NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology Studies of CIMSTAR 3800 in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of CIMSTAR 3800 in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Inhalation Studies)

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NTP Technical Report on the Toxicology Studies of CIMSTAR 3800 in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of CIMSTAR 3800 in Wistar Han [CrI:WI(Han)] Rats and B6C3F1/N Mice (Inhalation Studies)

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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, 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 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.

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.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

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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.

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology Studies of CIMSTAR 3800 in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of CIMSTAR 3800 in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Inhalation Studies)* on May 22, 2014, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers had 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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Abstract

CIMSTAR 3800 is a metalworking fluid used as a lubricant and coolant liquid on the surface of the worked piece to remove heat and fine swarf and to provide corrosion inhibition at the newly cut surface. CIMSTAR 3800 is used in the general machining and grinding of automotive aluminum parts and on light to moderate machining and grinding of light steel, stainless steels, hardened steels, and other materials. CIMSTAR 3800 was nominated by the National Institute for Occupational Safety and Health for study by the National Toxicology Program because of its high production volume, the large number of occupationally exposed workers, the lack of carcinogenicity and chronic toxicology data, and because epidemiologic data indicate an increased incidence of laryngeal cancer in workers exposed to metalworking fluids. Male and female F344/NTac rats and B6C3F1/N mice were exposed to CIMSTAR 3800 by inhalation for 3 months, and male and female Wistar Han [Crl:WI (Han)] rats (referred to as Wistar Han rats) and B6C3F1/N mice were exposed to CIMSTAR 3800 by inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and rat and mouse peripheral blood erythrocytes.

Three-month Study in F344/NTac Rats

Groups of 10 male and 10 female F344/NTac rats were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3 for 6 hours plus T_{90} (18 minutes) per day, 5 days per week for 14 weeks. All rats survived to the end of the study, and the mean body weights of all exposed groups were similar to those of the chamber controls. All exposed males and most exposed females had goblet cell hyperplasia, hyaline droplet accumulation, and suppurative inflammation of the olfactory epithelium and hyaline droplet accumulation and suppurative inflammation of the respiratory epithelium in the nose. In the larynx, there were significantly increased incidences of squamous metaplasia, hyperplasia of the squamous epithelium, and chronic active inflammation in all exposed groups. In the lung, there were significantly increased incidences of alveolus histiocytic cellular infiltration in males and females exposed to 200 or 400 mg/m^3.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3 for 6 hours plus T_{90} (18 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. The final mean body weights and mean body weight gains of 400 mg/m^3 males and females and the final mean body weight of 200 mg/m^3 females were significantly less than those of the chamber controls. The absolute and relative lung weights of males exposed to 100 mg/m^3 or greater were significantly greater than those of the chamber controls. In the nose, there were significantly increased incidences of hyaline droplet accumulation of the olfactory and respiratory epithelia in all exposed groups. In the larynx, there were significantly increased incidences of squamous metaplasia in all exposed groups, hyperplasia of the squamous epithelium in 200 and 400 mg/m^3 males and females, chronic active inflammation in 50 mg/m^3 males and males and females exposed to 100 mg/m^3 or greater, and epiglottis dysplasia in 400 mg/m^3 males and females. In the lung, there were significantly increased incidences of bronchiole hyperplasia in all exposed groups, perivascular chronic active inflammation in 200 and 400 mg/m^3 males and 100 and 200 mg/m^3 females, and arteriole hypertrophy in 400 mg/m^3 males and 200 mg/m^3 females.

Two-year Study in Wistar Han Rats

Groups of 50 male and 50 female Wistar Han rats were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 10, 30, or 100 mg/m^3 for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival of all exposed groups was similar to that of the chamber control groups. Mean body weights of exposed groups of male and female rats were similar to those of the chamber controls throughout the study.

In males, there were slightly increased incidences of prostate gland adenoma in the 100 mg/m^3 group, and adenoma or carcinoma (combined) in the 30 and 100 mg/m^3 groups.

In females, there was a positive trend in the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin. There was also a nonsignificant increase in the incidence of uterine adenocarcinoma or mixed malignant Müllerian tumor (combined) (original and residual longitudinal evaluation) in the 100 mg/m^3 group.

In the nose, there were chemical-related, significantly increased incidences of goblet cell hyperplasia, hyperplasia of olfactory epithelium glands, and hyaline droplet accumulation of the olfactory and respiratory epithelia in all exposed groups of males and females.

There were significantly increased incidences of squamous metaplasia in the larynx in all exposed groups.

In the lung, there were exposure concentration-related, significantly increased incidences of lymphohistiocytic inflammation in all exposed groups and of lymphohistiocytic hyperplasia of bronchus-associated lymphoid tissue in 30 mg/m^3 males and 100 mg/m^3 males and females. There was also a significantly increased incidence of alveolar epithelium hyperplasia in 100 mg/m^3 males.

In the bronchial lymph nodes, there were exposure concentration-related, significantly increased incidences of lymphohistiocytic hyperplasia in 30 and 100 mg/m^3 males and females. In the mediastinal lymph nodes, there were exposure concentration-related, significantly increased incidences of lymphohistiocytic hyperplasia in 100 mg/m^3 males and females.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 10, 30, or 100 mg/m^3 for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival of all exposed groups was similar to that of the chamber control groups. Mean body weights of exposed groups of male and female mice were similar to those of the chamber controls throughout the study.

There was a positive trend in the incidences of follicular cell carcinoma in the thyroid gland of females. There were also exposure concentration-related increased incidences of follicular cell hyperplasia.

In the lung, there was a positive trend in the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in females, and the incidence was significantly increased in the 100 mg/m^3 group. There was also a positive trend in the incidences of alveolar/bronchiolar carcinoma in females. Significantly increased incidences of bronchiole hyperplasia occurred in 30 and 100 mg/m^3 males and females. The incidences of alveolar epithelium hyperplasia were slightly increased in 30 mg/m^3 males and females and 100 mg/m^3 males. The incidences of histiocytic cellular infiltration were slightly increased in 100 mg/m^3 males and females. In

100 mg/m^3 females, there was a significantly increased incidence of chronic active perivascular inflammation.

In the nose, there were significantly increased incidences of hyaline droplet accumulation of the olfactory and respiratory epithelia in all exposed groups of mice and respiratory metaplasia of the olfactory epithelium in all exposed groups of males and 30 and 100 mg/m^3 females. There were increased incidences of olfactory epithelium atrophy in 100 mg/m^3 males and chronic active inflammation in 10 and 100 mg/m^3 males.

In the larynx, the incidences of squamous metaplasia were significantly increased in all exposed groups of males and females. There were significantly increased incidences of chronic active inflammation in 100 mg/m^3 males and females.

Genetic Toxicology

CIMSTAR 3800 was mutagenic in *E. coli* strain WP2 *uvr*A/pKM101 in the absence of exogenous metabolic activation (S9); no mutagenic activity was observed in *S. typhimurium* strains TA98 and TA100, with or without S9, or in the *E. coli* strain with S9.

In vivo, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were observed in peripheral blood samples from male and female F344/NTac rats or B6C3F1/N mice exposed to CIMSTAR 3800 via inhalation for 3 months.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; see summary of the peer review panel comments and the public discussion on this Technical Report in Appendix K) of CIMSTAR 3800 in male Wistar Han rats based on the incidences of prostate gland adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of CIMSTAR 3800 in female Wistar Han rats based on the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin and adenocarcinoma or mixed malignant Müllerian tumor (combined) of the uterus. There was *no evidence of carcinogenic activity* of CIMSTAR 3800 in male B6C3F1/N mice exposed to 10, 30, or 100 mg/m^3. There was *some evidence of carcinogenic activity* of CIMSTAR 3800 in female B6C3F1/N mice based on the incidences of follicular cell carcinoma of the thyroid gland and alveolar/bronchiolar adenoma or carcinoma (combined) of the lung.

Exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice.

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of CIMSTAR 3800

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in air	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3
Body weights	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group
Survival rates	33/50, 34/50, 34/50, 33/50	35/50, 33/50, 36/50, 30/50	39/50, 31/50, 36/50, 40/50	39/50, 37/50, 37/50, 33/50
Nonneoplastic effects	Nose: goblet cell, hyperplasia (0/50, 20/50, 25/50, 34/50); glands, olfactory epithelium, hyperplasia (1/50, 39/50, 47/50, 50/50); olfactory epithelium, accumulation, hyaline droplet (19/50, 50/50, 50/50, 50/50); respiratory epithelium, accumulation, hyaline droplet (0/50, 17/50, 25/50, 29/50) Larynx: metaplasia, squamous (1/50, 47/50, 50/50, 50/50) Lung: inflammation, lymphohistiocytic (6/50, 14/50, 41/50, 47/50); bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic (0/50, 1/50, 5/50, 19/50); alveolar epithelium, hyperplasia (4/50, 6/50, 11/50, 13/50) Lymph node, bronchial: hyperplasia, lymphohistiocytic (0/42, 0/40, 10/37, 28/35) Lymph node, mediastinal: hyperplasia, lymphohistiocytic (0/46, 0/45, 4/50, 29/49)	Nose: goblet cell, hyperplasia (0/50, 25/50, 34/50, 42/50); glands, olfactory epithelium, hyperplasia (1/50, 32/50, 48/50, 49/50); olfactory epithelium, accumulation, hyaline droplet (16/50, 50/50, 50/50, 50/50); respiratory epithelium, accumulation, hyaline droplet (1/50, 24/50, 31/50, 34/50) Larynx: metaplasia, squamous (1/50, 50/50, 50/50, 50/50) Lung: inflammation, lymphohistiocytic (3/50, 20/50, 42/50, 50/50); bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic (0/50, 0/50, 0/50, 5/50) Lymph node, bronchial: hyperplasia, lymphohistiocytic (0/38, 0/35, 7/32, 30/35) Lymph node, mediastinal: hyperplasia, lymphohistiocytic (0/49, 0/46, 4/45, 23/47)	Lung: bronchiole, hyperplasia (11/50, 11/50, 32/50, 44/50); alveolar epithelium, hyperplasia (4/50, 4/50, 6/50, 7/50); infiltration cellular, histiocyte (5/50, 5/50, 1/50, 9/50) Nose: olfactory epithelium, accumulation, hyaline droplet (4/50, 31/50, 43/50, 49/50); respiratory epithelium, accumulation, hyaline droplet (7/50, 36/50, 50/50, 50/50); olfactory epithelium, metaplasia, respiratory (7/50, 15/50, 25/50, 37/50); olfactory epithelium, atrophy (1/50, 0/50, 0/50, 4/50); inflammation, chronic active (6/50, 8/50, 4/50, 12/50) Larynx: metaplasia, squamous (0/50, 50/50, 49/49, 50/50); inflammation, chronic active (0/50, 2/50, 3/49, 8/50)	Thyroid gland: follicular cell, hyperplasia (1/50, 1/48, 2/50, 3/50) Lung: bronchiole, hyperplasia (7/50, 4/50, 22/50, 41/50); alveolar epithelium, hyperplasia (4/50, 2/50, 5/50, 4/50); infiltration cellular, histiocyte (4/50, 1/50, 1/50, 6/50); perivascular, inflammation, chronic active (4/50, 3/50, 0/50, 11/50) Nose: olfactory epithelium, accumulation, hyaline droplet (25/50, 40/49, 50/50, 49/50); respiratory epithelium, accumulation, hyaline droplet (34/50, 48/49, 50/50, 50/50); olfactory epithelium, metaplasia, respiratory (3/50, 4/49, 12/50, 23/50) Larynx: metaplasia, squamous (1/49, 49/49, 50/50, 50/50); inflammation, chronic active (0/49, 0/49, 0/50, 10/50)

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Neoplastic effects	None	None	None	Thyroid gland: follicular cell carcinoma (0/50, 0/48, 0/50, 3/50) Lung: alveolar/bronchiolar carcinoma (4/50, 1/50, 4/50, 8/50); alveolar/bronchiolar adenoma or carcinoma (4/50, 5/50, 6/50, 12/50)
Equivocal findings	Prostate gland: adenoma (1/50, 1/50, 1/50, 3/50); adenoma or carcinoma (1/50, 1/50, 2/50, 3/50)	Skin: squamous cell papilloma or keratoacanthoma (0/50, 0/50, 0/50, 4/50) Uterus: adenocarcinoma or mixed malignant Müllerian tumor (original and residual longitudinal evaluation combined) (1/50, 4/50, 5/50, 6/50)	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	Equivocal evidence	No evidence	Some evidence
Genetic toxicology				
Bacterial gene mutations:		Positive in *E. coli* strain WP2 *uvrA*/pKM101 without S9; negative in *S. typhimurium* strains TA98 and TA100 with and without S9; negative in *E. coli* with S9		
Micronucleated erythrocytes				
Rat peripheral blood in vivo:		Negative in males and females		
Mouse peripheral blood in vivo:		Negative in males and females		

Overview

The class of compounds known as metalworking fluids was nominated by the National Institute of Occupational Safety and Health for toxicologic testing by the National Toxicology Program. Individual metalworking fluids are formulated based upon the intended use, and hundreds of metalworking fluid formulations are believed to be in use. Selection of specific metalworking fluids for toxicologic testing based upon use and production data was not possible because these data are considered proprietary by the industry and were not available. For this reason, selection of specific metalworking fluids was initiated by selecting 30 heavily marketed soluble oils and semisynthetic fluids. These two classes, soluble oils and semisynthetic fluids, encompass the majority of metalworking fluids in use. The number of candidate metalworking fluids was reduced to 18 based on commercial availability, Material Safety Data Sheets, and elimination of redundant formulations. The 18 products underwent chemical analysis and the number of potential test compounds was reduced to nine by selecting from a minimum of three major producers a combination of representative formulations and complex, unique formulations. The remaining nine products underwent further chemical analysis and genetic toxicity assessment prior to selecting four formulations for inhalation studies. CIMSTAR 3800, TRIM VX, Syntilo 1023, and TRIM SC210 were selected for 3-month inhalation studies. Based on the results of the 3-month studies, CIMSTAR 3800 and TRIM VX were selected for 2-year studies.

Introduction

Chemical and Physical Properties

CIMSTAR 3800 and all metalworking fluids are highly complex mixtures of chemicals. Only limited information exists about the chemical components of individual metalworking fluids because of the highly competitive and proprietary nature of the metalworking fluid industry. The identity and proportion of chemical species in these mixtures are dependent on a number of factors including the manufacturer and the cooling and lubrication requirements of the machining process. Metalworking fluids are classified into the general categories of straight oils, soluble oils, semisynthetic fluids, or synthetic fluids based upon the amount of highly refined oil they contain[1-3]. Straight oils contain 60% to 100% oil. The petroleum oils used in straight oil metalworking fluids are usually mineral oils from highly refined napthenic or paraffinic oils. The soluble oils (emulsifiable) and semisynthetic metalworking fluids contain 5% to 85% oil, and the synthetic fluids contain no oil.

CIMSTAR 3800 is classified as a semisynthetic metalworking fluid because it contains lower amounts of oil and higher amounts of water than the soluble or straight oil metalworking fluids. The manufacturer recommends diluting the CIMSTAR 3800 concentrate to 5% to 10% in water. The concentrated semisynthetic metalworking fluids generally are diluted to 5% to 20% in water. These solutions form oil-water emulsions with the water acting as a cooling agent and the oil providing lubrication. The semisynthetic metalworking fluids may also be formulated with mineral oils, fatty acids, sulfur, chlorine, and phosphorus derivatives to provide enhanced lubrication for higher speeds and feed rates[4]. Other additives can include detergents to lower surface tension, polyol ester as an “oiliness agent,” petroleum sulfonate as an emulsifier, alkanolamines to provide reserve alkalinity and inhibit corrosion, chlorinated paraffins as “extreme pressure agents,” long chain fatty alcohols as defoamers, triazoles as a metal passivator, antioxidants, dyes, and biocides[5].

The composition of in-use metalworking fluids is considerably different from unused metalworking fluids. Because metalworking fluids are expensive, single use is not economically feasible. The useable lifetime of metalworking fluids is extended by recycling the fluids in a circulating system that filters out solid contaminants. However, physical and chemical effects can cause the components of in-use metalworking fluids to deteriorate. Environmental contaminants such as wear debris, rust, weld spatter, metal chips and abrasives, as well as contaminants entering through broken seals, dirty oil filter pipes, and chemical residue on metal parts can accelerate metalworking fluid breakdown. In addition, excessive temperatures can cause oxidation of metalworking fluid oils and constituents leading to the formation of acids, resins, varnishes, sludges, and carbonaceous deposits. Additives may be depleted with use, requiring routine product addition or supplemental additions to maintain metalworking fluid performance.

Water-based metalworking fluids are excellent nutritional sources for many kinds of bacteria and fungi, and contamination of in-use metalworking fluids is a problem in the metalworking industries. Bacteria, bacterial endotoxins, and fungi in metalworking fluid aerosols are a concern because of potential respiratory effects when inhaled by workers and skin infections following dermal contact. Antimicrobial agents are often added to metalworking fluids to control the

growth of microbes and to extend the usable life of these fluids. Some microbiocidal or microbiostatic activities of antimicrobial agents occur through the release of formaldehyde. Formaldehyde is an airway irritant and a recognized cause of occupational asthma[6]. Studies suggest that exposure to certain antimicrobial agents can cause allergic or irritant contact dermatitis[7].

