						
Creatine kinase (IU/L)						
Day 3	446 ± 54	360 ± 22	546 ± 73	401 ± 38	455 ± 32	451 ± 34
Day 23	336 ± 38	318 ± 30	352 ± 34	286 ± 20	339 ± 33	370 ± 30
Week 14	236 ± 22	184 ± 13	185 ± 17	181 ± 21	170 ± 15	168 ± 15
Sorbitol dehydrogenase (IU/L)						
Day 3	15 ± 1	15 ± 1	15 ± 1	15 ± 0	17 ± 1	13 ± 1
Day 23	16 ± 1	17 ± 1	15 ± 1	17 ± 1	15 ± 1	16 ± 1
Week 14	20 ± 1	21 ± 1	22 ± 1	20 ± 1	20 ± 1	20 ± 1
Bile salts (µmol/L)						
Day 3	5.4 ± 0.5	8.1 ± 1.6	6.4 ± 0.7	7.3 ± 1.4	6.9 ± 1.2	7.2 ± 1.8
Day 23	5.9 ± 0.7	6.1 ± 1.2	3.7 ± 0.3	4.8 ± 0.8	3.8 ± 0.3*	4.1 ± 0.4*
Week 14	3.5 ± 0.2	4.0 ± 0.7	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.5 ± 0.4
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	9	10	9	9	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 3	48.2 ± 0.6	48.7 ± 0.5	48.1 ± 0.6	49.3 ± 1.0	48.4 ± 0.6	49.4 ± 0.5
Day 23	49.9 ± 0.6	48.5 ± 0.4	48.6 ± 0.4	47.5 ± 0.5*	48.5 ± 0.4	49.4 ± 0.5
Week 14	47.5 ± 0.5	47.7 ± 0.4	48.5 ± 0.3	48.8 ± 0.4	48.8 ± 0.3	48.0 ± 0.3
Packed cell volume (mL/dL)						
Day 3	47.6 ± 0.4	48.4 ± 0.6	47.6 ± 0.5	48.4 ± 1.2	47.8 ± 0.6	48.8 ± 0.5
Day 23	49.3 ± 0.6	47.7 ± 0.5	48.3 ± 0.4	47.0 ± 0.4*	47.9 ± 0.3	48.5 ± 0.4
Week 14	47.6 ± 0.5	47.5 ± 0.6	48.3 ± 0.3	49.0 ± 0.4	48.6 ± 0.4	47.7 ± 0.4
Hemoglobin (g/dL)						
Day 3	14.8 ± 0.2	15.1 ± 0.3	14.7 ± 0.2	15.3 ± 0.4	15.1 ± 0.2	15.4 ± 0.2
Day 23	16.0 ± 0.2	15.6 ± 0.1	15.6 ± 0.1	15.2 ± 0.1**	15.5 ± 0.1	15.7 ± 0.1
Week 14	15.5 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	16.0 ± 0.1*	16.0 ± 0.1*	15.7 ± 0.1
Erythrocytes (10^6/µL)						
Day 3	7.78 ± 0.07	8.02 ± 0.13	7.75 ± 0.10	8.09 ± 0.20	7.97 ± 0.12	8.16 ± 0.13
Day 23	8.23 ± 0.12	8.13 ± 0.08	8.04 ± 0.06	7.92 ± 0.08	8.02 ± 0.07	8.17 ± 0.07
Week 14	8.44 ± 0.07	8.43 ± 0.10	8.55 ± 0.06	8.69 ± 0.07*	8.62 ± 0.06	8.50 ± 0.05
Reticulocytes (10^6/µL)						
Day 3	0.24 ± 0.02	0.28 ± 0.02	0.23 ± 0.02	0.26 ± 0.02	0.20 ± 0.02	0.28 ± 0.03
Day 23	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.20 ± 0.02	0.19 ± 0.01
Week 14	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Nucleated erythrocytes/100 leukocytes						
Day 3	0.5 ± 0.2	0.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Day 23	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
Week 14	0.5 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.5 ± 0.2
Mean cell volume (fL)						
Day 3	61.2 ± 0.3	60.3 ± 0.4	61.4 ± 0.3	59.8 ± 0.3*	60.1 ± 0.3	59.9 ± 0.6
Day 23	59.9 ± 0.5	58.6 ± 0.4	60.1 ± 0.3	59.3 ± 0.3	59.7 ± 0.4	59.3 ± 0.4
Week 14	56.4 ± 0.2	56.3 ± 0.2	56.5 ± 0.1	56.3 ± 0.2	56.4 ± 0.2	56.2 ± 0.2

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Female (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	9	10	9	9	10	10
Week 14	10	10	10	10	10	10
Mean cell hemoglobin (pg)						
Day 3	18.9 ± 0.1	18.8 ± 0.1	19.1 ± 0.1	18.9 ± 0.1	19.0 ± 0.1	18.9 ± 0.1
Day 23	19.4 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.2 ± 0.1
Week 14	18.4 ± 0.1	18.6 ± 0.1	18.5 ± 0.0	18.4 ± 0.0	18.5 ± 0.0	18.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.0 ± 0.1	31.2 ± 0.2	31.0 ± 0.2	31.6 ± 0.3*	31.6 ± 0.1*	31.5 ± 0.2*
Day 23	32.5 ± 0.3	32.8 ± 0.2	32.3 ± 0.3	32.3 ± 0.2	32.5 ± 0.2	32.4 ± 0.2
Week 14	32.7 ± 0.2	33.1 ± 0.3	32.8 ± 0.1	32.6 ± 0.1	32.8 ± 0.1	32.9 ± 0.1
Platelets (10^3/μL)						
Day 3	860.4 ± 29.1	814.1 ± 40.1	849.1 ± 24.0	848.7 ± 28.0	816.0 ± 29.5	835.0 ± 30.7
Day 23	762.0 ± 28.6	743.8 ± 23.8	784.3 ± 26.0	805.8 ± 16.8	796.3 ± 25.7	792.7 ± 8.9
Week 14	691.3 ± 13.7	679.2 ± 40.1	678.9 ± 13.4	657.2 ± 26.0	671.1 ± 12.9	686.1 ± 14.4
Leukocytes (10^3/μL)						
Day 3	13.54 ± 0.33	12.84 ± 0.60	12.85 ± 0.47	13.44 ± 0.55	11.74 ± 0.55*	10.02 ± 0.22**
Day 23	9.19 ± 0.52	9.82 ± 0.95	9.53 ± 0.70	7.84 ± 0.77	8.31 ± 0.78	8.38 ± 0.75
Week 14	6.54 ± 0.23	7.13 ± 0.40	6.91 ± 0.45	7.13 ± 0.36	7.23 ± 0.45	7.80 ± 0.50
Segmented neutrophils (10^3/μL)						
Day 3	1.22 ± 0.05	1.22 ± 0.06	1.38 ± 0.09	1.32 ± 0.08	1.21 ± 0.07	1.06 ± 0.04
Day 23	1.67 ± 0.19	1.31 ± 0.18	1.58 ± 0.17	1.16 ± 0.15	1.35 ± 0.15	1.32 ± 0.12
Week 14	1.18 ± 0.04	1.25 ± 0.05	1.31 ± 0.12	1.13 ± 0.08	1.25 ± 0.17	1.33 ± 0.09
Bands (10^3/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 3	12.07 ± 0.29	11.39 ± 0.56	11.20 ± 0.44	11.86 ± 0.47	10.27 ± 0.49*	8.74 ± 0.21**
Day 23	7.35 ± 0.48	8.29 ± 0.83	7.75 ± 0.61	6.49 ± 0.61	6.82 ± 0.64	6.90 ± 0.63
Week 14	5.14 ± 0.22	5.64 ± 0.37	5.35 ± 0.38	5.80 ± 0.31	5.71 ± 0.33	6.20 ± 0.43
Monocytes (10^3/μL)						
Day 3	0.07 ± 0.01	0.08 ± 0.02	0.12 ± 0.03	0.11 ± 0.01	0.13 ± 0.03	0.12 ± 0.02
Day 23	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01
Week 14	0.10 ± 0.03	0.11 ± 0.04	0.14 ± 0.04	0.09 ± 0.02	0.16 ± 0.06	0.10 ± 0.02
Basophils (10^3/μL)						
Day 3	0.017 ± 0.004	0.010 ± 0.003	0.007 ± 0.002	0.020 ± 0.012	0.007 ± 0.003	0.010 ± 0.002
Day 23	0.006 ± 0.002	0.004 ± 0.002	0.009 ± 0.004	0.006 ± 0.002	0.003 ± 0.002	0.005 ± 0.002
Week 14	0.002 ± 0.001	0.004 ± 0.002	0.005 ± 0.002	0.001 ± 0.001	0.002 ± 0.001	0.004 ± 0.002
Eosinophils (10^3/μL)						
Day 3	0.16 ± 0.03	0.14 ± 0.02	0.14 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.09 ± 0.01*
Day 23	0.11 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.02
Week 14	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	0.16 ± 0.02