Compositions of metalworking fluids are being continuously changed to improve various functions and reduce potential toxicity. Efforts to reduce exposures to potential carcinogens in metalworking fluids have been ongoing. Removal of polycyclic aromatic hydrocarbons (PAHs) from metalworking fluids began in the 1950s, and United States Environmental Protection Agency (USEPA) regulations in the 1980s were directed at reducing nitrosamine exposures. In addition, efforts to reduce volatile organic compounds have resulted in the increased use of water as a major component of currently used metalworking fluids. For these reasons, toxicity and carcinogenicity data on metalworking fluids obtained prior to the 1980s may not apply to metalworking fluids currently in use. Although many carcinogenic components have been removed, the newer metalworking fluids have not been tested so the cancer risk is not clear.

Production, Use, and Human Exposure

CIMSTAR 3800 is a semisynthetic metalworking fluid manufactured by one of the four largest metalworking fluid manufacturers and was considered a high-production formulation based on selection criteria used by the National Institute for Occupational Safety and Health (NIOSH). Metalworking fluid production and use data are considered proprietary information by the metalworking fluid manufacturers and these data are not available. The Independent Lubricant Manufacturers Association reported that 95 to 103 million gallons of metalworking fluids were produced annually in the United States from 1994 to 1999[8]. In 1999, five companies each reported producing more than 5 million gallons of these fluids. The largest category of metalworking fluids in terms of industry consumption is the metal removal fluids with more than 100 million gallons consumed annually[8]. Metal removal fluids are typically classified as either nonmiscible or miscible with water. Nonmiscible metal removal fluids are straight oils or neat oils and typically mineral oils. Miscible metal removal fluids are subdivided into water-soluble oils, semisynthetic fluids, and synthetic fluids. It is estimated that 80 million gallons of miscible metal removal fluids are sold each year in the United States and that this constitutes approximately 75% of the total metal removal fluid market.

Metalworking operations require application of lubricant and coolant liquids on the surface of the workpiece and the tool to remove heat, remove fine swarf (metal chips), and provide corrosion inhibition at the newly cut surface. CIMSTAR 3800 is recommended for use in general machining and grinding of automotive aluminum parts. It is also used on light to moderate machining and grinding of light steel, stainless steels, hardened steels, and other materials.

The National Occupational Exposure Survey[9] estimates that 1.2 million workers are potentially exposed to metalworking fluids. A more recent exposure survey has not been conducted; however, health hazard evaluations from the NIOSH[10] indicate that exposures to metalworking fluid aerosols have decreased over time.

Exposure to metalworking fluids occurs primarily from inhalation of aerosols and dermal contact with aerosols or wetted equipment. Workers can receive significant exposure to metalworking

fluids by inhalation of aerosols[11]. Aerosols form when a metalworking fluid is subjected to high shear forces or excess heat during use. The characteristics of these aerosols are dependent upon the particular metalworking fluid and the machining process for which it is being used; however, some aerosols can be stable and long lasting[12]. Dermal exposure to metalworking fluids can occur through contact with contaminated equipment, from splashes, or exposure to aerosols. The components and alkalinity of metal-working fluids are well-known causes of dermatitis in workers[13; 14]. CIMSTAR 3800 concentrate is diluted with water prior to use, and the aerosols generated in the workplace are composed of 90% to 95% water. In the current studies, it was not feasible to generate liquid aerosols containing up to 90% water without saturating the chamber air; therefore, aerosols were generated from the undiluted concentrate and diluted with clean air to produce the desired concentrations. As a result, the exposure concentrations in the current studies were considerably higher than those encountered in the workplace.

Regulatory Status

NIOSH recommends an exposure limit (REL) for all metalworking fluid aerosols of 0.4 mg/m^3 for thoracic particulate mass as a time-weighted average concentration for up to 10 hours per day during a 40-hour work week. Because of the limited availability of thoracic samplers, measurement of total particulate mass is an acceptable substitute. The NIOSH REL for total particulate mass is 0.5 mg/m^3[15].

Absorption, Distribution, Metabolism, and Excretion

No studies evaluating the absorption, distribution, metabolism, or excretion of metalworking fluids in experimental animals or humans were found in the literature.

Toxicity

Experimental Animals

Animal models have been used to evaluate the respiratory toxicity of different classes of metalworking fluids. Schaper and Detwiler[16] used a mouse model of pulmonary irritation[17] to compare the effects of different classes of metalworking fluids on pulmonary function of mice. Inhalation of straight oil aerosols did not cause as great a response (increased breathing rate) as exposure to semisynthetic fluids, synthetic fluids, or soluble oils. There were no significant differences in pulmonary irritation caused by used versus unused metalworking fluids. In a subsequent study[18], the same mouse model was used to evaluate the sensory irritation and pulmonary irritation properties of 10 metalworking fluids and to identify the contribution of three major components. Sensory irritation was caused mainly by tall oil fatty acids, whereas pulmonary irritation was caused primarily by sodium sulfonate. Paraffinic oils decreased the irritant properties of both tall oil fatty acids and sodium sulfonate.

Lim et al.[19] investigated the respiratory toxicity of a water-soluble metalworking fluid in F344 rats exposed to aerosol concentrations of 0, 20, 60, or 180 mg/m^3 6 hours per day, 5 days per week for 13 weeks. Pulmonary inflammation consisting of increases in lung weights, bronchoalveolar lavage (BAL) neutrophils, and foamy macrophages and thickening of the alveolar walls was observed at the two highest concentrations.

The effects of metalworking fluids contaminated with endotoxins have been investigated in experimental animals by several groups of investigators. Acute inhalation exposure of mice to a high concentration of in-use metalworking fluid aerosol contaminated with endotoxin caused a significant elevation of neutrophils, TNF-α, and IL-6 in BAL fluid[20]. Acute exposure of rats to a low concentration of metalworking fluid containing endotoxin also caused an increase in BAL neutrophils[21]. Lim et al.[22] investigated the effects of repeated exposure to a water-soluble metalworking fluid with and without endotoxin for 2 weeks. Endotoxin-contaminated metalworking fluid caused a significantly elevated inflammatory response in the lung relative to uncontaminated metalworking fluid.

Humans

Occupational exposure to metalworking fluid aerosols is associated with a variety of nonmalignant respiratory conditions. Hypersensitivity pneumonitis involves an immunologic reaction in the lung to inhaled antigen and may require prior sensitization to the antigen. This disease is characterized in its acute phase by alveolar inflammation and influenza-like symptoms. In its chronic phase (following repeated exposures), it is characterized by pulmonary fibrosis associated with respiratory impairment. Two cases of hypersensitivity pneumonitis associated with metalworking fluids were reported during a 3-year period to an occupational respiratory disease surveillance program operating in the United Kingdom[23]. Many more cases in North America have been recently recognized[24-27].

A variety of components, additives, and contaminants of metalworking fluids are sensitizers or irritants known to induce new-onset asthma, aggravate preexisting asthma, or irritate the airways of non-asthmatic workers. These sensitizers, irritants, or toxicants include ethanolamine and other amines, colophony, pine oil, tall oil, metals and metallic salts (e.g., chromium, nickel, cobalt, and tungsten carbide), castor oil, formaldehyde, chlorine, various acids, and fungal and other microbial contaminants (including gram-negative bacterial endotoxin)[28-30]. However, only a few of these agents have been documented as causes of metalworking fluid-associated asthma.

Considered in aggregate, several studies provide evidence indicative of an elevated risk of asthma among workers exposed to metalworking fluid aerosol concentrations currently found in large metalworking shops. As suggested by published clinical case reports, asthma induced by metalworking fluids appears to involve known sensitizers in some cases; however, various other agents acting possibly through irritant or inflammatory mechanisms may be responsible for a high proportion of metalworking fluid-associated asthma cases. Some evidence from cross-sectional studies strongly suggests a tendency for affected workers to transfer away from jobs with exposure to metalworking fluids.

With respect to metalworking fluid type, exposure to metalworking fluid aerosols in operations using synthetic fluids has been associated with asthma[31-33]. There is also evidence suggesting a causal association between asthma and exposure to soluble metalworking fluid aerosols, but it is somewhat less consistent than that for synthetic metalworking fluid exposures. Case reports have documented asthma caused by exposure to soluble oil metalworking fluids[28; 34] or to common components of soluble oil metalworking fluids[35]. A surveillance program in Michigan received 13 case reports of occupational asthma attributed to soluble oil metalworking fluids during 1988 to 1994[33], although some of the plants in which these patients worked may have also been using straight oil metalworking fluids. Of the seven relevant epidemiologic studies, results consistent

with statistically significant elevated risk estimates were presented only by Greaves et al.[32] (for cumulative exposure) and Rosenman et al.[33]. Findings of three of the other five studies indicated elevated, though not statistically significant, risk estimates for asthma, with point estimates ranging upward from 2.1[36-39]. Thus, the overall evidence also suggests an association between asthma and exposure to straight oil metalworking fluid aerosol[33; 34; 40].

Epidemiologic studies of respiratory symptoms present generally consistent, and in the case of the more recent studies, compelling evidence that occupational exposure to metalworking fluid aerosols causes symptoms consistent with airway irritation, chronic bronchitis, and asthma. The evidence suggests that at least three classes of metalworking fluids (straight oil, soluble oil, and synthetic) are capable of inducing respiratory symptoms at aerosol exposure concentrations that are currently typical of large metalworking shops. To date, there is no convincing evidence that identifies any particular component or components of metalworking fluid aerosol as the predominant cause of these symptoms, although some irritant components of metalworking fluid are clearly suspect[41]. One large multiplant study in the United States with mean exposures for the major types of metalworking fluids ranging from 0.41 to 0.55 mg/m^3 (thoracic fraction) found statistically significant quantitative exposure-response relationships between cumulative concentration of metalworking fluid aerosols and respiratory symptoms[32]. Likewise, another United States study found significant exposure-response relationships between aerosol exposure concentration and chest symptoms[41]. In addition, other studies have shown the onset or worsening of many symptoms in workers over the course of a work shift[33; 36; 41] and substantial symptomatic improvement in affected workers when away from work[32]. Thus, controlling worker exposures can prevent chronic effects induced by metalworking fluid aerosol exposure and may reverse early metalworking fluid-induced airway effects.

Reproductive and Developmental Toxicity

No studies on metalworking fluid reproductive or developmental toxicity in experimental animals or humans were found in the literature.

Carcinogenicity

Experimental Animals

There have been no carcinogenicity studies of CIMSTAR 3800 in animals. Six studies have examined the carcinogenicity of metalworking fluids in experimental animals[42-47]. Three of these studies reported findings related only to the skin[43-45]. Of these three studies, one examined unrefined cutting oil[43], one examined solvent-extracted cutting oil[45], and the third did not specify how the cutting oil was refined, although the cutting oil was probably highly refined as the PAH content was only 5.22%[44]. The study by Gilman and Vesselinovitch[43] found that among mice receiving skin applications of soluble cutting oils formulated from unrefined distillates three times weekly for 310 days, 61% developed skin tumors (22% of these had carcinomas) while no tumors occurred in the unexposed control group. Jepsen et al.[45] reported that among mice receiving skin applications of solvent-extracted cutting oils, 80% developed papillomas after exposure to undiluted soluble oil while there were no papillomas in mice exposed to diluted soluble oil. Jepsen et al.[45] also studied paraffin- and naphthalene-based straight oil metalworking fluids and found that 45% or 0% of mice developed papillomas after exposure to unused or used paraffin-based solvent-refined straight oil metalworking fluid (level of refining unspecified),

respectively. These investigators also reported that 40% or 100% of mice developed papillomas after exposure to unused or used naphthalene-based straight oil metalworking fluid (level of refining unspecified), respectively. Another study found that both unused and used cutting oils were potent skin tumor initiators[44]. These investigators reported that among mice given a single application of a cutting oil and thrice weekly application of the tumor promoting agent 12-*O*-tetradecanoyl-phorbol-13-acetate, 90% or 60% of mice developed benign skin cancers after exposure to unused or used cutting oil, respectively.

Three other animal studies examined the carcinogenic effects of metalworking fluid exposure on the skin and other organs[42; 46; 47]. In one study of 20 mice receiving skin applications of used cutting oils (type of refining unspecified) one to three times weekly for 6 months, two mice developed pulmonary cancer, and one of the two mice also developed skin cancer; none of the control mice developed cancer[42]. In a 2-year study in Wistar rats, pancreatic carcinoma occurred in nine of 40 rats dosed with undiluted rustproof cutting fluid consisting of sodium nitrite, triethanolamine (TEA), and polyethylene glycol, while none of the control rats developed pancreatic cancer[47]. All three of the components reported to be in this rustproof cutting fluid can be found in some metalworking fluids used in the United States. A third study found no evidence of carcinogenicity from solvent-extracted cutting oils[46].

Specific components of metalworking fluids [e.g., diethanolamine (DEA), TEA, nitrosoamines, and formaldehyde] have been evaluated for carcinogenic potential on an individual basis. Variable amounts of alkanolamines are present in many metalworking fluids. The carcinogenicity of TEA was investigated because of its potential conversion to the carcinogen *N*-nitrosodiethanolamine (NDELA). Following dermal exposure for 2 years, there was equivocal evidence of carcinogenic activity of TEA in male F344/N rats based on marginally increased incidences of renal tubule cell adenoma and no evidence of carcinogenicity in female rats[48]. In a 2-year dermal study of TEA in B6C3F1 mice, there was equivocal evidence of carcinogenicity in males based on the occurrence of liver hemangiosarcoma and some evidence of carcinogenicity in females based on increased incidences of hepatocellular adenoma[49]. The carcinogenicity of DEA was also studied in F344/N rats and B6C3F1 mice in a 2-year dermal exposure study[50]. There was no evidence of carcinogenic activity in rats; however, there was clear evidence of carcinogenic activity of DEA in mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males. DEA is not present in CIMSTAR 3800.

Humans

There have been no epidemiologic studies to evaluate the carcinogenicity of CIMSTAR 3800 in humans. Substantial evidence exists for increased risk of squamous cell skin cancer[51-53] especially of the scrotum[54-56] following exposure to metalworking fluids. The International Agency for Research on Cancer[57] designated unused and mildly used mineral oil as carcinogenic to humans based on squamous cell carcinoma of the skin, sinonasal cancer, and bladder cancer. NIOSH[15] concluded that substantial evidence exists for an increased risk of cancer at several tissue sites (larynx, rectum, pancreas, skin, scrotum, and urinary bladder) associated with at least some of the metalworking fluids used before the mid-1970s. The inconsistencies between studies with respect to the tissue sites that were affected, and the variation in the strength of association between the surrogates of exposure and specific sites are most likely related to the diverse nature of the metalworking fluid mixtures studied, the absence of detailed exposure information, and

the limitations of the epidemiologic tools with which metalworking fluid exposures have been studied. The evidence is equivocal for an association between metalworking fluid exposure and cancer at several other sites including the stomach, esophagus, lung, prostate gland, brain, colon, and hematopoietic system.

Given the small number of epidemiologic studies that have adequate exposure characterization, the specific metalworking fluid constituents or contaminants responsible for the various site-specific cancer risks remain to be determined. The study with the most statistical power and detailed exposure information included more than 46,000 autoworkers in Michigan[58]. Results of this study suggest that specific classes of metalworking fluids are associated with cancer at certain sites. However, within these metalworking fluid classes, the specific formulations responsible for the elevated cancer risks remain to be identified. Within the Tolbert et al.[58] study, straight oil exposure was modestly associated with increased risks for laryngeal and rectal cancers, and there was limited evidence that synthetic metalworking fluid exposure was associated with an increased risk for pancreatic cancer. Subsequent case-control studies based on the original cohort have confirmed the association of laryngeal cancer with straight oil metalworking fluids[59], and the association of pancreatic cancer with synthetic metalworking fluids[60]. The Tolbert et al.[58] study found less evidence that soluble oil metalworking fluid exposure is associated with cancer at any specific site. The most recent extension of this study up to 2005 added a follow-up for incident cancer. The cohort was limited to white males hired after 1938. The results of this evaluation provided evidence that oil-based metalworking fluids, especially straight mineral oils, were associated with an increased incidence of malignant melanoma[61]. In another evaluation of the same incident cohort, long-term dermal exposure to oil-based metalworking fluids was reported to cause an increased risk of nonseminomatous testicular germ cell cancer[62; 63].

The studies that provide most of the data suggesting an association between metalworking fluid exposure and cancer involved workers employed as early as the 1930s and as late as the mid-1980s. Because there is a latency period of 10 to 20 years between initial exposure to a carcinogen and the initial appearance of a solid-organ cancer caused by that carcinogen, the excess cancer mortality observed in these cohort studies most likely reflects the cancer risk associated with exposure conditions in the mid-1970s and earlier. Over the last several decades, substantial changes have been made in the metalworking industry, including changes in metalworking fluid composition, reduction of impurities, and reduction of exposure concentrations. These changes have likely reduced the cancer risks. However, since the epidemiologic data do not usually identify the metalworking fluid composition and impurities associated with the cancer risks observed in earlier cohorts, there are insufficient data to conclude that these changes will have eliminated all carcinogenic risks. The risk of cancer from metalworking fluid exposures in the mid-1970s and later remains to be determined because a definitive study has not yet been conducted on workers entering metalworking fluid-exposed jobs during this period. Thus, there is an unclear potential for current metalworking fluids to pose a similar carcinogenic hazard.

Genetic Toxicity

One epidemiologic study was identified in the literature that evaluated levels of DNA strand breaks, measured by alkaline elution, in 65 metalworkers occupationally exposed to synthetic cutting fluids in seven plants in Germany[64]. In this study, DNA damage levels were correlated

with airborne concentrations of NDELA, a genotoxic contaminant present in some metalworking fluids, and with duration of daily exposure to cutting fluids. Workers employed in areas with high concentrations of NDELA (>500 ng/m^3) had a significantly ($P < 0.01$) elevated mean number of DNA strand breaks in mononuclear blood cells compared with workers employed in areas with less than 50 ng NDELA/m^3 (1.69 ± 0.34 versus 0.76 ± 0.05, respectively). In addition, of the 37 nonsmokers among the 65 metalworkers, those who worked with cutting fluids more than 4.5 hours/day had a significantly ($P < 0.02$) elevated mean number of DNA strand breaks compared with those who worked less than 4.5 hours/day (1.34 ± 0.12 versus 0.91 ± 0.12, respectively). NDELA can be formed in metalworking fluids when DEA or TEA, present in these fluids, react with a nitrosating agent. However, in 1984, the USEPA prohibited the addition of nitrosating agents to metalworking fluids, which precludes extrapolation of this finding to CIMSTAR 3800. Fuchs et al.[64] did not provide details regarding the composition of the various metalworking fluids to which workers were exposed in their study, and therefore, it is not possible to determine if the correlation between DNA damage and increasing duration of exposure might have been due to ingredients other than NDELA that may also be present in the CIMSTAR 3800 formulation.

Study Rationale

CIMSTAR 3800 is one of two semisynthetic metalworking fluids nominated by NIOSH for study by the National Toxicology Program. The nomination of CIMSTAR 3800 for inhalation studies is based upon its high production volume, the large number of occupationally exposed workers, the lack of carcinogenicity and chronic toxicology data for this class of complex mixtures, and epidemiologic data indicating an increased incidence of laryngeal cancer in workers exposed to metalworking fluids.

Materials and Methods

Procurement and Characterization of CIMSTAR 3800

CIMSTAR 3800 was obtained from Milacron (Cincinnati, OH) in three lots (60224BBN, 71205BN, and 90317JN). Lot 60224BBN was used during the 3-month studies, and lots 71205BN and 90317JN were used during the 2-year studies. Characterization and stability analyses of the test material were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA) (Appendix H). Reports on analyses performed in support of the CIMSTAR 3800 studies are on file at the National Institute of Environmental Health Sciences.

Spectra of lots 60224BBN, 71205BN, and 90317JN of the test material, a yellow-orange liquid, were obtained by the analytical chemistry laboratory using Fourier transform infrared (FTIR) spectroscopy. FTIR was used to estimate the relative presence of various functional groups and create a reference for future comparison for the same lot or different lots. In general, the spectra were consistent with the presence of organic amines, and the spectra of the three lots were similar to each other.

Analyses for all lots included Karl Fischer titration for water content; the determination of pH, specific gravity, and refractive index; elemental analysis for carbon, hydrogen, nitrogen, and sulfur using a C, H, N, S analyzer; and elemental and metal analysis using inductively coupled plasma/optical emission spectrometry (ICP/OES); chloride, nitrate, and nitrite analysis by ion chromatography; and iodide by ion specific electrode titration. The study laboratory initially identified general organic components using gas chromatography (GC) with flame ionization detection (FID) and identified the major components using GC with mass spectrometry (MS) (lot 60224BBN). Alkanolamines were identified and quantified using high performance liquid chromatography (HPLC) with MS detection. Methanol was quantified using GC/FID (lot 60224BBN) or GC/MS (lots 71205BN and 90317JN). The oil fraction was assessed using GC/MS and GC/FID. The total amount of hexane extractable material was determined gravimetrically. The amounts of bacteria and fungi were determined using a Sani-Check BF® test kit (Biosan Laboratories, Warren, MI). Samples were diluted to 10% with sterile water and applied to paddles coated on one side with media for the determination of bacteria and on the other side for the determination of fungi, then incubated for 7 days at 25° to 30°C.

The test material was determined to be primarily composed of water, alkanolamines, and oil. Boron was present at approximately 0.6%. The material was basic with a pH of approximately 9.0. In general, the FTIR spectra were consistent with the presence of organic amines, which contained less than 0.01% of water soluble nitrates, nitrites, or chlorides. The mass balance percentages based on elemental and compound contributions resulted in 96% and 96% (lot 60224BBN) and 92% and 96% (lot 71205BN) coverage, respectively. There were no significant differences between the three lots. Taken together, these data indicate that all three lots of the test material were CIMSTAR 3800 with the expected composition of organic amines (Table H-3).

To ensure stability, the bulk test material was stored at approximately 63°F, protected from light, in metal drums. Periodic reanalyses of lots 71205BN and 90317JN were performed by the analytical chemistry laboratory and the study laboratory at least every 6 months during the 2-year studies. For each lot, FTIR spectra were obtained and compared to reference spectra of the same

lot and other lots; determinations were made for the pH, specific gravity, and refractive index; determination and quantitation of alkanolamines were performed using HPLC/MS; assessment of fatty acid methyl esters was performed using GC/FID; and the amounts of bacteria and fungi were determined. No degradation of the bulk test material was detected.

Aerosol Generation and Exposure System

The generation and delivery system used in the 3-month and 2-year studies consisted of two generator assemblies configured together so that the output from each assembly containing multi-jet nebulizers was directed to a common distribution line. CIMSTAR 3800 was continuously pumped to the liquid reservoir from the chemical cabinet reservoir by metering pumps during the aerosol generation process. A constant volume of test material was maintained in each assembly by a siphon tube inserted near the top of the assembly and connected to a vacuum source. Ports in the generator assembly introduced heated compressed air to drive the nebulizers and heated dilution air to transport aerosol to the distribution line.

High velocity compressed air created a vacuum in the liquid uptake tube that drew test material from the liquid reservoir into the multi-jet nebulizer streams where shear force broke the resultant liquid filaments into droplets. Large droplets were impacted on the impaction plate of the nebulizer or the generator assembly walls and were returned to the liquid reservoir; smaller droplets were drawn into the heated dilution air and transported to the common distribution line. The common distribution line was divided into two branches to supply aerosol to exposure chambers located on both sides of the exposure room; each branch line was insulated and terminated in a filter protecting the flowmeter controlling the line via the house vacuum supply. During exposures, at each chamber position, aerosol was removed from the distribution line and injected into a tee fitting and directed to the inlet of the exposure chamber where it was mixed with conditioned air to achieve the desired exposure concentration.

Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table H-4 and Table H-5. The concentration of boron in CIMSTAR 3800 was monitored using real-time aerosol monitors (RAMs). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m^3 chamber or the room, a HEPA-filtered room air blank, and two exposure chambers. The output voltage of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and boron in CIMSTAR 3800 concentrations that were determined by analyzing tandem filters collected daily from the exposure chambers.

For the 3-month studies and from April to July 24, 2008, in the 2-year studies, boron in CIMSTAR 3800 was extracted from the filters, shaken on an orbital shaker table, filtered and analyzed using ICP/OES. The ICP/OES instrument was calibrated against serially diluted CIMSTAR 3800 and a NIST-traceable spectrometric internal standard of yttrium.

After July 24, 2008, to the end of the study, the calibration of the RAMs was based on gravimetric determination of the amount of test material on the filters. Samples were acidified, serially extracted with *n*-hexane, chemically dried, solvent evaporated, desiccated, and weighed.

Chamber Atmosphere Characterization

Particle size distribution in each chamber was determined prior to the start of all studies and monthly during the studies. Impactor samples were taken from each exposure chamber using a Mercer-style seven-stage impactor and the stages were initially collected on glass coverslips lightly coated with silicone (stages 1–7) or filters (stage 8) and were analyzed by ICP/OES for boron in CIMSTAR 3800 for the 3-month studies and from April 2008 until July 23, 2008, for the 2-year studies. However, for the remainder of the 2-year studies, gravimetric samples were collected using stainless steel coverslips (stages 1–7) or glass fiber filters coated with Teflon® (stage 8) and analyzed gravimetrically as described previously. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis[65]. The resulting estimates of the mass median aerodynamic diameter and the geometric standard deviation of each set of samples are given in Table H-6, Table H-7, and Table H-8.

Buildup and decay rates for chamber aerosol concentrations were determined with and without animals present in the chambers. At a chamber air flow rate of 15 cubic feet per minute, the theoretical values for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) was approximately 9.4 minutes for the 3-month and 2-year studies. T_{90} values of 18 minutes and 12 minutes were selected for the 3-month and 2-year studies, respectively.

The uniformity of aerosol concentration in the inhalation exposure chambers with animals present was measured once during the 3-month studies and was evaluated before the 2-year studies began without animals present and every 3 months during the 2-year studies. RAM measurements were taken from 12 different chamber positions. Chamber concentration uniformity was acceptable throughout the 3-month and 2-year studies.

The persistence of CIMSTAR 3800 in the exposure chambers was monitored after aerosol delivery ended by monitoring the concentration overnight in the 400 mg/m^3 rat and mouse chambers during the 3-month studies and the 100 mg/m^3 rat and mouse chambers during the 2-year studies with and without animals present. The average CIMSTAR 3800 concentration decreased to 1% of the target concentration within 18 (rats and mice, 3-month studies), 14 (male rats, 2-year studies), 16 (female rats and mice, 2-year studies), or 15 (without animals, 2-year studies) minutes.

Stability studies of the test material in the generation and exposure system were performed before and during the studies by the study laboratory and the analytical chemistry laboratory. For the 3-month and the 2-year stability studies without animals present in the exposure chambers, the relative amounts of the major constituents in the exposure atmosphere generally reflected that of the bulk test material. The concentrations of all constituents were higher in the liquid reservoirs at the end of the exposure day indicating the loss of water. Boron, triethanolamine, and fatty acid methyl esters were present primarily as particulates while ethanolamine and 1-amino-

2-propanol had significant vapor phase contributions. The percentage of methanol in the liquid reservoirs was lower than that in the bulk test material.

Approximately 4 months into the 2-year studies, exposure chamber analyses with animals present indicated a shift in the relative amounts of several of the constituents relative to boron. Characterization of the test material was performed and compared with the results obtained prior to the study; all results were consistent, indicating that the test material was stable. Further investigation indicated that boron was not a good marker for the determination of CIMSTAR 3800 concentrations. The decision was made to calibrate the RAMs using CIMSTAR 3800 concentrations based on gravimetric determinations for the remainder of the 2-year studies.

Samples were collected, prepared, and analyzed gravimetrically. Instruments were calibrated for each method using gravimetrically prepared dilutions of the bulk test material. Constituents were within approximately 30% of the on-line monitor results in the distribution line and were comparable to the results observed during prestart assays. Triethanolamine concentrations were 95% to 107% of the on-line monitor results in the chamber atmosphere samples; fatty acid methyl ester concentrations were within 35% in the 10 mg/m^3 chambers and 20% in 100 mg/m^3 chambers. The relative proportions for boron and the volatile alkanolamines were reduced in the 100 mg/m^3 chambers and the reductions were greater in the rat/mouse chambers than in the male rat chambers. The greatest degree of reduction was seen in the 10 mg/m^3 rat/mouse chambers where boron was 34%, ethanolamine was 16%, and 1-amino-2-propanol was 30% of the expected values.

Methanol concentrations were increased in the reservoirs as well as the distribution line and exposure chambers. However, other volatile constituents (ethanolamine and 1-amino-2-propanol) were comparable in the reservoirs as well as the distribution line compared to prestart measurements while decreasing in the exposure chamber atmosphere with decreasing exposure concentrations, most significantly in the 10 mg/m^3 exposure chambers.

The concentrations of all constituents were 94% to 108% relative to the bulk test material in the chemical cabinet reservoir at the end of the exposure day. Concentrations of these constituents were 117% to 150% higher in the liquid reservoirs indicating the loss of water, comparable to prestart measurements.

Animal Source

Male and female F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc. (Germantown, NY), and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for the 3-month studies. Male and female Wistar Han [Crl:WI (Han)] rats were obtained from Charles River Laboratories (Raleigh, NC) and male and female B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for use in the 2-year studies. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax, and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative

purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP time to evaluate different rat models between 2005 and 2006[66]. The Wistar Han rat, an outbred rat stock, was then selected because it was projected to have a long lifespan, resistance to disease, large litter size, and low neonatal mortality.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Toxicology Northwest Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to CIMSTAR 3800 and to determine the appropriate exposure concentrations to be used in the 2-year studies.

On receipt, rats were 3 to 4 weeks old and mice were 4 to 5 weeks old. Animals were quarantined for 11 days and were 5 to 6 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. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix J).

Groups of 10 male and 10 female rats and mice were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3, 6 hours plus T_{90} (18 minutes) per day, 5 days per week for 14 weeks. Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Clinical findings were recorded after exposure on day 1, twice daily on days 2 through 5, 8 through 12, and 19, weekly thereafter, and at study termination. The animals were weighed initially, on days 6, 13, and 19, weekly thereafter, 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 rats and the retroorbital sinus of mice at the end of the studies for hematology and clinical chemistry (rats only) analyses. For the hematology samples, blood was collected in tubes containing potassium EDTA; for the clinical chemistry samples, the blood was collected in a tube devoid of anticoagulant but containing a separator gel for serum. An Abbott Cell-Dyn 3700 (Abbott Diagnostics Systems, Abbott Park, IL) was used to determine packed cell volume; hemoglobin concentration; erythrocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration. Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge, Heraeus Holding GmbH; Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to Cell-Dyn values for packed cell volume. Blood smears were stained with a Romanosky-type aqueous stain in a Wescor 7100 slide stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts were based on classifying

a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as reticulocyte:erythrocyte ratio using the Miller disc method[67]. Blood samples for clinical chemistry analyses were analyzed using a Roche Hitachi 912 System (Roche Diagnostic Corporation, Indianapolis, IN). The hematology and clinical chemistry parameters measured are listed in Table 1.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination 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. Complete histopathologic examinations were performed by the study laboratory pathologist on chamber control and 400 mg/m^3 rats and mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to an NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[68] and Boorman et al.[69].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed by whole body inhalation to CIMSTAR 3800 aerosol at concentrations of 0, 10, 30, or 100 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks.

Rats were quarantined for 13 days 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 were approximately 6 weeks old and mice 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 J).

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

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks and at study termination. Body weights were recorded on day 1, weekly for 13 weeks, every 4 weeks thereafter and at study termination.

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 and testes were first fixed in a modified 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. A special study of the uterus was conducted to examine the residual uterine tissue for additional lesions. In the original evaluation, a transverse section through each uterine horn, approximately 0.5 cm distal to the cervix, was collected for histopathologic evaluation. For the residual evaluation, all remaining cervices, vaginas, and uterine remnants were processed, sectioned longitudinally (i.e., parallel to the long axis), and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 1.

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 data 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 QA pathologist evaluated slides from all tumors and all potential target organs, which included the lung, larynx, and nose of rats and mice; bronchial and mediastinal lymph nodes, prostate gland, and testis of rats; and trachea of mice.

The QA 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 QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. 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[68] and Boorman et al.[69]. 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.[70].