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Diethylamine

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Female (continued)						
Clinical Chemistry						
n						
Day 3	9	10	10	10	10	10
Day 23	9	10	10	9	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	11.7 ± 0.4[b]	11.9 ± 0.6	10.1 ± 0.5	11.3 ± 0.8	9.4 ± 0.7**	11.4 ± 0.5
Day 23	13.3 ± 0.5	11.3 ± 0.5*	13.1 ± 0.6	11.6 ± 0.3	12.3 ± 0.4	11.7 ± 0.3
Week 14	15.2 ± 0.5	15.3 ± 0.4	15.4 ± 0.3	15.1 ± 0.3	16.1 ± 0.3	16.2 ± 0.4
Creatinine (mg/dL)						
Day 3	0.26 ± 0.02	0.28 ± 0.01	0.24 ± 0.02	0.25 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Day 23	0.59 ± 0.02	0.59 ± 0.01	0.61 ± 0.01	0.58 ± 0.01	0.57 ± 0.03	0.56 ± 0.02
Week 14	0.67 ± 0.02	0.63 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	0.66 ± 0.02	0.66 ± 0.02
Total protein (g/dL)						
Day 3	6.2 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	6.0 ± 0.1
Day 23	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Week 14	7.4 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.0 ± 0.1
Albumin (g/dL)						
Day 3	4.5 ± 0.1	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.3 ± 0.0**	4.4 ± 0.0*
Day 23	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.7 ± 0.0	4.6 ± 0.1
Week 14	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.0 ± 0.0*
Globulin (g/dL)						
Day 3	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
Day 23	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
Week 14	2.1 ± 0.1	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.0 ± 0.0
Albumin/globulin ratio						
Day 3	2.7 ± 0.0	2.8 ± 0.0	2.8 ± 0.0	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.0
Day 23	2.5 ± 0.1	2.6 ± 0.0	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.0	2.5 ± 0.0
Week 14	2.5 ± 0.0	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.0	2.5 ± 0.0
Alkaline phosphatase (IU/L)						
Day 3	560 ± 10	564 ± 7	552 ± 8	537 ± 11	522 ± 12*	531 ± 11*
Day 23	302 ± 8	311 ± 6	312 ± 7	305 ± 9	315 ± 6	327 ± 6
Week 14	203 ± 13	189 ± 8	203 ± 4	192 ± 7	211 ± 4	202 ± 6
Creatine kinase (IU/L)						
Day 3	580 ± 69	509 ± 49[c]	521 ± 66	508 ± 47	558 ± 87	568 ± 47
Day 23	362 ± 27	419 ± 44	438 ± 37	367 ± 77	384 ± 50	390 ± 35
Week 14	220 ± 17	289 ± 30	299 ± 44	228 ± 26	236 ± 21	333 ± 31
Sorbitol dehydrogenase (IU/L)						
Day 3	14 ± 1[b]	16 ± 1	14 ± 1	14 ± 1	13 ± 1	12 ± 1
Day 23	17 ± 1	16 ± 1	17 ± 1	17 ± 1	18 ± 1	17 ± 1
Week 14	19 ± 1	19 ± 1	18 ± 1	20 ± 1	20 ± 1	18 ± 1
Bile salts (μmol/L)						
Day 3	7.9 ± 2.0	7.7 ± 1.9	5.5 ± 0.7	6.1 ± 1.1	5.1 ± 0.3	5.5 ± 0.6
Day 23	7.2 ± 1.1	5.8 ± 0.6	6.8 ± 0.9	5.9 ± 1.1	6.2 ± 0.6	6.6 ± 1.4
Week 14	7.0 ± 0.7	7.8 ± 0.8	6.1 ± 0.6	5.0 ± 0.3	6.2 ± 0.7	7.8 ± 1.6