Table 1. Experimental Design and Materials and Methods in the Inhalation Studies of CIMSTAR 3800

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
F344/NTac rats B6C3F1/N mice	Wistar Han rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
11 days	Rats: 13 days Mice: 12 days
Average Age When Studies Began	
5 to 6 weeks	Rats: 6 weeks Mice: 5 to 6 weeks
Date of First Exposure	
June 12, 2006	Rats: April 14, 2008 Mice: May 5, 2008
Duration of Exposure	
6 hours plus T_{90} (18 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure	
Rats: September 11 (males) or 12 (females), 2006 Mice: September 13 (males) or 14 (females), 2006	Rats: April 11–13 (males) or 13–15 (females), 2010 Mice: May 2–4 (males) or 4–6 (females), 2010
Necropsy Dates	
Rats: September 12 (males) or 13 (females) 2006 Mice: September 14 (males) or 15 (females), 2006	Rats: April 12–14 (males) or 14–16 (females), 2010 Mice: May 3–5 (males) or 5–7 (females), 2010
Average Age at Necropsy	
Rats: 18 to 19 weeks Mice: 19 to 20 weeks	Rats: 110 to 111 weeks Mice: 109 to 111 weeks
Size of Study Groups	
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 3-month studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo

Three-month Studies	Two-year Studies
Diet	
Irradiated NTP-2000 wafers (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure periods, changed weekly	Same as 3-month studies
Water	
Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 3-month studies
Cages	
Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly, rotated on study days 5 through 12 then weekly within chambers	Same as 3-month studies, except rotated weekly within chambers
Cageboard	
Techboard® untreated paper excreta pan liner (Shepherd Specialty Papers, Kalamazoo, MI), changed daily	Techboard® Ultra untreated paper excreta pan liner (Shepherd Specialty Papers, Watertown, TN), changed daily
Chamber Air Supply Filters	
Single HEPA, new at study start; charcoal (RSE, Inc., New Baltimore, MI), new at study start; Purafil (Environmental Systems, Lynnwood, WA), new at study start	Same as 3-month studies, except HEPA changed annually
Chambers	
Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE), changed weekly, excreta pans changed daily	Same as 3-month studies
Chamber Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour
Exposure Concentrations	
0, 25, 50, 100, 200, and 400 mg/m^3	0, 10, 30, and 100 mg/m^3
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, on days 6, 13 and 19, weekly thereafter, and at the end of the studies; clinical findings were recorded on day 1 after exposure, twice daily on days 2 through 5, 8 through 12, and 19, weekly thereafter, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies; clinical findings were recorded monthly and at the end of the studies.
Method of Kill	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	

Three-month Studies	Two-year Studies
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the studies for hematology and clinical chemistry (rats only). Hematology: hematocrit; packed cell volume; hemoglobin; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; Howell-Jolly bodies; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, globulin, glucose, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids	None
Histopathology	
Complete histopathology was performed on chamber control and 400 mg/m^3 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), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (bronchial, mandibular, mediastinal, 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), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, 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.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[71] 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[72] method for testing two groups for equality and Tarone's[73] 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 Table A-1, Table A-3, Table B-1, Table B-3, Table C-1, Table C-4, Table D-1, and Table D-5 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 (Table A-2, Table B-2, Table C-2, and Table D-2) 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, tooth, and Zymbal's gland) 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. Table A-2, Table B-2, Table C-2, and Table D-2 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 kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[74-76] 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 kill; if the animal died prior to terminal kill 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[74]. 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[74] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1/N mice[77]. Bailer and Portier[74] 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[78].

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-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 dosed 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[79] and Williams[80; 81]. Hematology and clinical chemistry data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[82] (as modified by Williams[83]) and Dunn[84]. Jonckheere's test[85] 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[86] were examined by NTP personnel, and implausible values were eliminated from the analysis.

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 control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[87-89]. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. The current study is the only inhalation study in Wistar Han rats in the historical control database; therefore, only historical control incidences for all routes and all vehicles are used for Wistar Han rats in this Technical Report.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[90]. In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment 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 CIMSTAR 3800 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 rat and 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[91; 92]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by 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[93] and the somatic mutation theory of cancer[94; 95]. 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[96]. 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)[97; 98]. 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[99; 100]. 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[101]. 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

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-586.

Three-month Study in F344/NTac Rats

All rats survived to the end of the study and mean body weights of exposed groups of rats were similar to those of chamber control rats (Table 2; Figure 1). Clinical findings in male and female rats exposed to 200 or 400 mg/m^3 consisted mainly of lethargy, ruffled fur, and nasal/eye discharge.

Table 2. Survival and Body Weights of F344/NTac Rats in the Three-month Inhalation Study of CIMSTAR 3800[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	85 ± 2	349 ± 6	264 ± 6	
25	10/10	85 ± 2	353 ± 9	269 ± 9	101
50	10/10	84 ± 2	354 ± 7	271 ± 7	102
100	10/10	85 ± 2	354 ± 7	269 ± 7	102
200	10/10	84 ± 2	352 ± 5	268 ± 4	101
400	10/10	84 ± 2	350 ± 6	266 ± 5	100
Female					
0	10/10	76 ± 1	188 ± 5	112 ± 5	
25	10/10	78 ± 1	190 ± 5	113 ± 4	101
50	10/10	77 ± 1	195 ± 5	118 ± 5	104
100	10/10	77 ± 1	189 ± 5	112 ± 4	100
200	10/10	76 ± 1	185 ± 5	110 ± 5	98
400	10/10	77 ± 2	184 ± 4	107 ± 4	98

[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.

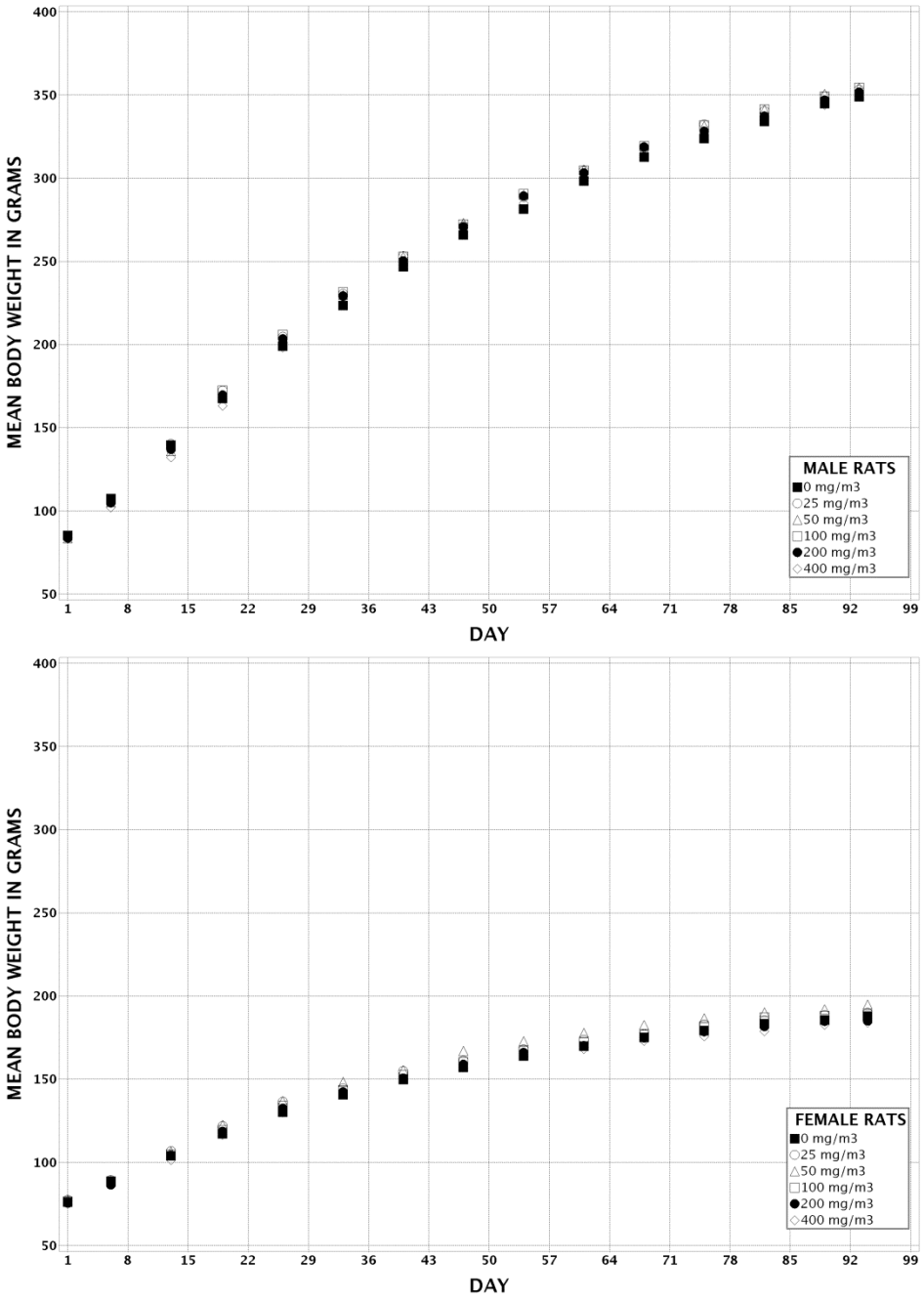


Figure 1. Growth Curves for F344/NTac Rats Exposed to CIMSTAR 3800 by Inhalation for Three Months

There were no changes in hematology parameters attributable to CIMSTAR 3800 exposure (Table F-1).

At 3 months, there was a minimal to mild (up to 23%) exposure concentration-related decrease in serum activity of alanine aminotransferase in all exposed groups of male rats (Table F-1). There was also a mild (up to 43%) exposure concentration-related decrease in sorbitol dehydrogenase activity in all exposed groups of male rats and in females exposed to 100 mg/m^3 or greater. The biological significance or mechanism of these decreases is unknown, but these changes may represent decreased hepatocellular enzyme production or release.

There were no biologically significant changes in absolute or relative organ weights (Table G-1).

Treatment-related changes were seen only in the respiratory system. In the nose, the incidences of goblet cell hyperplasia, olfactory epithelium and respiratory epithelium hyaline droplet accumulation, and olfactory epithelium and respiratory epithelium suppurative inflammation in all exposed groups of male and female rats were significantly greater than the chamber control incidences (Table 3). These lesions did not occur in chamber controls, except one male had hyaline droplet accumulation in the olfactory epithelium. In both males and females, there were slight, generally exposure concentration-related increases in the severities of goblet cell hyperplasia and olfactory epithelium and respiratory epithelium suppurative inflammation. A single 400 mg/m^3 male had focal necrosis of the olfactory epithelium and focal hyperplasia of the respiratory epithelium and one 400 mg/m^3 female had focal squamous metaplasia of the respiratory epithelium.

Goblet cell hyperplasia was minimal or mild, and involved the respiratory epithelium covering the ventral half of the nasal septum, ventral meatus, medial surface of nasoturbinates, and nasopharyngeal duct. Goblet cells were usually hypertrophic as well as hyperplastic. The hyaline droplet accumulation in the respiratory and olfactory epithelia was characterized by the presence of variably sized, eosinophilic globules within the cytoplasm of the epithelial cells. Suppurative inflammation consisted of increased numbers of neutrophils, many degenerate, scattered within the lamina propria associated with both the olfactory and respiratory epithelia. Occasionally, there were small numbers of degenerate neutrophils within glandular lumina, as well.

In the larynx of all exposed groups of males and females, there were significantly increased incidences of squamous metaplasia of the respiratory epithelium, hyperplasia of the squamous epithelium lining the arytenoid processes, and chronic active inflammation in the lamina propria (Table 3). These lesions did not occur in chamber controls except for chronic active inflammation in a single male. There were also generally exposure concentration-related increases in the severities of these lesions in both sexes.

Squamous metaplasia of the larynx was characterized by replacement of the respiratory epithelium by three to 13 cell layers of well-differentiated squamous epithelial cells with variable amounts of keratin. The lesion was located predominantly at the base of the epiglottis but was occasionally seen within the ventral pouch and ventrolateral laryngeal wall as well, particularly in the higher exposure concentration groups. Hyperplasia of the squamous epithelium lining the arytenoid processes had a similar appearance, but because these processes are normally covered by squamous epithelium, hyperplasia was deemed a more appropriate term. The hyperplasia was minimal to mild with up to seven layers of epithelial cells. Chronic active inflammation was characterized by variable numbers of neutrophils with fewer lymphocytes, plasma cells, and

occasional macrophages in the lamina propria, most often at the base of the epiglottis, but also in the ventral pouch and vocal folds.

In the lung of 200 and 400 mg/m^3 males and females, there were significantly increased incidences of alveolus histiocytic cellular infiltration (Table 3). This lesion was characterized by an increase in the number of alveolar macrophages in the lung compared to the lung of chamber control rats. Often, these macrophages were larger than those in the chamber control rats and contained several clear, cytoplasmic vacuoles.

Exposure Concentration Selection Rationale: The exposure concentrations selected for the 2-year inhalation study in rats were 10, 30, and 100 mg/m^3. The 10 mg/m^3 concentration was selected based on the occurrence of significant lesions in the nose and larynx in groups exposed to 25 mg/m^3 or greater in the 3-month study. The 100 mg/m^3 exposure concentration was selected based on exposure concentration-related increased severity of squamous metaplasia of the respiratory epithelium in the larynx, and olfactory epithelium necrosis in one 400 mg/m^3 male. In addition, the observation of nasal and eye discharge in groups exposed to 200 or 400 mg/m^3 indicated that exposure to these concentrations of the alkaline aerosol for 2 years might result in undue animal stress and discomfort.

Table 3. Incidences of Nonneoplastic Lesions of the Respiratory System in F344/NTac Rats in the Three-month Inhalation Study of CIMSTAR 3800

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
Nose[a]	10	10	10	10	10	10
Goblet Cell, Hyperplasia[b]	0	10** (1.3)[c]	10** (1.6)	10** (1.7)	10** (1.8)	10** (1.9)
Olfactory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	10** (3.0)	10** (3.0)	10** (3.0)	10** (3.0)	10** (3.0)
Olfactory Epithelium, Inflammation, Suppurative	0	10** (1.2)	10** (1.3)	10** (1.3)	10** (1.6)	10** (1.7)
Olfactory Epithelium, Necrosis	0	0	0	0	0	1 (1.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.6)	10** (1.8)	10** (2.4)	10** (2.8)	10** (2.8)
Respiratory Epithelium, Hyperplasia	0	0	0	0	0	1 (1.0)
Respiratory Epithelium, Inflammation, Suppurative	0	10** (1.2)	10** (1.3)	10** (1.3)	10** (1.6)	10** (1.7)
Larynx	10	10	10	10	10	10
Inflammation, Chronic Active	1 (1.0)	9** (1.1)	10** (1.3)	10** (1.5)	10** (2.0)	10** (2.5)
Metaplasia, Squamous	0	10** (3.1)	10** (3.9)	10** (4.0)	10** (4.0)	10** (4.0)

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Squamous Epithelium, Hyperplasia	0	6** (1.0)	5* (1.0)	10** (1.3)	10** (1.6)	10** (1.7)
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	7** (1.0)	10** (1.0)
Female						
Nose	10	10	10	10	10	10
Goblet cell, Hyperplasia	0	8** (1.5)	10** (1.5)	9** (1.6)	10** (1.7)	10** (1.8)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.8)	10** (3.0)	10** (2.9)	10** (3.0)	10** (3.0)
Olfactory Epithelium, Inflammation, Suppurative	0	9** (1.0)	10** (1.1)	10** (1.3)	10** (1.6)	10** (1.4)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.6)	10** (2.8)	10** (2.6)	10** (2.8)	10** (2.7)
Respiratory Epithelium, Inflammation, Suppurative	0	9** (1.0)	10** (1.1)	10** (1.3)	10** (1.6)	10** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	1 (1.0)
Larynx	10	10	10	10	10	10
Inflammation, Chronic Active	0	10** (1.0)	10** (1.1)	10** (1.6)	10** (1.7)	10** (2.2)
Metaplasia, Squamous	0	10** (2.9)	10** (3.4)	10** (4.0)	10** (4.0)	10** (4.0)
Squamous Epithelium, Hyperplasia	0	9** (1.1)	7** (1.0)	10** (1.4)	9** (1.3)	10** (1.7)
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	10** (1.0)	10** (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.

Two-year Study in Wistar Han Rats

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of male and female rats was similar to that of the chamber control groups.

Body Weights and Clinical Findings

The mean body weights of all exposed groups of males and females were similar to those of the chamber control groups throughout the study (Table 5, Table 6; Figure 3). Clinical findings included thinness and torso/ventral mass in all exposed and chamber control groups, torso/dorsal mass or torso/dorsal ulcer/abscess in 30 and 100 mg/m^3 males, ruffled fur in 10 and 100 mg/m^3 males, and abnormal breathing and torso/lateral mass in 100 mg/m^3 males.

Table 4. Survival of Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	17	12	13	15
Natural deaths	0	4	3	2
Animals surviving to study termination	33	34	34	33
Percent probability of survival at end of study[a]	66	68	68	66
Mean survival (days)[b]	688	681	703	683
Survival analysis[c]	P = 1.000	P = 1.000	P = 0.818N	P = 1.000
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	1	0	0
Moribund	13	16	14	19
Natural deaths	2	0	0	1
Animals surviving to study termination	35[e]	33[e]	36	30[e]
Percent probability of survival at end of study	70	65	72	58
Mean survival (days)	700	693	711	707
Survival analysis	P = 0.380	P = 0.738	P = 0.926N	P = 0.416

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[73] is in the chamber control column, and the results of the life table pairwise comparisons[72] with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by N.
[d]Censored from survival analyses.
[e]Includes one animal that died during the last week of the study.

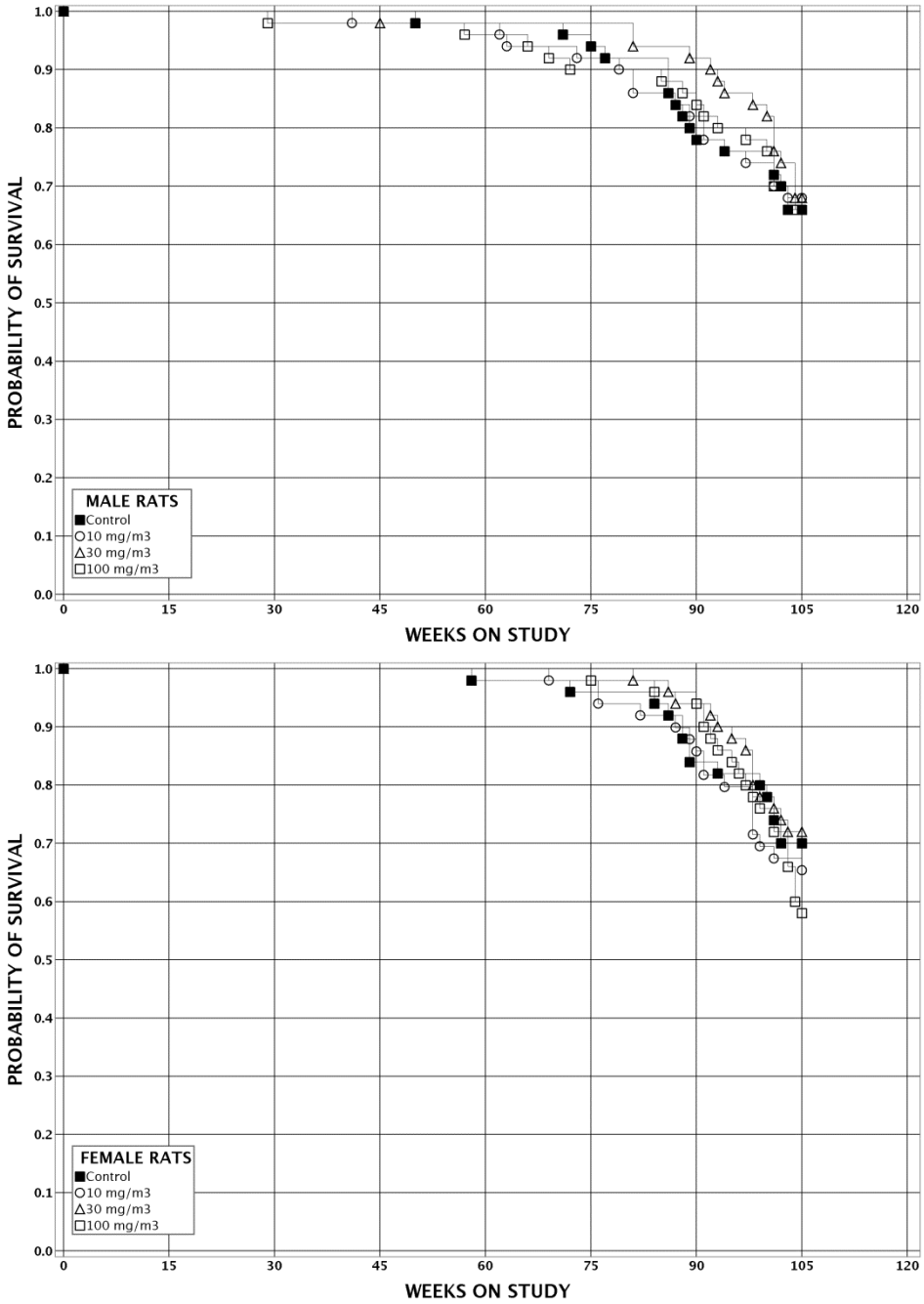


Figure 2. Kaplan-Meier Survival Curves for Wistar Han Rats Exposed to CIMSTAR 3800 by Inhalation for Two Years

Table 5. Mean Body Weights and Survival of Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

Day	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	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	138	50	138	100	50	137	99	50	137	99	50
10	192	50	193	100	50	189	98	50	186	97	50
17	232	50	234	101	50	231	100	50	227	98	50
24	265	50	269	102	50	266	100	50	261	99	50
31	291	50	296	102	50	293	101	50	289	99	50
38	314	50	321	102	50	316	101	50	312	99	50
45	335	50	342	102	50	338	101	50	334	100	50
52	351	50	357	102	50	355	101	50	350	100	50
59	363	50	370	102	50	367	101	50	362	100	50
66	375	50	383	102	50	379	101	50	374	100	50
73	385	50	394	102	50	389	101	50	385	100	50
80	396	50	405	102	50	395	100	50	395	100	50
87	405	50	413	102	50	405	100	50	405	100	50
115	430	50	437	102	50	431	100	50	427	99	50
143	450	50	458	102	50	450	100	50	446	99	50
171	464	50	474	102	50	465	100	50	460	99	50
199	478	50	488	102	50	478	100	50	472	99	50
227	492	50	502	102	50	489	99	50	488	99	49
255	504	50	516	102	50	501	99	50	499	99	49
283	517	50	527	102	50	511	99	50	510	99	49
311	524	50	537	103	49	519	99	49	517	99	49
339	535	50	550	103	49	529	99	49	529	99	49
367	545	49	558	102	49	535	98	49	534	98	49
395	555	49	568	102	49	543	98	49	545	98	48
423	565	49	578	102	49	553	98	49	551	98	48
451	572	49	580	102	47	562	98	49	556	97	48
479	578	49	589	102	47	569	98	49	568	98	46
507	584	48	597	102	46	575	98	49	578	99	45
535	590	46	604	102	46	582	99	49	585	99	45
563	593	46	606	102	44	587	99	49	589	99	45
591	593	46	613	103	43	593	100	47	591	100	45
619	597	40	612	102	42	593	99	47	595	100	43
647	597	39	621	104	39	596	100	44	600	101	40
675	602	38	622	103	38	605	101	43	602	100	39
703	603	38	622	103	37	610	101	41	596	99	38
Mean for Weeks											
1–13	311	–	317	102	–	312	100	–	309	99	–
14–52	488	–	499	102	–	486	100	–	483	99	–
53–101	583	–	598	103	–	577	99	–	576	99	–

Table 6. Mean Body Weights and Survival of Female Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

Day	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	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	118	50	118	100	50	118	100	50	117	99	50
10	143	50	143	100	50	143	100	50	141	99	50
17	158	50	159	101	50	158	100	50	157	99	50
24	172	50	174	101	50	174	101	50	170	99	50
31	182	50	183	101	50	184	101	50	180	99	50
38	191	50	191	100	50	193	101	50	189	99	50
45	199	50	199	100	50	200	101	50	197	99	50
52	204	50	206	101	50	208	102	50	202	99	50
59	209	50	211	101	50	213	102	50	208	99	50
66	211	50	215	102	50	215	102	50	213	101	50
73	217	50	220	101	50	221	102	50	217	100	50
80	222	50	225	101	50	226	102	50	220	99	50
87	227	50	230	101	50	230	102	50	225	99	50
115	237	50	240	101	50	241	102	50	235	99	50
143	246	50	249	101	50	252	103	50	243	99	50
171	253	50	256	101	50	259	103	50	250	99	50
199	258	50	262	102	50	267	103	50	257	100	50
227	265	50	271	102	50	272	103	50	264	100	50
255	271	50	275	102	50	278	102	50	268	99	50
283	278	50	284	102	50	286	103	50	275	99	50
311	288	50	290	101	50	293	102	50	280	98	50
339	295	50	299	101	50	299	101	50	285	97	50
367	300	50	303	101	50	307	103	50	289	96	50
395	313	50	313	100	50	317	102	50	300	96	50
423	322	49	326	101	50	328	102	50	296	92	50
451	334	49	336	101	50	339	102	50	316	95	50
479	341	49	339	99	50	348	102	50	326	96	50
507	344	48	348	101	49	359	104	50	336	98	50
535	355	48	363	102	47	370	104	50	344	97	49
563	362	48	369	102	46	380	105	49	348	96	49
591	367	47	373	101	45	385	105	49	358	97	48
619	371	42	377	102	43	396	107	47	361	97	48
647	375	41	380	101	40	396	106	45	359	96	43
675	385	41	384	100	39	401	104	43	360	94	40
703	377	37	396	105	33	398	106	39	370	98	36
Mean for Weeks											
1–13	189	–	190	101	–	191	101	–	187	99	–
14–52	266	–	270	102	–	272	102	–	262	99	–
53–101	350	–	354	101	–	363	104	–	336	96	–

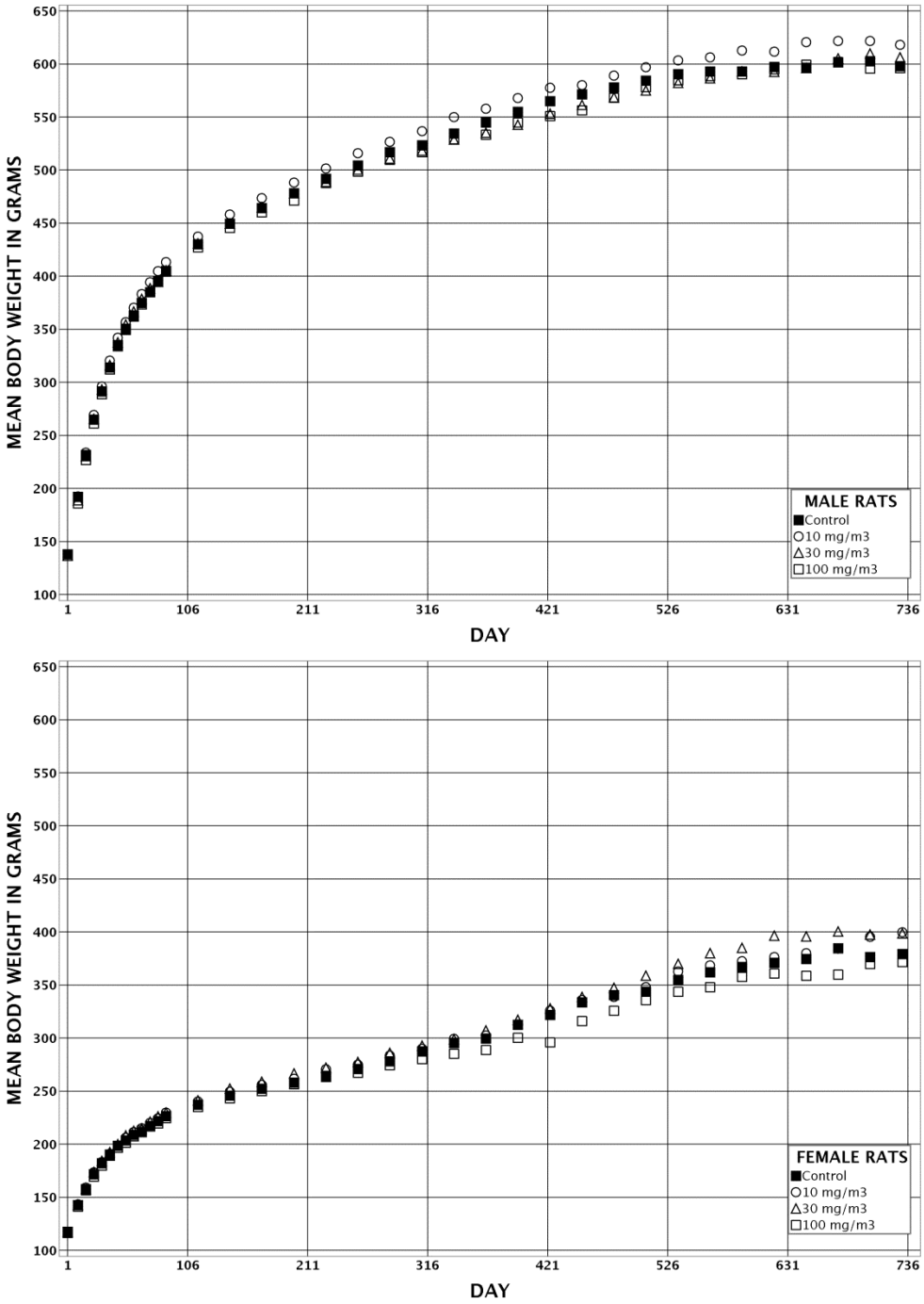


Figure 3. Growth Curves for Wistar Han Rats Exposed to CIMSTAR 3800 by Inhalation for Two Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the prostate gland, brain, uterus, skin, nose, larynx, lung, bronchial and mediastinal lymph nodes, and thyroid gland. 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.

Prostate Gland: There were increased incidences of prostate gland adenoma or carcinoma (combined) in 30 and 100 mg/m^3 males compared to chamber controls (Table 7, Table A-1, and Table A-2); however, the increases were not statistically significant. The increases were mainly attributable to increased incidences of adenoma, as there was a single carcinoma in the 30 mg/m^3 group. In three studies using Wistar Han rats (including the current study), there were no carcinomas and there was a single adenoma in the 150 control animals. In the current study, there were five adenomas and one carcinoma in the exposed groups.

All neoplasms of the prostate gland were located in the ventral prostatic lobe. The adenomas were small masses that occupied one to three minimally expanded acini and caused minimal compression of the adjacent acini. The neoplastic cells grew in a cribriform pattern and the epithelium was generally two to five cell layers thick with occasional solid areas. The cells were often jumbled and the nuclei lacked basal polarity. They had moderate amounts of basophilic to amphophilic cytoplasm and round to oval nuclei with one to two small nucleoli. There was scant connective tissue within the adenomas. The carcinoma was much larger, effacing nearly the entire prostate gland and invading into surrounding tissues. There were numerous, often dilated, glands lined by one to three layers of cuboidal to flattened neoplastic epithelial cells with basophilic to amphophilic cytoplasm. The nuclei were round to oval with an open chromatin pattern. The glands were often filled with degenerate neutrophils and cell debris or mucinous material with fewer inflammatory cells. There was abundant fibrous stroma separating the glands with scattered mononuclear inflammatory cells and fewer neutrophils.

Table 7. Incidences of Neoplasms of the Prostate Gland in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Number Examined Microscopically	50	50	50	50
Adenoma, multiple[a]	1	0	1	1
Adenoma (includes multiple)[b]	1	1	1	3
Carcinoma[c]	0	0	1	0

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Adenoma or Carcinoma (includes multiple)[b]				
Overall rate[d]	1/50 (2%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate[e]	2.3%	2.3%	4.3%	6.9%
Terminal rate[f]	1/33 (3%)	1/34 (3%)	1/34 (3%)	3/33 (9%)
First incidence (days)	729 (T)	729 (T)	684	729 (T)
Poly-3 test[g]	P = 0.187	P = 0.757	P = 0.520	P = 0.304

(T) = Terminal kill.
[a]Number of animals with neoplasm.
[b]Historical control incidence for all routes of 2-year studies: 1/150.
[c]Historical control incidence: 0/150.
[d]Number of animals with neoplasm per number of animals with prostate gland examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]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 differential mortality in animals that do not reach terminal kill.

Brain: The occurrences of benign or malignant granular cell tumor (combined) were slightly increased in all exposed male groups compared to chamber controls (Table 8, Table A-1, and Table A-2), with the highest incidence in the 10 mg/m^3 group. Although not statistically significant, the incidence of benign or malignant granular cell tumor (combined) in the 10 mg/m^3 group exceeded the historical control range (0% to 4%) for all exposure routes.

The benign granular cell tumors were located in the meninges of the cerebellum or cerebrum. The malignant granular cell tumors were located within or adjacent to the lateral and fourth ventricles and distorted the brain architecture. There was also evidence of invasion into the adjacent neuropil. The neoplastic cells in the benign and malignant granular cell tumors had a similar appearance. They were large, round cells with abundant, deeply eosinophilic, granular cytoplasm. The nuclei were generally oval with finely stippled chromatin and a single, small nucleolus.

Uterus: In the female rats, there was a small increase in the incidence of uterine adenocarcinoma and a single mixed malignant Müllerian tumor (MMMT) in the 100 mg/m^3 group (Table 9, Table B-1, and Table B-2). In an effort by NTP to evaluate rat uteri more thoroughly, additional evaluations were conducted on the residual uterine tissue from this and other studies. The residual uterine tissue was sectioned longitudinally (i.e., parallel to long axis) and included the cervix and vagina. In the residual evaluation for CIMSTAR 3800, additional uterine neoplasms were identified in the exposed groups, but not in the control group, and there were significantly increased incidences of uterine adenocarcinoma and adenocarcinoma or MMMT (combined) in the 30 and 100 mg/m^3 groups (Table 9 and Table B-2). The trend statistic for these lesions was not significant. When combined with the data from the original evaluation in which there was a single adenocarcinoma in the chamber control group, the incidences of adenocarcinoma and adenocarcinoma or MMMT (combined) in the 30 and 100 mg/m^3 groups were not statistically significant. In two other studies in which residual uterine tissue from Wistar Han rats was evaluated, there were eight adenocarcinomas in controls from one study and four adenocarcinomas in controls from the other study[102; 103]. Although the total number of female rats in the Wistar Han historical control database is low (150), this suggests that uterine epithelial

neoplasms are not uncommon in Wistar Han rats. Furthermore, the eight adenocarcinomas in controls from the one study[103] exceeds the total incidence of adenocarcinoma or MMMT (combined) in the 100 mg/m^3 group from the current study.