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=9

TABLE F2
Hematology Data for Mice in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
Male						
n	9	10	10	10	10	10
Hematocrit (%)	50.6 ± 0.4	51.0 ± 0.8	50.9 ± 0.3	50.9 ± 0.4	50.5 ± 0.3	50.4 ± 0.3
Packed cell volume (%)	51.2 ± 0.4	51.0 ± 0.8	51.4 ± 0.4	51.1 ± 0.3	51.0 ± 0.3	50.6 ± 0.3
Hemoglobin (g/dL)	16.1 ± 0.1	16.2 ± 0.3	16.3 ± 0.1	16.3 ± 0.1	16.2 ± 0.1	16.1 ± 0.1
Erythrocytes (10^6/µL)	10.46 ± 0.11	10.45 ± 0.16	10.54 ± 0.07	10.56 ± 0.07	10.47 ± 0.07	10.41 ± 0.06
Reticulocytes (10^6/µL)	0.18 ± 0.02	0.18 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	0.22 ± 0.02	0.17 ± 0.02
Nucleated erythrocytes /100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean cell volume (fL)	48.9 ± 0.3	48.7 ± 0.2	48.8 ± 0.3	48.5 ± 0.2	48.7 ± 0.2	48.6 ± 0.2
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.5 ± 0.1	15.4 ± 0.1	15.4 ± 0.0	15.5 ± 0.1	15.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.1	31.9 ± 0.1	31.7 ± 0.2	31.9 ± 0.1	31.7 ± 0.1	31.9 ± 0.1*
Platelets (10^3/µL)	926.6 ± 27.5	903.1 ± 22.2	878.3 ± 27.8	946.3 ± 15.5	870.6 ± 36.9	854.8 ± 24.2
Leukocytes (10^3/µL)	2.81 ± 0.26	3.34 ± 0.33	3.24 ± 0.26	3.00 ± 0.29	3.41 ± 0.28	3.45 ± 0.33
Segmented neutrophils (10^3/µL)	0.36 ± 0.05	0.45 ± 0.04	0.42 ± 0.05	0.41 ± 0.05	0.43 ± 0.08	0.37 ± 0.04
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.35 ± 0.23	2.79 ± 0.30	2.74 ± 0.23	2.52 ± 0.24	2.88 ± 0.25	3.00 ± 0.31
Monocytes (10^3/µL)	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.02
Basophils (10^3/µL)	0.017 ± 0.004	0.014 ± 0.003	0.013 ± 0.003	0.010 ± 0.004	0.015 ± 0.003	0.014 ± 0.003
Eosinophils (10^3/µL)	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
Female						
n	10	10	10	10	10	10
Hematocrit (%)	49.7 ± 0.3	50.1 ± 0.3	50.0 ± 0.4	50.3 ± 0.3	50.6 ± 0.3*	51.0 ± 0.4**
Packed cell volume (%)	50.2 ± 0.2	50.6 ± 0.4	50.1 ± 0.4	50.7 ± 0.3	50.9 ± 0.4	51.3 ± 0.4
Hemoglobin (g/dL)	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.3 ± 0.1	16.5 ± 0.1
Erythrocytes (10^6/µL)	10.11 ± 0.06	10.17 ± 0.08	10.06 ± 0.09	10.21 ± 0.05	10.20 ± 0.07	10.37 ± 0.11
Reticulocytes (10^6/µL)	0.20 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	0.20 ± 0.01	0.21 ± 0.02	0.23 ± 0.02
Nucleated erythrocytes /100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean cell volume (fL)	49.7 ± 0.2	49.8 ± 0.1	49.8 ± 0.2	49.7 ± 0.3	49.8 ± 0.2	49.4 ± 0.2
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.0 ± 0.1	31.9 ± 0.1	32.1 ± 0.1	31.8 ± 0.1	32.0 ± 0.1	32.1 ± 0.1
Platelets (10^3/µL)	802.9 ± 17.2	825.5 ± 23.1	814.6 ± 28.9	834.3 ± 15.1	829.7 ± 13.8	758.7 ± 23.5
Leukocytes (10^3/µL)	2.85 ± 0.27	2.80 ± 0.19	3.46 ± 0.35	2.99 ± 0.19	3.27 ± 0.34	2.89 ± 0.17
Segmented neutrophils (10^3/µL)	0.43 ± 0.05	0.32 ± 0.04	0.38 ± 0.04	0.32 ± 0.04	0.40 ± 0.05	0.33 ± 0.05
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.35 ± 0.23	2.42 ± 0.17	3.00 ± 0.30	2.60 ± 0.18	2.78 ± 0.31	2.50 ± 0.15
Monocytes (10^3/µL)	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Basophils (10^3/µL)	0.012 ± 0.002	0.008 ± 0.001	0.011 ± 0.003	0.009 ± 0.001	0.011 ± 0.002	0.009 ± 0.003
Eosinophils (10^3/µL)	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the chamber control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	173 ± 4	171 ± 5	171 ± 4	158 ± 3*	128 ± 3**	103 ± 2**
Heart						
Absolute	0.66 ± 0.03	0.62 ± 0.03	0.62 ± 0.02	0.59 ± 0.02*	0.51 ± 0.02**	0.46 ± 0.01**
Relative	3.791 ± 0.093	3.632 ± 0.088	3.618 ± 0.039	3.698 ± 0.056	3.986 ± 0.096	4.487 ± 0.140**
R. Kidney						
Absolute	0.68 ± 0.03	0.71 ± 0.03	0.68 ± 0.02	0.66 ± 0.01	0.57 ± 0.02**	0.51 ± 0.01**
Relative	3.931 ± 0.064	4.122 ± 0.086	3.956 ± 0.022	4.168 ± 0.042*	4.440 ± 0.063**	4.974 ± 0.115**
Liver						
Absolute	7.83 ± 0.37	7.37 ± 0.20	7.71 ± 0.31	7.13 ± 0.33	5.31 ± 0.12**	4.49 ± 0.10**
Relative	45.105 ± 1.145	43.110 ± 0.123	44.952 ± 1.053	44.969 ± 1.484	41.658 ± 0.418	43.698 ± 0.529
Lung						
Absolute	1.11 ± 0.03	1.26 ± 0.09	1.29 ± 0.10	1.20 ± 0.08	0.92 ± 0.02	0.75 ± 0.04**
Relative	6.424 ± 0.149	7.424 ± 0.589	7.572 ± 0.792	7.541 ± 0.451	7.213 ± 0.303	7.265 ± 0.326
R. Testis						
Absolute	0.977 ± 0.025	1.013 ± 0.038	1.060 ± 0.026	1.012 ± 0.019	0.931 ± 0.023	0.637 ± 0.061**
Relative	5.643 ± 0.104	5.921 ± 0.120	6.201 ± 0.215	6.398 ± 0.168	7.308 ± 0.042*	6.231 ± 0.621*
Thymus						
Absolute	0.484 ± 0.021	0.452 ± 0.014	0.447 ± 0.008	0.393 ± 0.015**	0.309 ± 0.011**	0.123 ± 0.008**
Relative	2.790 ± 0.077	2.649 ± 0.105	2.612 ± 0.044	2.477 ± 0.072*	2.437 ± 0.129**	1.200 ± 0.068**
Female						
Necropsy body wt	124 ± 3	124 ± 3	125 ± 3	120 ± 2	101 ± 2**	86 ± 3**
Heart						
Absolute	0.50 ± 0.02	0.50 ± 0.01	0.52 ± 0.01	0.49 ± 0.01	0.45 ± 0.01**	0.43 ± 0.01**
Relative	4.037 ± 0.098	4.085 ± 0.082	4.193 ± 0.125	4.065 ± 0.075	4.403 ± 0.077*	5.041 ± 0.104**
R. Kidney						
Absolute	0.52 ± 0.02	0.52 ± 0.01	0.57 ± 0.02	0.54 ± 0.01	0.50 ± 0.02	0.44 ± 0.01**
Relative	4.158 ± 0.115	4.215 ± 0.078	4.532 ± 0.049**	4.531 ± 0.039**	4.970 ± 0.092**	5.151 ± 0.088**
Liver						
Absolute	5.07 ± 0.23	4.94 ± 0.17	5.28 ± 0.12	4.93 ± 0.11	4.43 ± 0.08**	4.31 ± 0.08**
Relative	40.795 ± 1.119	39.990 ± 1.164	42.279 ± 0.522	41.239 ± 0.489	43.705 ± 0.911*	50.023 ± 1.200**
Lung						
Absolute	0.88 ± 0.03	1.02 ± 0.11	1.00 ± 0.04	1.21 ± 0.14	0.94 ± 0.07	0.90 ± 0.12
Relative	7.118 ± 0.160	8.303 ± 0.926	8.064 ± 0.370	10.116 ± 1.010*	9.330 ± 0.663*	10.398 ± 1.246*
Thymus						
Absolute	0.394 ± 0.022	0.387 ± 0.008	0.433 ± 0.008	0.371 ± 0.015	0.282 ± 0.005**	0.164 ± 0.023**
Relative	3.171 ± 0.125	3.135 ± 0.082	3.480 ± 0.134	3.099 ± 0.120	2.788 ± 0.091	1.890 ± 0.243**

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** Significantly different (P≤0.01) from the chamber control group by Williams' or Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	347 ± 6	344 ± 4	357 ± 6	350 ± 7	355 ± 7	338 ± 6
Heart						
Absolute	0.94 ± 0.01	0.93 ± 0.02	0.96 ± 0.03	0.93 ± 0.02	0.94 ± 0.02	0.91 ± 0.02
Relative	2.721 ± 0.039	2.704 ± 0.033	2.696 ± 0.062	2.661 ± 0.029	2.641 ± 0.036	2.707 ± 0.033
R. Kidney						
Absolute	1.03 ± 0.02	1.07 ± 0.02	1.06 ± 0.02	1.06 ± 0.03	1.05 ± 0.02	1.03 ± 0.02
Relative	2.976 ± 0.038	3.111 ± 0.040	2.976 ± 0.046	3.020 ± 0.033	2.966 ± 0.055	3.045 ± 0.034
Liver						
Absolute	11.04 ± 0.28	11.23 ± 0.20	11.74 ± 0.28	11.44 ± 0.40	11.62 ± 0.25	11.27 ± 0.33
Relative	31.794 ± 0.335	32.663 ± 0.316	32.834 ± 0.321	32.602 ± 0.619	32.794 ± 0.371	33.294 ± 0.396*
Lung						
Absolute	1.84 ± 0.12	1.70 ± 0.03	1.72 ± 0.07	1.81 ± 0.09	1.76 ± 0.05	1.76 ± 0.07
Relative	5.310 ± 0.355	4.942 ± 0.114	4.806 ± 0.193	5.159 ± 0.185	4.961 ± 0.150	5.212 ± 0.193
R. Testis						
Absolute	1.449 ± 0.019	1.434 ± 0.018	1.427 ± 0.033	1.428 ± 0.030	1.446 ± 0.027	1.407 ± 0.026
Relative	4.183 ± 0.070	4.174 ± 0.054	4.002 ± 0.111	4.078 ± 0.051	4.084 ± 0.066	4.167 ± 0.050
Thymus						
Absolute	0.311 ± 0.012	0.347 ± 0.017	0.326 ± 0.017	0.323 ± 0.012	0.307 ± 0.016	0.291 ± 0.010
Relative	0.896 ± 0.029	1.012 ± 0.053	0.911 ± 0.039	0.920 ± 0.024	0.864 ± 0.037	0.863 ± 0.034
Female						
Necropsy body wt	204 ± 6	199 ± 3	197 ± 3	200 ± 4	202 ± 3	201 ± 5
Heart						
Absolute	0.63 ± 0.01	0.63 ± 0.01	0.64 ± 0.01	0.64 ± 0.01	0.63 ± 0.01	0.66 ± 0.02
Relative	3.081 ± 0.053	3.166 ± 0.060	3.226 ± 0.041	3.212 ± 0.042	3.140 ± 0.037	3.283 ± 0.043*
R. Kidney						
Absolute	0.62 ± 0.02	0.66 ± 0.01	0.65 ± 0.01	0.64 ± 0.01[b]	0.66 ± 0.01	0.66 ± 0.01
Relative	3.068 ± 0.049	3.295 ± 0.051**	3.308 ± 0.036**	3.227 ± 0.032[b]	3.265 ± 0.045*	3.312 ± 0.068**
Liver						
Absolute	6.23 ± 0.20	6.16 ± 0.08	6.18 ± 0.14	6.09 ± 0.16	6.31 ± 0.15	6.28 ± 0.18
Relative	30.564 ± 0.422	30.950 ± 0.460	31.381 ± 0.505	30.368 ± 0.496	31.286 ± 0.429	31.263 ± 0.287
Lung						
Absolute	1.12 ± 0.04	1.12 ± 0.02	1.17 ± 0.02	1.14 ± 0.03	1.16 ± 0.03	1.16 ± 0.03
Relative	5.513 ± 0.176	5.633 ± 0.099	5.938 ± 0.152	5.684 ± 0.131	5.752 ± 0.095	5.800 ± 0.233
Thymus						
Absolute	0.287 ± 0.009	0.264 ± 0.010	0.281 ± 0.012	0.273 ± 0.008	0.278 ± 0.009	0.288 ± 0.008
Relative	1.416 ± 0.058	1.325 ± 0.053	1.430 ± 0.054	1.361 ± 0.034	1.379 ± 0.038	1.443 ± 0.051

* Significantly different (P≤0.05) from the chamber control group by Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Inhalation Study of Diethylamine[a]

	Chamber Control	31 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
n	5	5	5	5	5	3
Necropsy body wt	28.3 ± 0.4	26.7 ± 0.5	27.4 ± 0.3	24.6 ± 0.4**	20.8 ± 0.6**	16.6 ± 1.2**
Heart						
Absolute	0.15 ± 0.00	0.12 ± 0.00*	0.14 ± 0.01*	0.12 ± 0.00**	0.11 ± 0.00**	0.09 ± 0.00**
Relative	5.231 ± 0.162	4.658 ± 0.173	5.100 ± 0.241	5.045 ± 0.086	5.306 ± 0.131	5.650 ± 0.302
R. Kidney						
Absolute	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.18 ± 0.01**	0.15 ± 0.02**
Relative	8.479 ± 0.238	8.878 ± 0.537	9.113 ± 0.209	9.287 ± 0.222	8.757 ± 0.244	9.194 ± 0.274
Liver						
Absolute	1.49 ± 0.05	1.33 ± 0.04	1.43 ± 0.02	1.17 ± 0.03**	0.98 ± 0.04**	0.72 ± 0.07**
Relative	52.427 ± 1.113	49.873 ± 0.604	52.120 ± 0.804	47.615 ± 0.420**	47.050 ± 0.744**	43.202 ± 1.670**
Lung						
Absolute	0.19 ± 0.00	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.16 ± 0.01**	0.15 ± 0.01**
Relative	6.849 ± 0.071	7.222 ± 0.316	6.708 ± 0.200	7.247 ± 0.088	7.703 ± 0.219*	8.869 ± 0.443**
R. Testis						
Absolute	0.105 ± 0.002	0.106 ± 0.003	0.103 ± 0.002	0.107 ± 0.002	0.100 ± 0.005	0.112 ± 0.021
Relative	3.711 ± 0.094	3.983 ± 0.084	3.750 ± 0.111	4.348 ± 0.120	4.823 ± 0.152*	6.689 ± 1.106**
Thymus						
Absolute	0.059 ± 0.004	0.041 ± 0.006	0.060 ± 0.005	0.050 ± 0.003	0.022 ± 0.004**	0.014 ± 0.003**
Relative	2.089 ± 0.169	1.549 ± 0.259	2.182 ± 0.183	2.037 ± 0.106	1.092 ± 0.219**	0.832 ± 0.099**
Female						
n	5	5	5	5	5	2
Necropsy body wt	22.3 ± 0.2	22.5 ± 0.5	22.6 ± 0.3	20.3 ± 0.4**	18.0 ± 0.3**	15.2 ± 0.5**
Heart						
Absolute	0.13 ± 0.00	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00**	0.09 ± 0.00**	0.09 ± 0.01**
Relative	5.752 ± 0.172	5.155 ± 0.107*	5.292 ± 0.186	5.410 ± 0.056	4.902 ± 0.097**	5.906 ± 0.464
R. Kidney						
Absolute	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.14 ± 0.01*	0.14 ± 0.01
Relative	7.093 ± 0.217	7.719 ± 0.181*	7.679 ± 0.236	7.778 ± 0.129*	7.793 ± 0.247*	8.880 ± 0.037**
Liver						
Absolute	1.16 ± 0.03	1.13 ± 0.03	1.17 ± 0.02	1.03 ± 0.02**	0.87 ± 0.02**	0.76 ± 0.04**
Relative	52.004 ± 0.811	50.078 ± 0.498	51.767 ± 0.680	50.598 ± 0.351	48.642 ± 0.734**	49.649 ± 0.669
Lung						
Absolute	0.17 ± 0.00	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.15 ± 0.00	0.15 ± 0.00
Relative	7.459 ± 0.123	7.725 ± 0.140	8.049 ± 0.415	8.752 ± 0.270**	8.589 ± 0.293**	9.879 ± 0.325**
Thymus						
Absolute	0.080 ± 0.005	0.079 ± 0.002	0.075 ± 0.004	0.060 ± 0.004**	0.039 ± 0.003**	0.015 ± 0.003**
Relative	3.598 ± 0.197	3.506 ± 0.126	3.302 ± 0.149	2.970 ± 0.200*	2.206 ± 0.212**	0.981 ± 0.165**