Table 8. Incidences of Neoplasms of the Brain in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Number Examined Microscopically	50	50	50	50
Benign Granular Cell Tumor[a,b]	0	2	0	1
Malignant Granular Cell Tumor[c]	0	1	1	0
Benign or Malignant Granular Cell Tumor[b]				
Overall rate[d]	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate[e]	0.0%	7.0%	2.2%	2.3%
Terminal rate[f]	0/33 (0%)	2/34 (6%)	0/34 (0%)	1/33 (3%)
First incidence (days)	–[h]	704	684	729 (T)
Poly-3 test[g]	P = 0.593N	P = 0.116	P = 0.511	P = 0.500

T = terminal kill.
[a]Number of animals with neoplasm.
[b]Historical control incidence for all routes of 2-year studies: 3/150.
[c]Historical control incidence: 0/150.
[d]Number of animals with neoplasm per number of animals with brain examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]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 differential mortality in animals that do not reach terminal kill. A negative trend is indicated by **N**.
[h]Not applicable; no neoplasms in animal group.

Table 9. Incidences of Neoplasms of the Uterus in Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Number Necropsied	50	50	50	50
Original Evaluation				
Adenocarcinoma[a,b]	1	1	1	2
Mixed Malignant Müllerian Tumor[c]	0	0	0	1
Adenocarcinoma or Mixed Malignant Müllerian Tumor[b]	1	1	1	3
Residual Longitudinal Evaluation				
Adenocarcinoma				
Overall rate[d]	0/50 (0%)	4/50 (8%)	5/50 (10%)	5/50 (10%)
Adjusted rate[e]	0.0%	9.2%	10.7%	11.0%
Terminal rate[f]	0/35 (0%)	4/32 (13%)	3/36 (8%)	4/29 (14%)
First incidence (days)	–[h]	731 (T)	646	725
Poly-3 test[g]	P = 0.126	P = 0.057	P = 0.034	P = 0.032
Mixed Malignant Müllerian Tumor	0	0	0	1
Adenocarcinoma or Mixed Malignant Müllerian Tumor				
Overall rate	0/50 (0%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	0.0%	9.2%	10.7%	13.1%
Terminal rate	0/35 (0%)	4/32 (13%)	3/36 (8%)	5/29 (17%)
First incidence (days)	–	731 (T)	646	725
Poly-3 test	P = 0.059	P = 0.057	P = 0.034	P = 0.016
Original and Residual Longitudinal Evaluation				
Adenocarcinoma	1	4	5	5
Mixed Malignant Müllerian Tumor	0	0	0	1
Adenocarcinoma or Mixed Malignant Müllerian Tumor				
Overall rate	1/50 (2%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	2.2%	9.2%	10.7%	13.1%
Terminal rate	1/35 (3%)	4/32 (13%)	3/36 (8%)	5/29 (17%)
First incidence (days)	731 (T)	731 (T)	646	725
Poly-3 test	P = 0.102	P = 0.170	P = 0.111	P = 0.058

T = terminal kill.
[a]Number of animals with neoplasm.
[b]Historical control incidence for all routes of 2-year studies: 7/150.
[c]Historical control incidence for all routes of 2-year studies: 0/150.
[d]Number of animals with neoplasm per number of animals necropsied.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]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 differential mortality in animals that do not reach terminal kill.
[h]Not applicable; no neoplasms in animal group.

Uterine adenocarcinomas were generally small and poorly demarcated masses composed of cuboidal epithelial cells. The neoplastic cells were large and polymorphic and often had basophilic cytoplasm. They were arranged in branching papillary patterns and often formed tubular or acinar structures. There were often multiple layers of neoplastic epithelial cells lining these structures. The MMMT was a large mass and had a similar epithelial component, but also had a mesenchymal component that was also considered neoplastic. The mesenchymal cells were enlarged with large, elongated nuclei and scattered mitotic figures and were arranged in streaming bundles.

Skin: In females, there was a positive trend in the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin (Table 10, Table B-1 and Table B-2); however, the incidence in the 100 mg/m^3 group was not significantly greater than the chamber control incidence. The majority of these neoplasms were squamous cell papillomas, but there was also one keratoacanthoma. No squamous cell papillomas or keratoacanthomas have occurred in the 150 female Wistar Han historical controls from all routes of study.

Table 10. Incidences of Neoplasms of the Skin in Female Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Number of Animals Necropsied	50	50	50	50
Squamous Cell Papilloma[a]				
Overall rate[b]	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[c]	0.0%	0.0%	0.0%	6.5%
Terminal rate[d]	0/35 (0%)	0/32 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	–[f]	–	–	673
Poly-3 test[e]	P = 0.008	–[g]	–	P = 0.122
Keratoacanthoma[a,h]	0	0	0	1
Squamous Cell Papilloma or Keratoacanthoma[a]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	8.7%
Terminal rate	0/35 (0%)	0/32 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	–	–	–	673
Poly-3 test	P = 0.002	–	–	P = 0.063

[a]Historical control incidence for all routes of 2-year studies: 0/150.
[b]Number of animals with neoplasm per number of animals necropsied.
[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[d]Observed incidence at terminal kill.
[e]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 differential mortality in animals that do not reach terminal kill.
[f]Not applicable; no neoplasms in animal group.
[g]Value of statistic cannot be computed.
[h]Number of animals with neoplasm.

Nose: In the nose, there were chemical-related, nonneoplastic lesions consistent with an irritant effect. In all exposed male and female groups, there were significantly increased incidences of goblet cell hyperplasia, hyperplasia of submucosal glands subjacent to the olfactory epithelium, and hyaline droplet accumulation in the respiratory and olfactory epithelia (Table 11, Table A-3, and Table B-3).

Goblet cell hyperplasia of the respiratory epithelium was histologically characterized by minimally to mildly increased number and size of goblet cells, particularly along the nasal septum, ethmoturbinates, and the epithelium lining the nasopharyngeal duct. Hyperplasia of the olfactory epithelium submucosal glands was characterized by an increase in the size and number of the glands, and the cytoplasm of the glandular cells was often distended by concurrent hyaline droplet accumulations. The volumetric expansion of the submucosal glands often resulted in variable elevation of the overlying olfactory epithelium. A few neutrophils were sometimes present in the hyperplastic gland lumens or adjacent submucosal stroma, but this was not a prominent feature. Hyaline droplet accumulation of the respiratory and olfactory epithelia typically occurred in the ventral nasal cavity, on the ethmoturbinates, and along the margin of the ventromedial nasal cavity adjacent to the nasopharynx. Histologically, this lesion was characterized by aggregates of hyalinized, eosinophilic material distending the cytoplasm of olfactory and respiratory epithelial cells, though the severity was much greater in the olfactory epithelium.

Larynx: In the larynx of all exposed groups of males and females, there were significantly increased incidences of squamous metaplasia of the respiratory epithelium (Table 11, Table A-3, and Table B-3). In the 30 and 100 mg/m^3 groups the severities of this lesion increased with increasing exposure concentration. In a few animals, there was concurrent inflammation (Table A-3 and Table B-3), but in the majority of the exposed rats, squamous metaplasia was the only lesion present. Squamous metaplasia of the laryngeal respiratory epithelium was also seen in the 3-month study. The difference in the severity of this lesion between the 3-month and 2-year studies was likely due to differences in the grading schemes.

Squamous metaplasia was most common along the ventral aspect of the most cranial section of larynx, at the base of the epiglottis, although in severely affected animals, the medial aspects of the arytenoid cartilages were also affected. Histologically, this lesion was characterized by replacement of the respiratory or transitional epithelium by flattened squamous epithelium.

Table 11. Incidences of Nonneoplastic Lesions of the Respiratory System in Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	3 mg/m^3	100 mg/m^3
Male				
Nose[a]	50	50	50	50
Glands, Olfactory Epithelium, Hyperplasia[b]	1 (1.0)[c]	39** (1.5)	47** (2.0)	50** (2.6)
Goblet Cell, Hyperplasia	0	20** (1.1)	25** (1.1)	34** (1.3)
Olfactory Epithelium, Accumulation, Hyaline Droplet	19 (1.6)	50** (2.8)	50** (3.0)	50** (3.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	17** (1.1)	25** (1.1)	29** (1.2)
Larynx	50	50	50	50
Metaplasia, Squamous	1 (2.0)	47** (1.5)	50** (2.2)	50** (2.7)
Lung	50	50	50	50
Alveolar Epithelium, Hyperplasia	4 (1.3)	6 (1.8)	11 (1.9)	13* (2.4)
Bronchus-associated Lymphoid Tissue, Hyperplasia, Lymphohistiocytic	0	1 (1.0)	5* (1.0)	19** (2.0)
Inflammation, Lymphohistiocytic	6 (1.2)	14* (1.0)	41** (1.1)	47** (1.8)
Lymph Node, Bronchial	42	40	37	35
Hyperplasia, Lymphohistiocytic	0	0	10** (1.1)	28** (2.1)
Lymph Node, Mediastinal	46	45	50	49
Hyperplasia, Lymphohistiocytic	0	0	4 (1.5)	29** (2.2)
Female				
Nose	50	50	50	50
Glands, Olfactory Epithelium, Hyperplasia	1 (1.0)	32** (1.6)	48** (2.0)	49** (2.6)
Goblet Cell, Hyperplasia	0	25** (1.0)	34** (1.1)	42** (1.2)
Olfactory Epithelium, Accumulation, Hyaline Droplet	16 (1.6)	50** (2.6)	50** (2.9)	50** (3.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	24** (1.1)	31** (1.1)	34** (1.2)
Larynx	50	50	50	50
Metaplasia, Squamous	1 (2.0)	50** (1.7)	50** (2.5)	50** (2.8)
Lung	50	50	50	50
Bronchus-associated Lymphoid Tissue, Hyperplasia, Lymphohistiocytic	0	0	0	5* (1.0)
Inflammation, Lymphohistiocytic	3 (1.0)	20** (1.0)	42** (1.0)	50** (1.9)
Lymph Node, Bronchial	38	35	32	35
Hyperplasia, Lymphohistiocytic	0	0	7** (1.1)	30** (1.6)
Lymph Node, Mediastinal	49	46	45	47
Hyperplasia, Lymphohistiocytic	0	0	4 (1.3)	23** (1.7)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 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.

Lung: In the lung, there were significant, exposure concentration-related increased incidences of lymphohistiocytic inflammation in all exposed groups of males and females and of lymphohistiocytic hyperplasia of bronchus-associated lymphoid tissue (BALT) in 30 mg/m^3 males and 100 mg/m^3 males and females (Table 11, Table A-3, and Table B-3). There were also increased incidences of alveolar epithelium hyperplasia in all exposed groups of males, but only the incidence in 100 mg/m^3 males was significantly increased compared to that in the chamber controls. The severities of lymphohistiocytic inflammation in the 100 mg/m^3 groups and BALT lymphohistiocytic hyperplasia in 100 mg/m^3 males were increased compared to the chamber controls. The severities of alveolar epithelium hyperplasia increased with exposure concentration in males.

Lymphohistiocytic inflammation was characterized histologically by accumulations of small lymphocytes and foamy, vacuolated macrophages (Figure 7). In some cases, the inflammatory cells were closely intermixed forming nodules, typically within alveoli. These nodules, reminiscent of small granulomas, were most often found in the subpleural region. The inflammatory cells also formed more dispersed accumulations where the macrophages and lymphocytes were not as closely associated. The macrophages were often within alveoli and the lymphocytes multifocally expanded the adjacent alveolar septa. The more dispersed pattern was present in all animals with inflammation, but the nodules were mainly present in the more severely affected rats in the 30 and 100 mg/m^3 groups. Lymphohistiocytic hyperplasia in the BALT was histologically similar to lymphohistiocytic inflammation in the lungs and was characterized by multifocal aggregates of foamy, vacuolated histiocytes within the BALT separated by increased numbers of small lymphocytes (Figure 8). Secondary follicles were generally absent. Alveolar epithelium hyperplasia consisted of poorly demarcated, noncompressive parenchymal foci in which alveolar septa were lined by plump, cuboidal epithelial cells.

Lymph Nodes: In the bronchial lymph nodes, there were significant, exposure concentration-related increased incidences of lymphohistiocytic hyperplasia in 30 and 100 mg/m^3 males and females (Table 11, Table A-3, and Table B-3). In the mediastinal lymph nodes, there were significant, exposure concentration-related increased incidences of lymphohistiocytic hyperplasia in 100 mg/m^3 males and females. The severities of these lesions also increased with increasing exposure concentration.

Lymphohistiocytic hyperplasia in the bronchial and mediastinal lymph nodes was characterized by multifocal aggregates of foamy, vacuolated histiocytes, particularly in the paracortical region, separated by increased numbers of small lymphocytes. Secondary follicles were generally absent throughout the cortex. The appearance of this lesion was similar to the appearance of the lymphohistiocytic inflammation of the lung and lymphohistiocytic hyperplasia of the BALT described above.

Thyroid Gland: In female rats, there were positive trends in the incidences of C-cell carcinoma (chamber control, 0/50; 10 mg/m^3, 0/50; 30 mg/m^3, 0/50; 100 mg/m^3, 3/49; Table B-1 and Table B-2) and C-cell adenoma or carcinoma (combined) (3/50, 2/50, 4/50, 7/49). The incidence of C-cell adenoma or carcinoma (combined) in the 100 mg/m^3 group did not exceed the historical control range (6% to 14%) for all routes; however, the incidence of C-cell carcinoma in the 100 mg/m^3 group did exceed the all routes historical control range (0% to 2%). Furthermore,

the incidences of C-cell carcinoma and C-cell adenoma or carcinoma (combined) in any exposed group were not statistically significant when compared to the chamber controls.

Mice

Three-month Study

All mice survived to the end of the study (Table 12). Final mean body weights and mean body weight gains of 400 mg/m^3 males and females were significantly less than those of the chamber controls; the final mean body weight of 200 mg/m^3 females was also significantly less (Table 12; Figure 4). Clinical findings associated with exposure to CIMSTAR 3800 consisted of lethargy in all 200 and 400 mg/m^3 mice and ruffled fur in all exposed groups of males and 400 mg/m^3 females.

There were no changes in hematology parameters attributable to CIMSTAR 3800 exposure (Table F-2).

The absolute and relative lung weights of males exposed to 100 mg/m^3 or greater were significantly greater than those of the chamber controls (Table G-2). The relative lung weights of 200 and 400 mg/m^3 females were also significantly greater than that of the chamber controls. Other organ weight differences were related to the reduced body weights.

Table 12. Survival and Body Weights of Mice in the Three-month Inhalation Study of CIMSTAR 3800[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	24.3 ± 0.5	39.5 ± 0.9	15.2 ± 0.7	
25	10/10	24.3 ± 0.6	38.1 ± 1.3	13.8 ± 1.1	97
50	10/10	24.3 ± 0.4	38.6 ± 0.7	14.3 ± 0.8	98
100	10/10	24.3 ± 0.5	37.4 ± 0.6	13.1 ± 0.4	95
200	10/10	24.2 ± 0.4	37.8 ± 0.6	13.6 ± 0.6	96
400	10/10	24.0 ± 0.5	32.9 ± 0.9**	8.9 ± 0.6**	83
Female					
0	10/10	20.0 ± 0.3	33.0 ± 1.2	13.0 ± 1.1	
25	10/10	19.5 ± 0.5	31.6 ± 0.7	12.1 ± 0.6	96
50	10/10	20.3 ± 0.4	31.8 ± 0.8	11.5 ± 0.9	96
100	10/10	19.4 ± 0.3	31.9 ± 0.7	12.5 ± 0.6	97
200	10/10	19.4 ± 0.2	30.5 ± 0.8*	11.1 ± 0.6	92
400	10/10	19.6 ± 0.5	28.1 ± 0.5**	8.6 ± 0.4**	85

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.

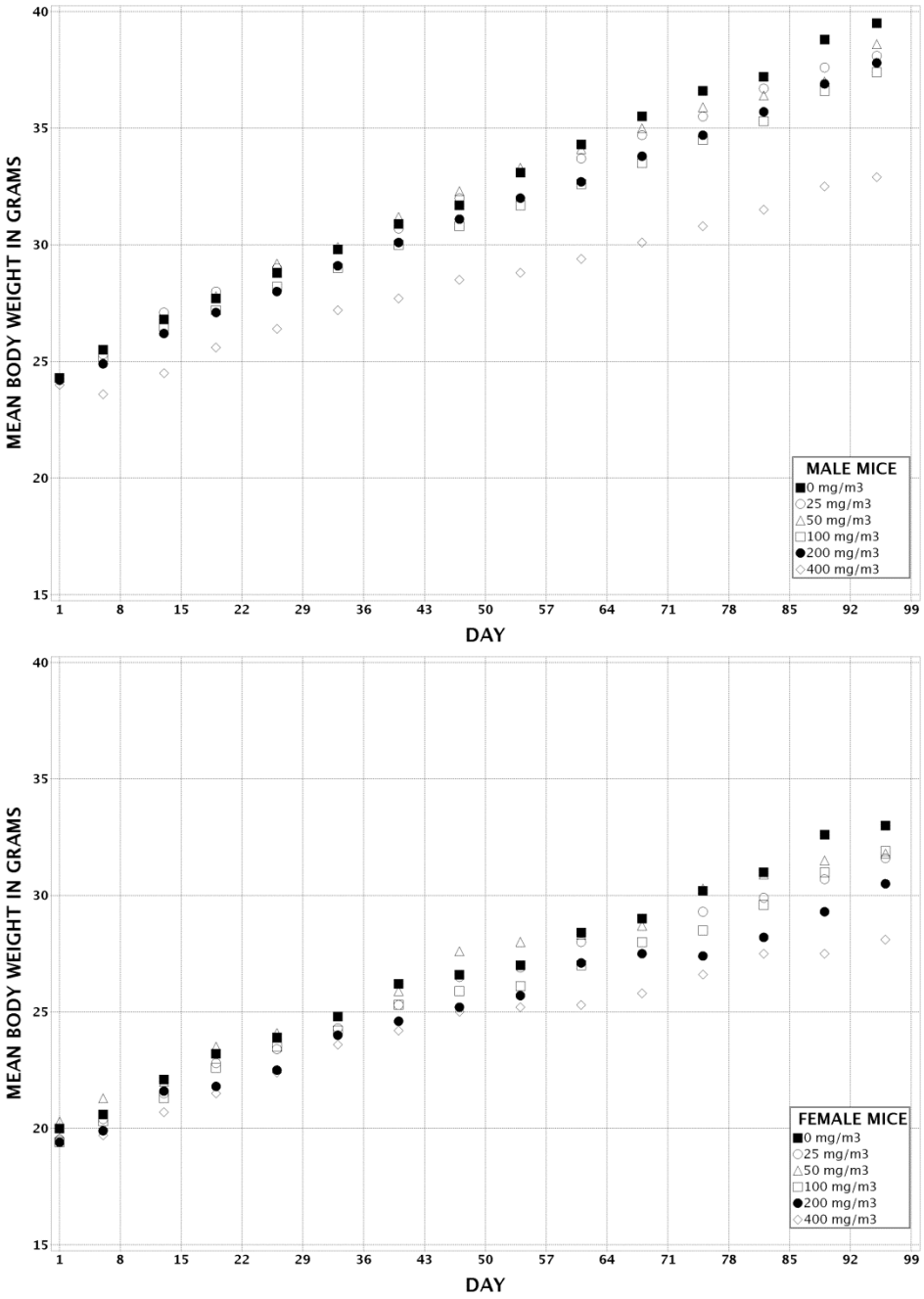


Figure 4. Growth Curves for Mice Exposed to CIMSTAR 3800 by Inhalation for Three Months

Treatment-related lesions were seen only in the respiratory system. In the nose of all exposed groups of mice, the incidences of hyaline droplet accumulation in the olfactory and respiratory epithelia were significantly greater than the chamber control incidences (Table 13). These lesions did not occur in chamber control mice. In both sexes, the severity of these lesions tended to increase with increasing exposure concentration. Hyaline droplet accumulation in the respiratory and olfactory epithelia was characterized by the presence of variably sized, eosinophilic globules within the cytoplasm of the epithelial cells, particularly on the ventral and lateral aspects of the

ethmoid turbinates, in the nasopharyngeal duct, and at the junctions of olfactory and respiratory epithelia.

In the larynx, there were significantly increased incidences of squamous metaplasia of the respiratory epithelium in all exposed groups of males and females, and the severity of this lesion increased with increasing exposure concentration (Table 13). In males and females exposed to 200 or 400 mg/m^3, there were significantly increased incidences of hyperplasia of the squamous epithelium lining the arytenoid processes. There were significantly increased incidences of chronic active inflammation of the lamina propria in males exposed to 50 mg/m^3 or greater and females exposed to 100 mg/m^3 or greater, and the severity increased with increasing exposure concentration. There were also significantly increased incidences of dysplasia of the epithelium at the level of the epiglottis in 400 mg/m^3 males and females. Squamous metaplasia was characterized by replacement of the respiratory epithelium by three to 15 cell layers of well-differentiated squamous epithelial cells with variable amounts of keratin. The lesion was located predominantly at the base of the epiglottis, but was occasionally seen on the ventrolateral laryngeal wall as well, particularly in the higher exposure concentration groups. Hyperplasia of the squamous epithelium lining the arytenoid processes had a similar appearance, but because these processes are normally covered by squamous epithelium, hyperplasia was deemed a more appropriate term. Hyperplasia was slightly less severe than metaplasia with up to seven layers of squamous epithelial cells. Chronic active inflammation was generally associated with squamous metaplasia and was characterized by variable numbers of neutrophils with fewer lymphocytes, plasma cells, and occasional macrophages in the lamina propria, most often at the base of the epiglottis, but also in the ventral pouch and vocal folds. Epithelial dysplasia of the epiglottis was associated with squamous metaplasia and was characterized by the extensive proliferation of metaplastic squamous epithelium resulting in irregular, disorganized epithelial surfaces (Figure 9). Proliferating epithelial cells formed tortuous invaginations into the subjacent submucosa and an undulating epithelial surface. The cytomegalic and karyomegalic dysplastic squamous epithelial cells were either randomly oriented or aligned perpendicular to the basal lamina and sometimes formed whorls. There were also dyskeratotic cells in the stratum granulosum, reflecting disorderly cellular maturation.

In the lung, there were significantly increased incidences of bronchiole hyperplasia in all exposed groups of males and females, and the severity was increased at 100 mg/m^3 or greater (Table 13). There were significantly increased incidences of chronic active perivascular inflammation in 200 and 400 mg/m^3 males and 100 and 200 mg/m^3 females. There were also significantly increased incidences of arteriole hypertrophy in 400 mg/m^3 males and 200 mg/m^3 females. Bronchiole hyperplasia was characterized histologically by increased numbers of cuboidal to columnar epithelial cells, with up to three layers of epithelial cells. In some instances, there were clusters of cuboidal to columnar epithelial cells that sometimes formed papillary projections into the airway lumen. Only the terminal bronchioles were involved in the minimally affected animals, but in the mildly affected animals, the terminal bronchioles and alveolar ducts were involved. Arteriole hypertrophy consisted of thickening of the tunica media of small pulmonary arterioles. Chronic active perivascular inflammation was characterized by the accumulation of small numbers of lymphocytes with fewer plasma cells and neutrophils around arterioles and, rarely, venules, and was observed in the presence and absence of arteriole hypertrophy.

Exposure Concentration Selection Rationale: The exposure concentrations selected for the 2-year inhalation study in mice were 10, 30, and 100 mg/m^3. The 10 mg/m^3 concentration was selected based on the occurrence of significant lesions of the larynx in groups exposed to 25 mg/m^3 or greater in the 3-month study. There were increased severities of chronic active inflammation of the larynx in males and females exposed to 50 mg/m^3 or greater. The 100 mg/m^3 concentration was selected based on significantly reduced body weights in 200 mg/m^3 females and 400 mg/m^3 males and females, increased incidences of hyperplasia of the squamous epithelium of the larynx in 200 and 400 mg/m^3 groups, and increased incidences of epithelial dysplasia of the epiglottis in 400 mg/m^3 groups.

Table 13. Incidences of Nonneoplastic Lesions of the Respiratory System in Mice in the Three-month Inhalation Study of CIMSTAR 3800

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
Nose[a]	10	10	10	10	10	10
Olfactory Epithelium, Accumulation, Hyaline Droplet[b]	0	7** (1.0)[c]	4* (1.0)	10** (1.9)	10** (1.7)	9** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	8** (1.0)	10** (1.0)	10** (2.0)	10** (1.7)	10** (1.9)
Larynx	10	10	10	10	10	10
Epiglottis, Dysplasia	0	0	0	0	0	6** (1.8)
Inflammation, Chronic Active	0	0	4* (1.3)	6** (1.3)	10** (1.9)	9** (1.9)
Metaplasia, Squamous	0	10** (2.0)	10** (2.2)	10** (2.9)	10** (3.9)	10** (4.0)
Squamous Epithelium, Hyperplasia	0	1 (1.0)	0	0	7** (1.9)	10** (2.2)
Lung	10	10	10	10	10	10
Arteriole, Hypertrophy	0	0	0	2 (1.0)	3 (1.3)	4* (1.0)
Bronchiole, Hyperplasia	0	10** (1.0)	10** (1.0)	10** (1.9)	10** (1.5)	10** (1.9)
Perivascular, Inflammation, Chronic Active	0	0	0	3 (1.0)	4* (1.0)	6** (1.2)
Female						
Nose	10	10	10	10	10	10
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	7** (1.0)	10** (1.0)	10** (1.8)	10** (2.0)	10** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.0)	10** (1.0)	10** (1.8)	10** (2.0)	10** (2.0)
Larynx	10	10	10	10	10	10
Epiglottis, Dysplasia	0	0	0	0	2 (1.0)	7** (1.7)
Inflammation, Chronic Active	0	0	1 (1.0)	9** (1.3)	10** (2.1)	10** (2.0)
Metaplasia, Squamous	0	10** (2.0)	10** (2.0)	10** (2.8)	10** (3.9)	10** (4.0)
Squamous Epithelium, Hyperplasia	0	0	0	1 (1.0)	10** (2.0)	10** (2.0)

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Lung	10	10	10	10	10	10
Arteriole, Hypertrophy	0	0	0	2 (1.0)	4* (1.0)	2 (1.0)
Bronchiole, Hyperplasia	0	10** (1.0)	10** (1.0)	10** (2.0)	10** (1.8)	10** (2.0)
Perivascular, Inflammation, Chronic Active	0	0	1 (1.0)	6** (1.0)	8** (1.0)	3 (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.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 5). Survival of all exposed groups of male and female mice was similar to that of the chamber control groups.

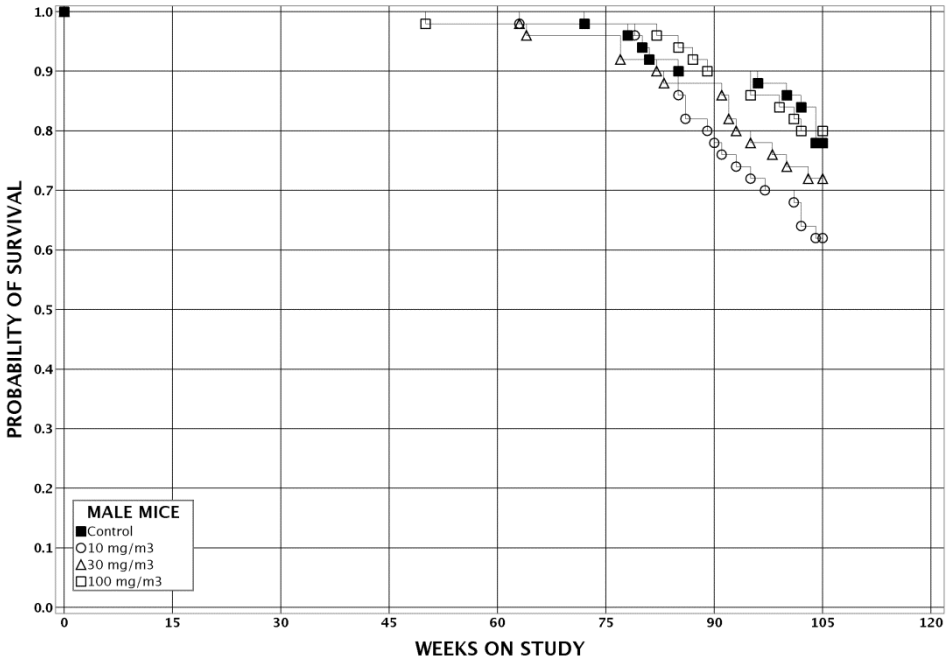
Body Weights and Clinical Findings

The mean body weights of all exposed groups of males and females were similar to those of the chamber control groups throughout the study (Figure 6; Table 15 and Table 16). Clinical findings included thinness in all exposed and chamber control groups of males and females, torso/ ventral mass in all exposed male groups, and torso/dorsal mass in 100 mg/m^3 males.

Table 14. Survival of Mice in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	5	12	7	4
Natural deaths	6	7	7	6
Animals surviving to study termination	39	31	36	40
Percent probability of survival at end of study[a]	78	62	72	80
Mean survival (days)[b]	709	688	693	706
Survival analysis[c]	P = 0.323N	P = 0.109	P = 0.558	P = 1.000
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	0	1
Moribund	7	8	10	11
Natural deaths	4	5	3	5
Animals surviving to study termination	39	37	37	33
Percent probability of survival at end of study	78	74	74	67
Mean survival (days)	696	706	706	688
Survival analysis	P = 0.308	P = 0.817	P = 0.847	P = 0.358

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[73] is in the chamber control column, and the results of the life table pairwise comparisons[72] with the chamber controls are in the exposed group columns. A negative trend is indicated by **N**.
[d]Censored from survival analyses.



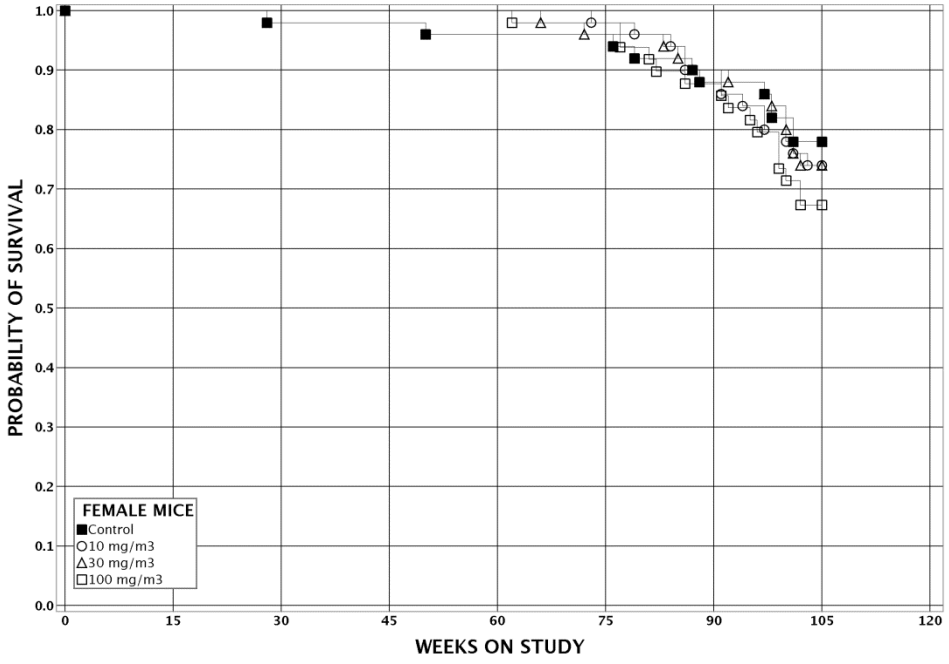


Figure 5. Kaplan-Meier Survival Curves for Mice Exposed to CIMSTAR 3800 by Inhalation for Two Years