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	8 ppm	16 ppm	32 ppm	62 ppm	125 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	39.3 ± 0.8	38.4 ± 1.0	37.8 ± 0.3	39.6 ± 0.9	39.3 ± 0.8	30.8 ± 0.5**
Heart						
Absolute	0.16 ± 0.01	0.17 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.15 ± 0.00
Relative	4.187 ± 0.123	4.305 ± 0.088	4.206 ± 0.072	4.130 ± 0.125	4.150 ± 0.077	4.875 ± 0.113**
R. Kidney						
Absolute	0.32 ± 0.01	0.31 ± 0.01	0.30 ± 0.00	0.32 ± 0.01	0.32 ± 0.01	0.28 ± 0.01**
Relative	8.108 ± 0.244	8.035 ± 0.168	8.022 ± 0.110	8.123 ± 0.165	8.073 ± 0.102	9.200 ± 0.141**
Liver						
Absolute	1.66 ± 0.04[b]	1.63 ± 0.04	1.64 ± 0.03	1.67 ± 0.05	1.66 ± 0.04	1.35 ± 0.04**
Relative	42.607 ± 0.731[b]	42.565 ± 0.706	43.296 ± 0.559	42.106 ± 0.651	42.167 ± 0.626	43.909 ± 0.797
Lung						
Absolute	0.21 ± 0.01	0.22 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Relative	5.366 ± 0.158	5.743 ± 0.437	5.953 ± 0.142	5.564 ± 0.146	5.656 ± 0.148	7.047 ± 0.239**
R. Testis						
Absolute	0.126 ± 0.002	0.116 ± 0.005*	0.119 ± 0.002	0.120 ± 0.002	0.119 ± 0.002	0.118 ± 0.002
Relative	3.228 ± 0.105	3.018 ± 0.143	3.162 ± 0.059	3.043 ± 0.058	3.043 ± 0.052	3.852 ± 0.064**
Thymus						
Absolute	0.049 ± 0.003	0.047 ± 0.003	0.047 ± 0.002	0.042 ± 0.004	0.048 ± 0.004	0.036 ± 0.003**
Relative	1.239 ± 0.083	1.227 ± 0.086	1.232 ± 0.054	1.061 ± 0.107	1.211 ± 0.085	1.151 ± 0.080
Female						
Necropsy body wt	32.6 ± 1.4	31.9 ± 1.5	34.3 ± 1.4	32.5 ± 1.1	31.7 ± 1.0	27.3 ± 0.3**
Heart						
Absolute	0.15 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.13 ± 0.00*
Relative	4.529 ± 0.157	4.538 ± 0.168	4.267 ± 0.160	4.536 ± 0.181	4.604 ± 0.107	4.915 ± 0.120
R. Kidney						
Absolute	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.19 ± 0.00**
Relative	6.696 ± 0.233	6.656 ± 0.211	6.226 ± 0.255	6.392 ± 0.174	6.670 ± 0.184	6.961 ± 0.109
Liver						
Absolute	1.47 ± 0.06	1.44 ± 0.06	1.54 ± 0.05	1.46 ± 0.05	1.45 ± 0.05	1.24 ± 0.02*
Relative	45.177 ± 1.190	45.349 ± 0.911	45.115 ± 1.229	45.068 ± 0.483	45.749 ± 1.081	45.504 ± 0.550
Lung						
Absolute	0.22 ± 0.00	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.00	0.22 ± 0.01
Relative	6.756 ± 0.289	7.045 ± 0.270	6.520 ± 0.287	7.016 ± 0.314	6.989 ± 0.181	8.134 ± 0.225**
Thymus						
Absolute	0.060 ± 0.002	0.060 ± 0.003	0.062 ± 0.004	0.058 ± 0.003	0.050 ± 0.002*	0.046 ± 0.003**
Relative	1.861 ± 0.055	1.874 ± 0.084	1.784 ± 0.056	1.815 ± 0.120	1.574 ± 0.037	1.685 ± 0.123

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
** (P≤0.01)
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	32 ppm	62 ppm	125 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	347 ± 6	350 ± 7	355 ± 7	338 ± 6
L. Cauda epididymis	0.1871 ± 0.0052	0.1802 ± 0.0061	0.1873 ± 0.0044	0.1736 ± 0.0043
L. Epididymis	0.4913 ± 0.0094	0.4861 ± 0.0119	0.4947 ± 0.0114	0.4662 ± 0.0069
L. Testis	1.5165 ± 0.0238	1.4772 ± 0.0350	1.5102 ± 0.0278	1.4473 ± 0.0264
Spermatid measurement				
Spermatid heads (10^3/mg testis)	123.55 ± 5.72	125.24 ± 5.86	125.76 ± 3.96	128.93 ± 4.48
Spermatid heads (10^6/testis)	171.00 ± 7.16	168.63 ± 9.72	173.38 ± 6.35	169.38 ± 4.01
Epididymal spermatozoal measurements				
Sperm motility (%)	93.01 ± 0.72	88.60 ± 1.45**	87.27 ± 1.57**	68.44 ± 2.78**
Sperm (10^3/mg cauda epididymis)	669 ± 37	660 ± 30	660 ± 22	598 ± 34
Sperm (10^6/cauda epididymis)	124.3 ± 5.7	118.0 ± 4.6	123.3 ± 3.6	103.9 ± 6.3

** Significantly different ($P \leq 0.01$) from the chamber control group by Shirley's test

[a] Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid, sperm/mg cauda epididymis, and sperm/cauda epididymis measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	32 ppm	62 ppm	125 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	204 ± 6	200 ± 4	202 ± 3	201 ± 5
Proportion of regular cycling females[b]	10/10	10/10	10/10	10/10
Estrous cycle length (days)	5.0 ± 0.05	5.0 ± 0.05	5.0 ± 0.00	5.0 ± 0.05
Estrous stages (% of cycle)				
Diestrus	58.3	54.2	53.3	55.0
Proestrus	16.7	15.8	16.7	14.2
Estrus	20.0	17.5	20.0	20.8
Metestrus	5.0	12.5	10.0	10.0

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among exposure groups and between the chamber control group and each exposed group indicated the exposed females did not have extended estrus or diestrus.

[b] Number of females with a regular cycle/number of females cycling

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	32 ppm	62 ppm	125 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.3 ± 0.8	39.6 ± 0.9	39.3 ± 0.8	30.8 ± 0.5**
L. Cauda epididymis	0.0185 ± 0.0014	0.0190 ± 0.0008	0.0179 ± 0.0007	0.0153 ± 0.0008*
L. Epididymis	0.0535 ± 0.0019	0.0554 ± 0.0011	0.0520 ± 0.0012	0.0498 ± 0.0009
L. Testis	0.1170 ± 0.0022	0.1154 ± 0.0019	0.1141 ± 0.0012	0.1106 ± 0.0026
Spermatid measurement				
Spermatid heads (10^3/mg testis)	190.79 ± 5.43	187.45 ± 6.09	188.23 ± 4.89	183.79 ± 8.95
Spermatid heads (10^6/testis)	20.72 ± 0.81	20.12 ± 0.73	19.63 ± 0.45	18.83 ± 1.13
Epididymal spermatozoal measurements				
Sperm motility (%)	86.66 ± 1.45	80.60 ± 1.41**	78.47 ± 1.51**	73.65 ± 2.04**
Sperm (10^3/mg cauda epididymis)	1,170 ± 117	991 ± 66	1,143 ± 57	1,235 ± 60
Sperm (10^6/cauda epididymis)	20.4 ± 0.8	18.4 ± 0.8	20.2 ± 0.6	18.7 ± 1.0

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** Significantly different (P≤0.01) from the chamber control group by Shirley's (sperm motility) or Williams' test (body weights)
[a] Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunn's test (spermatid, sperm/mg cauda epididymis and sperm/cauda epididymis measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of Diethylamine[a]

	Chamber Control	32 ppm	62 ppm	125 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	32.6 ± 1.4	32.5 ± 1.1	31.7 ± 1.0	27.3 ± 0.3**
Proportion of regular cycling females[b]	8/10	9/10	9/10	7/10
Estrous cycle length (days)	3.9 ± 0.11[c]	4.5 ± 0.50	4.0 ± 0.00	4.3 ± 0.11*
Estrous stages (% of cycle)				
Diestrus	31.7	30.0	25.0	26.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	44.2	47.5	50.0	50.0
Metestrus	22.5	22.5	25.0	23.3
Uncertain diagnoses	1.7	0.0	0.0	0.0

* Significantly different (P≤0.05) from the chamber control group by Shirley's test
** Significantly different (P≤0.01) from the chamber control group by Williams' test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among exposure groups and between the chamber control group and each exposed group indicated the exposed females did not have extended estrus or diestrus.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF DIETHYLAMINE

Diethylamine was obtained from Alkyl Amines Chemicals, Ltd. (Maharashtra, India), in one lot (BE/07/01) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Research Triangle Institute (RTI) (Research Triangle Park, NC), the study laboratory at Battelle Toxicology Northwest (Richland, WA), Galbraith Laboratories, Inc. (Knoxville, TN), and Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO). Reports on analyses performed in support of the diethylamine studies are on file at the National Institute of Environmental Health Sciences.

Lot BE/07/01, a colorless liquid with a strong ammonia odor, was identified as diethylamine by RTI and Chemir/Polytech Laboratory, Inc., using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy; in addition, lot BE/07/01 was identified as diethylamine by RTI using gas chromatography (GC) coupled with mass spectrometry. All spectra were consistent with literature reference spectra (*Aldrich*, 1981, 1993; NIST, Database 1A) of diethylamine. Representative IR and proton NMR spectra are presented in Figures I1 and I2.

Chemir/Polytech Laboratories, Inc., determined the moisture content of lot BE/07/01 using Karl Fischer titration, and Galbraith Laboratories, Inc., measured the purity of the bulk chemical by elemental analysis. The purity of lot BE/07/01 was also determined by RTI and the study laboratory using GC by systems A and B, respectively (Table I1).

For lot BE/07/01, Karl Fischer titration indicated 275 ppm water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for diethylamine. GC by systems A and B showed one major peak accounting for more than 99.9% of the total integrated area. The overall purity of lot BE/07/01 was determined to be approximately 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 2-week, 3-month, and 2-year studies using GC by system B, and no degradation of the bulk chemical was detected.

VAPOR GENERATION AND EXPOSURE SYSTEM

A diagram of the diethylamine vapor generation and delivery system used in the studies is shown in Figure I3. Diethylamine was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor into a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate and nitrogen flow rate. The pressure in the distribution manifold was kept fixed to ensure constant flow through the manifold and into all chambers as the flow of vapor to each chamber was adjusted.

Individual Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. A metering valve with a flow indicator at the manifold controlled the flow rate to each chamber. To initiate exposure, the chamber exposure valves were rotated to allow the vapor to flow to each exposure chamber inlet duct where it was further diluted with HEPA®-filtered, conditioned air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (model 3022A, TSI, Inc., St. Paul, MN) was used with and without animals in the exposure chambers to ensure that

diethylamine vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

VAPOR CONCENTRATION MONITORING

Summaries of the chamber vapor concentrations are given in Tables I2 through I4. Chamber and room concentrations of diethylamine were monitored by an on-line gas chromatograph (system C, Table I1). Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 30 (2-year studies) minutes during each 6-hour exposure period using Hastelloy-C stream-select and gas-sampling valves (VALCO Instruments Company, Houston, TX) in a separate, heated valve oven. The sample lines composing each sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of diethylamine in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was calibrated (and recalibrated whenever acceptance criteria were not met) by a comparison of chamber concentration data to data from grade samples that were collected with acrylic ester adsorbent gas sampling tubes (XAD®-7; SKC, Eighty Four, PA), extracted with methylene chloride containing triethylamine as an internal standard, and analyzed using an off-line gas chromatograph (system D). Known values of chamber atmosphere were sampled at a constant flow rate ensured by a calibrated orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of diethylamine and the internal standard (triethylamine) in methylene chloride.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. For rats and mice in the 2-week studies, T_{90} values ranged from 8 to 12 minutes with animals present; T_{10} values ranged from 9 to 11 minutes with animals present. For rats and mice in the 3-month studies, T_{90} values ranged from 9 to 15 minutes without animals present and from 12 to 17 minutes with animals present; T_{10} values ranged from 7 to 10 minutes without animals present and from 9 to 15 minutes with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 8 to 13 minutes without animals present and from 10 to 27 minutes with animals present; T_{10} values ranged from 7 to 9 minutes without animals present and from 12 to 21 minutes with animals present. A T_{90} value of 12 minutes was selected for the 2-week and 3-month studies. Due to the reactivity of diethylamine with large groups of exposed rats and mice, a T_{90} value of 15 minutes was used for the 2-year studies.

The uniformity of vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week and 3-month studies and approximately quarterly during the 2-year studies. The vapor concentration was measured using the on-line gas chromatograph (system C, Table I1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. During the 2-week studies and prior to the 3-month and 2-year studies, concentrations were measured at 12 chamber positions, one in front and one in back for each of the six possible animal cage unit positions per chamber. During the 3-month and 2-year studies, concentrations were measured at the regular monitoring port and from sample ports at levels where animals were present. Chamber concentration uniformity was maintained throughout the studies.

The persistence of diethylamine in the chambers after vapor delivery ended was determined by monitoring the vapor concentration in the 500 ppm chambers in the 2-week studies, the 125 ppm chambers in the 3-month studies, and the 125 ppm (rats) and 62.5 ppm (mice) chambers in the 2-year studies, with (all studies) and without (3-month and 2-year studies) animals present in the chambers. In the 2-week studies, the concentration decreased to 1% of the

target concentration within 31 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 29 minutes without animals present and within 59 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 23 (rat) and 16 (mice) minutes without animals present and within 98 (rats) and 42 (mice) minutes with animals present.

Samples of the test atmosphere from the distribution lines and the low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies and also at the beginning and end of one generation day during the 2-week, 3-month, and 2-year studies. The atmosphere samples were collected with adsorbent gas sampling tubes containing an acrylic ester (XAD®-7), followed by a tube containing activated coconut charcoal (ORBO™-32; Supelco, Inc., Bellefonte, PA), and extracted with methylene chloride. Additional samples were collected from the generator reservoir, and all of the samples were analyzed using GC by system B (Table I1) to measure the stability and purity of diethylamine in the generation and delivery system.

No evidence of degradation of diethylamine was noted in any part of the exposure system. Two impurity peaks with areas greater than 0.1% of the total peak areas were noted in some of the samples collected from the exposure chambers in the 3-month and 2-year studies. Additional collections of test atmosphere samples determined that only one of these impurity peaks was reproducible, and it was identified as *N,N*-diethylformamide using GC coupled with mass spectrometry. Parallel sampling with acetonitrile-filled bubblers and sorbent collection tubes demonstrated that the presence of *N,N*-diethylformamide in the samples was most likely due to artifact formation on the sorbent. No impurity peaks were resolved in the generator reservoir samples.

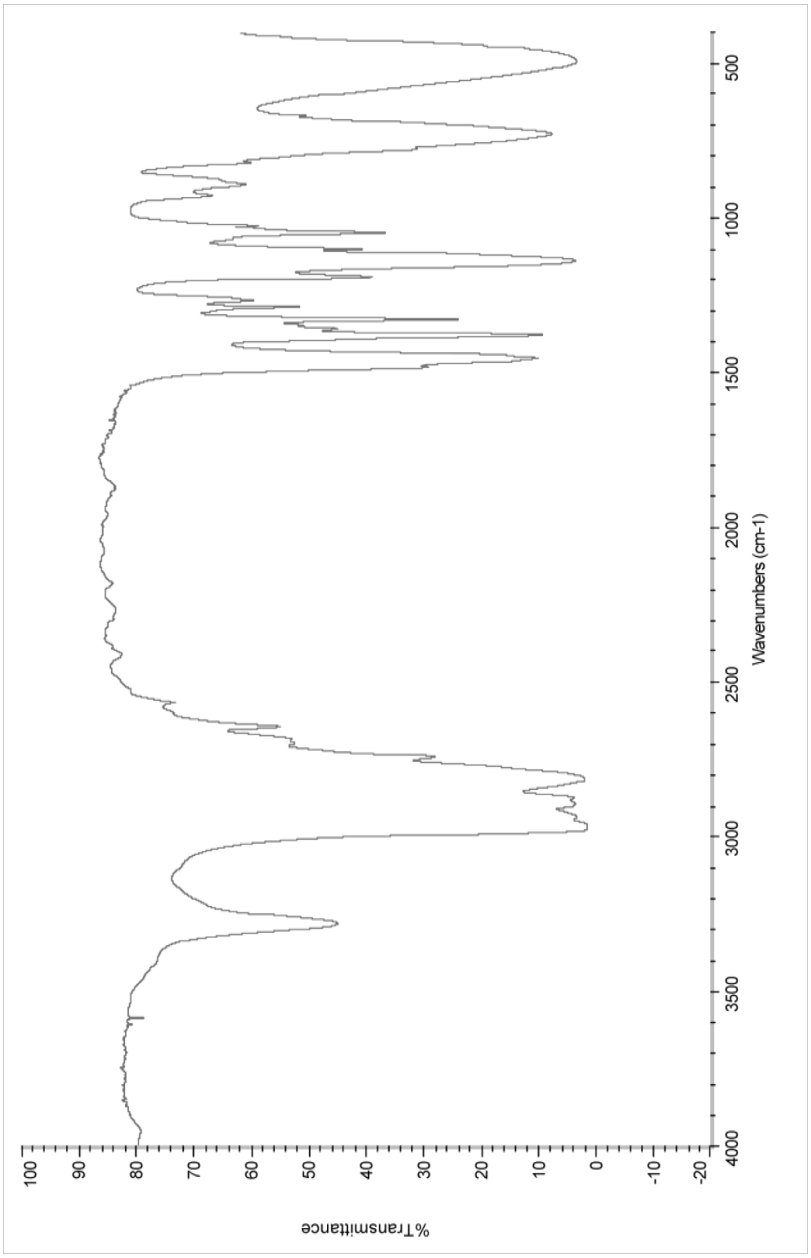

FIGURE I1
Infrared Absorption Spectrum of Diethylamine

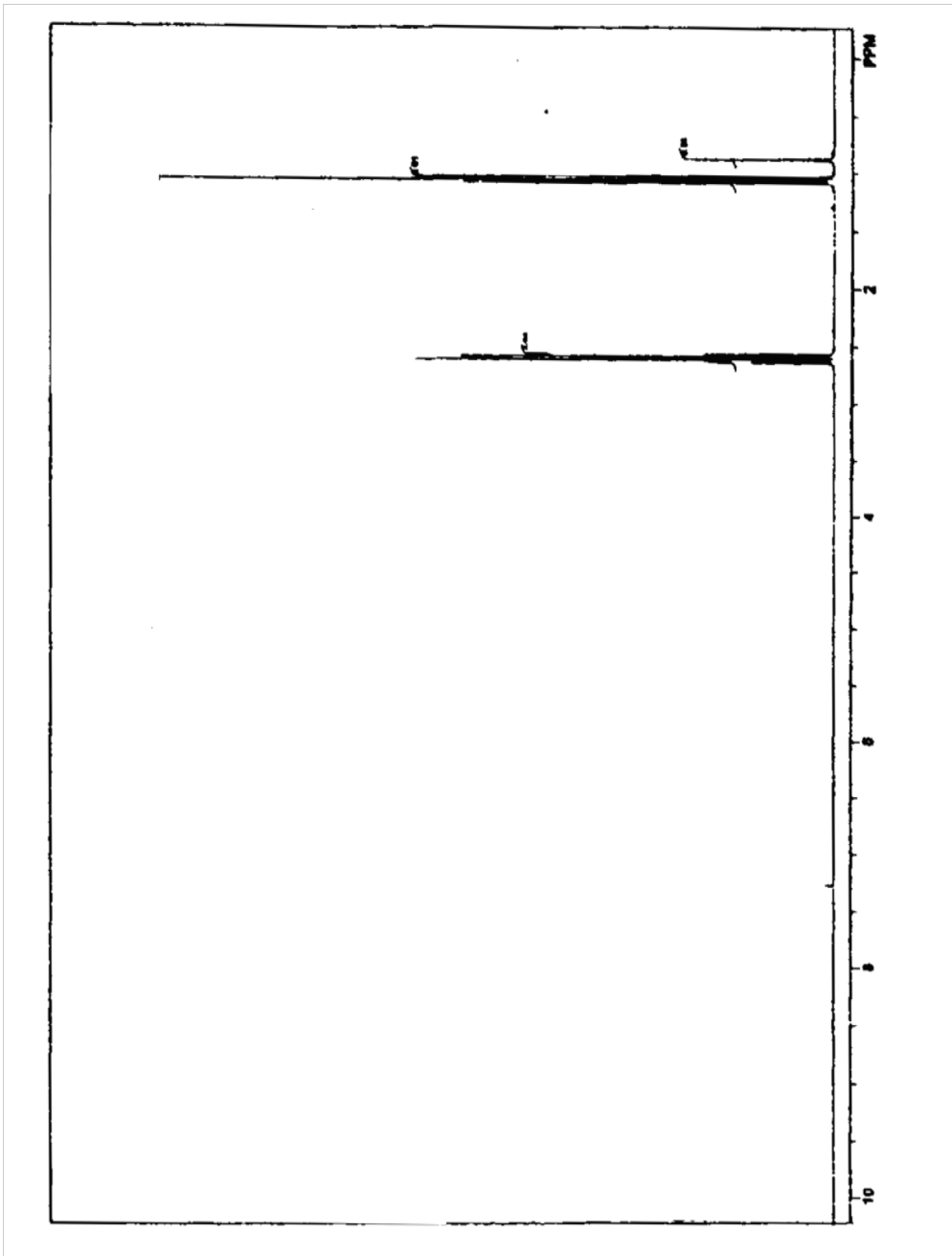

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Diethylamine

TABLE I1
Gas Chromatography Systems Used in the Inhalation Studies of Diethylamine[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	SPB™-1, 60 m × 0.32 mm, 1.0 µm film (Supelco, Inc., Bellefonte, PA)	Nitrogen at 1.0 mL/minute	50° C for 5 minutes, then 10° C/minute to 300° C, held for 10 minutes
System B			
Flame ionization	PTA-5, 30 m × 0.53 mm, 3.0 µm film (Supelco, Inc.)	Helium at 2.5 psi head pressure	35° C for 6 minutes, then 2° C/minute to 50° C, then 7°/minute to 260° C, held for 1 minute
System C			
Flame ionization	RTX-5, 15 m × 0.53 mm, 3 µm film (Restek, Bellefonte, PA)	Nitrogen at 15 mL/minute	Isothermal at 40° C
System D			
Flame ionization	PTA-5, 30 m × 0.53 mm, 3.0 µm film (Supelco, Inc.)	Helium at 4 psi head pressure	20° C for 4 minutes, then 2.5° C/minute to 35° C, then 15° C/minute to 150° C, held for 2 minutes

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA).

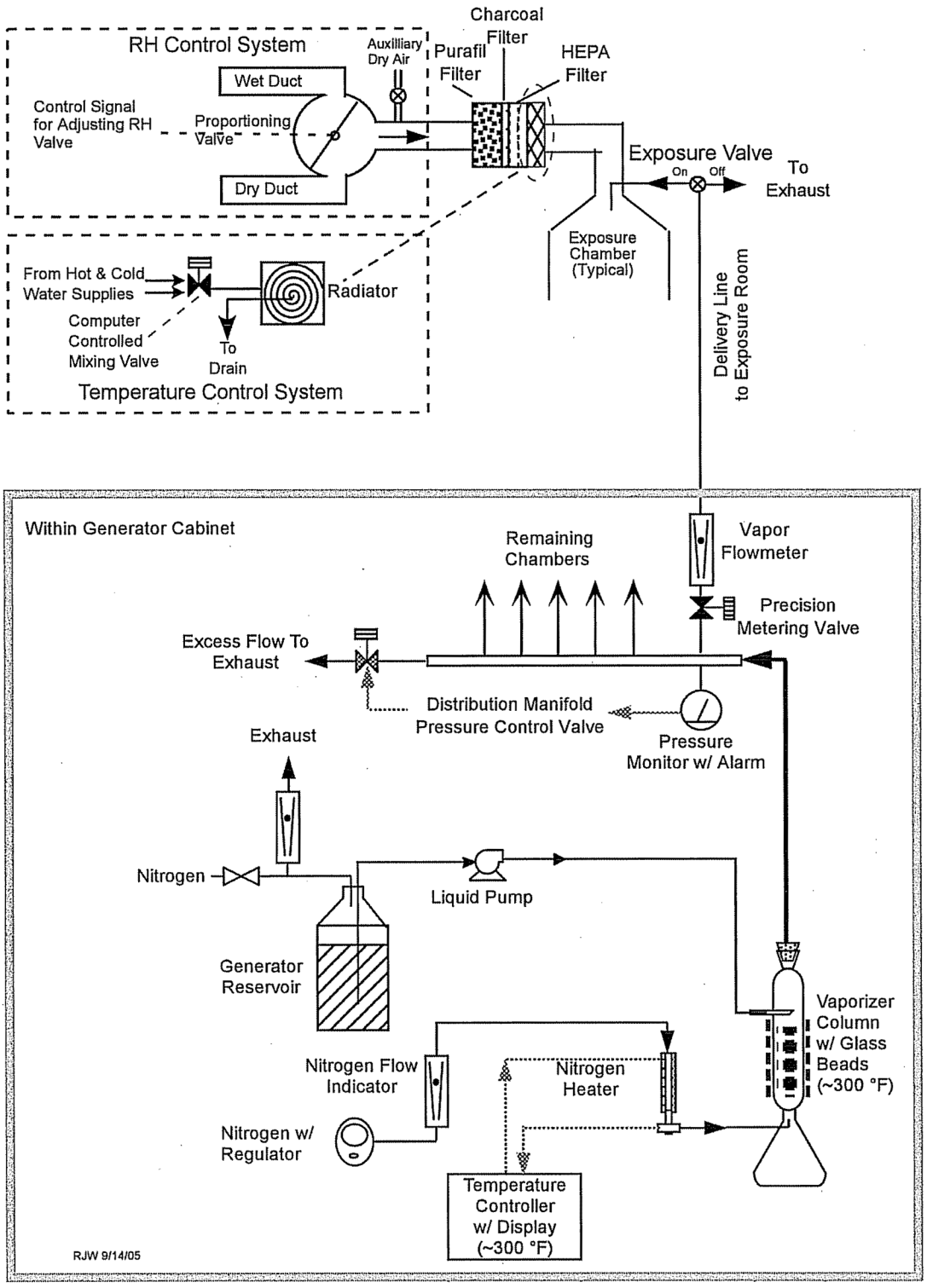

FIGURE I3
Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of Diethylamine

TABLE I2
Summary of Chamber Concentrations in the 2-Week Inhalation Studies of Diethylamine

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	31	225	30.9 ± 0.5
	62.5	224	62.9 ± 1.3
	125	226	125 ± 2
	250	225	252 ± 4
	500	225	499 ± 9
Mouse Chambers			
	31	245	30.9 ± 0.5
	62.5	244	62.8 ± 1.2
	125	246	125 ± 3
	250	245	252 ± 4
	500	245	499 ± 9

[a] Mean ± standard deviation

TABLE I3
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of Diethylamine

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	8	1,239	8.0 ± 0.3
	16	1,270	15.9 ± 0.6
	32	1,275	32.0 ± 1.3
	62	1,275	62.2 ± 2.3
	125	1,273	126 ± 5
Mouse Chambers			
	8	1,277	8.0 ± 0.3
	16	1,309	15.9 ± 0.6
	32	1,315	32.0 ± 1.3
	62	1,314	62.2 ± 2.3
	125	1,313	126 ± 5

[a] Mean ± standard deviation

TABLE I4
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Diethylamine

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	31	8,001	31.0 ± 1.1
	62.5	8,101	62.5 ± 2.3
	125	8,298	125 ± 4
Mouse Chambers			
	16	8,003	16.1 ± 0.6
	31	8,058	31.1 ± 1.0
	62.5	8,215	62.6 ± 1.9

[a] Mean ± standard deviation

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.66	13.7 – 16.1	23
Crude fat (% by weight)	8.1 ± 0.37	7.4 – 9.0	23
Crude fiber (% by weight)	9.2 ± 0.46	8.2 – 9.9	23
Ash (% by weight)	4.9 ± 0.24	4.4 – 5.4	23
Amino Acids (% of total diet)			
Arginine	0.770 ± 0.070	0.670 – 0.970	18
Cystine	0.225 ± 0.023	0.150 – 0.250	18
Glycine	0.706 ± 0.043	0.620 – 0.800	18
Histidine	0.362 ± 0.082	0.310 – 0.680	18
Isoleucine	0.542 ± 0.046	0.430 – 0.660	18
Leucine	1.087 ± 0.066	0.960 – 1.240	18
Lysine	0.712 ± 0.118	0.310 – 0.840	18
Methionine	0.407 ± 0.051	0.260 – 0.490	18
Phenylalanine	0.626 ± 0.043	0.540 – 0.720	18
Threonine	0.500 ± 0.046	0.430 – 0.610	18
Tryptophan	0.142 ± 0.024	0.110 – 0.200	18
Tyrosine	0.388 ± 0.058	0.280 – 0.540	18
Valine	0.667 ± 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.243	3.49 – 4.54	18
Linolenic	0.30 ± 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	4,784 ± 115	3,230 – 8,900	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	8.8 ± 3.79	6.4 – 25.2	23
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.964 ± 0.044	0.884 – 1.030	23
Phosphorus (%)	0.578 ± 0.027	0.535 – 0.623	23
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.110	0.14 – 0.50	23
Cadmium (ppm)	0.06 ± 0.022	0.04 – 0.10	23
Lead (ppm)	0.09 ± 0.022	0.06 – 0.13	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.22 ± 0.058	0.16 – 0.45	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	12.9 ± 4.14	7.89 – 24.4	23
Nitrite nitrogen (ppm)[c]	<0.61		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	10 ± 0	10	23
Coliform (MPN/gm)	3.0 ± 0.1	3.0 – 3.6	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.5 ± 1.75	2.3 – 8.5	23
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.31	1.1 – 5.6	23
N-Nitrosopyrrolidine (ppb)[e]	2.0 ± 0.73	1.0 – 4.1	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.120 ± 0.142	0.020 – 0.416	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.147 ± 0.157	0.020 – 0.551	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female chamber control rats and mice at the end of the 2-week studies. For the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at 2 weeks and five male and five female chamber control rats and mice at study termination. For the 2-year studies, serum samples were collected from five male and five female sentinel rats and mice at 2 weeks and 6, 12, and 18 months, and from five male and five female 125 ppm rats and 62.5 ppm mice at study termination. Fecal samples were taken from five male and four female mice at 18 months in the 2-year study for *Helicobacter spp.* by polymerase chain reaction testing. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
2-Week Study	
ELISA	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
3-Month Study	
ELISA	
H-1	2 weeks
KRV	2 weeks
Mycoplasma arthritidis	Study termination
M. pulmonis	2 weeks, study termination
PVM	2 weeks, study termination
RCV/SDA	2 weeks, study termination
Sendai	2 weeks, study termination
Immunofluorescence Assay	
Parvovirus	Study termination

Method and Test	Time of Collection
RATS	
2-Year Study	
ELISA	
H-1	2 weeks
KRV	2 weeks
M. arthritidis	Study termination
M. pulmonis	2 weeks, study termination
PVM	2 weeks, 6, 12, and 18 months, study termination
RCV/SDA	2 weeks, 6, 12, and 18 months, study termination
Sendai	2 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination
RCV/SDA	12 months
MICE	
2-Week Study	
ELISA	
GDVII (mouse encephalomyelitis virus)	Study termination
MVM (minute virus of mice)	Study termination
MHV (mouse hepatitis virus)	Study termination
M. pulmonis	Study termination
PVM	Study termination
Sendai	Study termination
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII	2 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
MVM	2 weeks
Mouse adenoma virus	Study termination
MHV	2 weeks, study termination
M. arthritidis	Study termination
M. pulmonis	2 weeks, study termination
PVM	2 weeks, study termination
Reovirus 3	Study termination
Sendai	2 weeks, study termination
Immunofluorescence Assay	
GDVII	Study termination
LCM	Study termination
Mouse adenoma virus-FL	Study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV	Study termination
M. arthritidis	Study termination
Parvovirus	Study termination
PVM	Study termination

Method and Test	Time of Collection
MICE	
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	2 weeks, 6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus	6, 12, and 18 months, study termination
MHV	2 weeks, 6, 12, and 18 months, study termination
MMV VP2 (mouse minute virus viral protein 2)	2 weeks, 12 and 18 months, study termination
MPV VP2 (mouse parvovirus viral protein 2)	12 and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	2 weeks, study termination
PVM	2 weeks, 6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	2 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Mouse adenoma virus 1	Study termination
Mouse adenoma virus-F1	18 months
MCMV	Study termination
MHV	6 months
Parvovirus	6 months
PVM	12 and 18 months
Polymerase Chain Reaction	
Heliobacter species	18 months

RESULTS

All test results were negative.