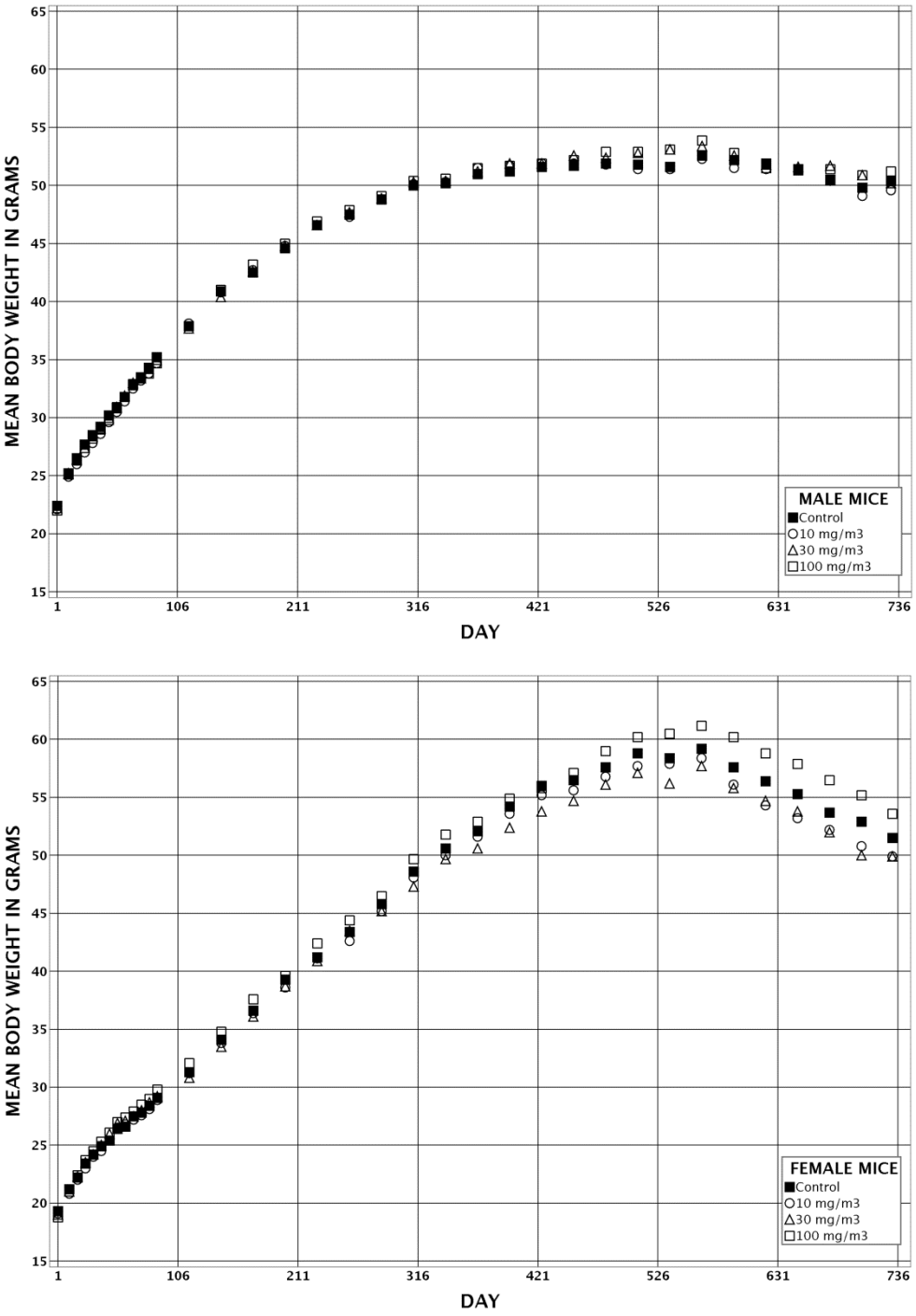


Figure 6. Growth Curves for Mice Exposed to CIMSTAR 3800 by Inhalation for Two Years

Table 15. Mean Body Weights and Survival of Male Mice in the Two-year Inhalation Study of CIMSTAR 3800

Day	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	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	22.4	50	22.1	99	50	22.2	99	50	22.0	98	50
11	25.2	50	24.9	99	50	25.2	100	50	25.1	99	50
18	26.5	50	26.0	98	50	26.4	99	50	26.3	99	50
25	27.7	50	27.0	98	50	27.4	99	50	27.4	99	50
32	28.5	50	27.8	98	50	28.4	100	50	28.2	99	50
39	29.2	50	28.6	98	50	29.1	99	50	29.0	99	50
46	30.2	50	29.6	98	50	30.0	99	50	29.8	99	50
53	30.8	50	30.5	99	50	30.9	100	50	30.9	100	50
60	31.8	50	31.4	99	50	31.9	100	50	31.8	100	50
67	32.8	50	32.5	99	50	33.0	101	50	32.9	100	50
74	33.5	50	33.2	99	50	33.4	100	50	33.5	100	50
81	34.3	50	33.8	99	50	34.2	100	50	33.8	99	50
88	35.2	50	34.7	99	50	35.0	100	50	34.7	99	50
116	37.9	50	38.1	101	50	37.7	100	50	37.9	100	50
144	40.9	50	40.8	100	50	40.4	99	50	41.0	100	50
172	42.5	50	42.7	101	50	42.6	100	50	43.2	102	50
200	44.6	50	44.8	100	50	44.8	101	50	45.0	101	50
228	46.6	50	46.6	100	50	46.6	100	50	46.9	101	50
256	47.5	50	47.3	100	50	47.7	101	50	47.9	101	50
284	48.8	50	48.8	100	50	48.9	100	50	49.1	101	50
312	50.0	50	50.1	100	50	50.3	100	50	50.4	101	50
340	50.2	50	50.3	100	50	50.4	100	50	50.6	101	50
368	51.0	50	51.5	101	50	51.2	101	50	51.5	101	49
396	51.2	50	51.7	101	50	51.9	101	50	51.7	101	49
424	51.6	50	51.9	101	50	51.9	101	50	51.9	101	49
452	51.7	50	51.9	101	49	52.6	102	48	52.2	101	49
480	51.9	50	51.8	100	49	52.4	101	48	52.9	102	49
508	51.8	49	51.4	99	49	52.8	102	48	52.9	102	49
536	51.6	49	51.4	100	49	53.1	103	46	53.1	103	49
564	52.6	47	52.3	99	47	53.4	102	46	53.9	102	49
592	52.2	45	51.5	99	43	52.6	101	44	52.8	101	47
620	51.9	45	51.4	99	40	51.8	100	44	51.5	99	46
648	51.4	45	51.3	100	37	51.6	100	40	51.3	100	45
676	50.5	44	50.4	100	36	51.7	102	39	51.4	102	43
704	49.8	43	49.1	99	35	50.9	102	37	50.9	102	42
Mean for Weeks											
1–13	29.9	–	29.4	98	–	29.8	100	–	29.6	99	–
14–52	45.4	–	45.5	100	–	45.5	100	–	45.8	101	–
53–101	51.5	–	51.4	100	–	52.2	101	–	52.2	101	–

Table 16. Mean Body Weights and Survival of Female Mice in the Two-year Inhalation Study of CIMSTAR 3800

Day	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	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	19.3	50	19.1	99	50	19.0	98	50	18.8	97	50
11	21.2	50	20.8	98	50	21.0	99	50	21.2	100	50
18	22.2	50	22.0	99	50	22.3	101	50	22.4	101	50
25	23.4	50	23.0	99	50	23.5	100	50	23.7	101	50
32	24.2	50	24.0	100	50	24.2	100	50	24.5	101	50
39	24.9	50	24.5	98	50	25.0	100	50	25.3	102	50
46	25.4	50	25.4	100	50	26.0	103	50	26.1	103	50
53	26.4	50	26.5	101	50	26.9	102	50	27.0	103	50
60	26.6	50	26.8	101	50	27.1	102	50	27.4	103	50
67	27.5	50	27.2	99	50	27.5	100	50	27.9	101	50
74	27.8	50	27.6	99	50	28.0	101	50	28.5	102	50
81	28.4	50	28.1	99	50	28.7	101	50	29.0	102	50
88	29.1	50	28.9	99	50	29.2	100	50	29.8	102	50
116	31.3	50	31.2	100	50	30.8	99	50	32.1	103	50
144	34.1	50	33.8	99	50	33.5	99	50	34.8	102	50
172	36.6	50	36.4	99	50	36.1	99	50	37.6	103	50
200	39.3	49	38.6	98	50	38.7	99	50	39.6	101	50
228	41.2	49	41.1	100	50	40.9	99	50	42.4	103	50
256	43.4	49	42.6	98	50	43.5	100	50	44.4	102	49
284	45.8	49	45.2	99	50	45.2	99	50	46.5	102	49
312	48.6	49	48.1	99	50	47.3	97	50	49.7	102	49
340	50.6	49	50.0	99	50	49.7	98	50	51.8	102	49
368	52.1	48	51.6	99	50	50.6	97	50	52.9	102	49
396	54.2	48	53.6	99	50	52.4	97	50	54.9	101	49
424	56.0	48	55.2	99	50	53.8	96	50	55.8	100	49
452	56.5	48	55.6	99	50	54.7	97	50	57.1	101	48
480	57.6	48	56.8	99	50	56.1	97	49	59.0	102	48
508	58.8	48	57.7	98	49	57.1	97	48	60.2	102	48
536	58.4	47	57.9	99	49	56.2	96	48	60.5	104	46
564	59.2	46	58.4	99	48	57.7	98	48	61.2	103	45
592	57.6	46	56.1	97	47	55.8	97	47	60.2	105	44
620	56.4	44	54.3	96	45	54.7	97	45	58.8	104	43
648	55.3	44	53.2	96	43	53.8	97	44	57.9	105	41
676	53.7	44	52.2	97	42	52.0	97	44	56.5	105	39
704	52.9	40	50.8	96	38	50.0	95	39	55.2	104	35
Mean for Weeks											
1–13	25.1	–	24.9	99	–	25.3	101	–	25.5	102	–
14–52	41.2	–	40.8	99	–	40.6	99	–	42.1	102	–
53–101	56.1	–	54.9	98	–	54.2	97	–	57.7	103	–

Pathology and Statistical Analyses

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

Thyroid Gland: There was an increased incidence of follicular cell carcinoma in 100 mg/m^3 females (Table 17, Table D-1, and Table D-2). Although the incidence was not statistically significant, there was a positive trend and the 100 mg/m^3 incidence exceeded the historical control ranges for inhalation studies (0% to 2%) and all routes of exposure (0% to 2%); follicular cell carcinoma occurred in three female mice exposed to 100 mg/m^3 in the current study but in only two of 942 historical controls (Table D-3). Although no follicular cell adenomas occurred in the current study, there were slight but exposure concentration-related increased incidences of follicular cell hyperplasia in females (Table 17 and Table D-5). Follicular cell hyperplasia is an uncommon background lesion and is thought to be a precursor to follicular cell neoplasia.

Follicular cell carcinomas were bilateral masses that replaced the normal thyroid gland parenchyma. They were composed of neoplastic follicular epithelial cells that formed variably sized follicular structures with infoldings and papillary projections of follicular cells into the follicles and little to no colloid. There were other areas with more solid nodules of follicular cells. The neoplastic cells were polygonal with moderate amounts of amphophilic cytoplasm and round to oval nuclei. In one instance, the neoplasm had invaded into a lymphatic duct. Follicular cell hyperplasia was characterized by increased numbers of normal follicular cells within follicles, sometimes appearing as a segment of the follicular epithelium with several layers of cells and other times as papillary infoldings of the follicular epithelium into the follicular lumen.

Table 17. Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Female Mice in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Number Examined Microscopically	50	48	50	50
Follicular Cell Hyperplasia[a]	1 (2.0)[b]	1 (2.0)	2 (2.0)	3 (2.3)
Follicular Cell Carcinoma[c]				
Overall rate[d]	0/50 (0%)	0/48 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[e]	0.0%	0.0%	0.0%	6.9%
Terminal rate[f]	0/39 (0%)	0/36 (0%)	0/37 (0%)	2/33 (6%)
First incidence (days)	–[h]	–	–	712
Poly-3 test[g]	P = 0.008	–[i]	–	P = 0.112

[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical incidence for 2-year inhalation studies with chamber controls (mean ± standard deviation): 1/299 (0.3% ± 0.8%), range 0%–2%; all routes: 2/942 (0.2% ± 0.6%), range 0%–2%.
[d]Number of animals with neoplasm per number of animals with thyroid gland examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]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 differential mortality in animals that do not reach terminal sacrifice.
[h]Not applicable; no neoplasm in animal group.
[i]Value of statistic cannot be computed.

Lung: Compared to that of the chamber controls, there was a significantly increased incidence of alveolar/ bronchiolar adenoma or carcinoma (combined) in 100 mg/m^3 females (Table 18, Table D-1, and Table D-2). There were also increased incidences of alveolar/bronchiolar carcinoma in 100 mg/m^3 females and of alveolar/bronchiolar adenoma in all exposed female groups, but the increases were not statistically significant. However, there were positive trends in the incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in female mice. The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in 100 mg/m^3 females exceeded the historical control ranges for inhalation studies and for all routes of exposure (Table 18 and Table D-4).

In male mice, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/ bronchiolar adenoma or carcinoma (combined) were slightly increased in the 100 mg/m^3 group, but the incidences and trends were not statistically significant, and the incidences did not exceed the historical control ranges for inhalation studies or for all routes (Table 18, Table C-1, Table C-2, and Table C-3). However, there was an increase in the number of animals with multiple carcinomas in the 100 mg/m^3 group relative to the concurrent control.

Alveolar/bronchiolar adenomas were well circumscribed and expansile, often compressing the adjacent lung parenchyma. The neoplasms were most often composed of cuboidal to low columnar epithelial cells that occasionally formed short projections into the alveolar spaces. The neoplastic cells were relatively monomorphic with slightly vacuolated cytoplasm. The mitotic index was low and necrosis was rare. Alveolar/bronchiolar carcinomas were similar to adenomas; however, the carcinomas were often locally invasive, were generally larger, had areas

of sarcomatous and squamous differentiation, and the neoplastic cells were occasionally anaplastic.

There were significantly increased incidences of bronchiole hyperplasia in 30 and 100 mg/m^3 males and females (Table 18, Table C-4, and Table D-5). It is not known whether this lesion is a precursor to alveolar/bronchiolar neoplasms. Hyperplasia of the alveolar epithelium, which may be a precursor to pulmonary neoplasia, occurred in all groups of males and females. While the incidences of this lesion in exposed groups were not significantly increased compared to the chamber controls, there was an exposure-related increase in the severity of the lesion in males. In both sexes, there were slightly increased incidences of histiocytic cellular infiltration in the 100 mg/m^3 groups, but the incidences were not statistically significant. In 100 mg/m^3 females, there was a significantly increased incidence of chronic active perivascular inflammation.

Bronchiole epithelium hyperplasia primarily involved small bronchioles and often extended into the alveolar ducts and spaces. Epithelial cells lining the affected small bronchioles formed one to two layers of cells that frequently had loss of basal nuclear orientation. In more severe cases, epithelial cells formed papillary projections that obscured the bronchiolar lumen. There was often an increase in fibrous connective tissue within the lesion. Alveolar epithelium hyperplasia was characterized by foci in which the alveolar septa were thickened by a single layer of cuboidal, type II alveolar epithelium cells. There was minimal inflammation and no involvement of the terminal bronchioles, thus this lesion was distinct from bronchiolar epithelium hyperplasia. Histiocyte infiltration was characterized by an increase in the number of alveolar macrophages, often large and with foamy cytoplasm, in the alveolar spaces. Chronic active perivascular inflammation was characterized by increased numbers of lymphocytes, macrophages, and neutrophils in the perivascular spaces.

Nose: In all exposed groups of males and females, there were significantly increased incidences of hyaline droplet accumulation in the respiratory and olfactory epithelia and respiratory metaplasia of the olfactory epithelium (except 10 mg/m^3 females) (Table 18, Table C-4, and Table D-5). There was a positive trend in the incidences of olfactory epithelium atrophy in males and a slightly increased incidence in the 100 mg/m^3 group. There were increased incidences of chronic active inflammation in 10 and 100 mg/m^3 males and squamous metaplasia of the respiratory epithelium occurred in a few males and females exposed to 10 or 100 mg/m^3; although the incidences of these lesions were not statistically significant, they were considered to be related to CIMSTAR 3800 exposure.

Olfactory and respiratory epithelium hyaline droplet accumulation were characterized by the accumulation of brightly eosinophilic, homogenous material within the cytoplasm of the epithelial cells. This change mainly affected the naso- and maxilloturbinates, the nasal septum, the glands at the junction of the respiratory and olfactory epithelia, the lateral wall, the nasopharyngeal duct, and the lateral aspects of the ethmoturbinates. Respiratory metaplasia of the olfactory epithelium is the replacement of the olfactory epithelium by ciliated respiratory epithelium, and olfactory epithelium atrophy was characterized by loss of cells and resultant attenuation of the olfactory epithelium. Both lesions were primarily seen in the dorsal meatus and ethmoturbinates. While the biologic significance of hyaline droplet accumulation is unknown, it is frequently seen in inhalation studies. Atrophy of the olfactory epithelium is a degenerative change and respiratory metaplasia of the olfactory epithelium is considered an

adaptive or protective change induced by prolonged, low-level exposure to nasal toxicants. In the current study, these lesions were minimal to mild.

Table 18. Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Mice in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Lung[a]	50	50	50	50
Alveolar Epithelium, Hyperplasia[b]	4 (1.3)[c]	4 (1.3)	6 (2.3)	7 (2.6)
Bronchiole, Hyperplasia	11 (1.0)	11 (1.1)	32** (1.2)	44** (1.1)
Infiltration Cellular, Histiocyte	5 (1.6)	5 (1.6)	1 (1.0)	9 (2.0)
Alveolar/bronchiolar Adenoma[d]	5	2	8	9
Alveolar/bronchiolar Carcinoma, Multiple	1	1	0	5
Alveolar/bronchiolar Carcinoma (includes multiple)[e]	8	8	8	10
Alveolar/bronchiolar Adenoma or Carcinoma[f]	13	9	14	17
Nose	50	50	50	50
Inflammation, Chronic Active	6 (1.3)	8 (2.1)	4 (1.5)	12 (1.7)
Olfactory Epithelium, Accumulation, Hyaline Droplet	4 (1.3)	31** (1.5)	43** (1.8)	49** (2.6)
Olfactory Epithelium, Atrophy	1 (1.0)	0	0	4 (1.0)
Olfactory Epithelium, Metaplasia, Respiratory	7 (1.1)	15* (1.3)	25** (1.2)	37** (1.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	7 (1.1)	36** (1.4)	50** (1.8)	50** (2.6)
Respiratory Epithelium, Metaplasia, Squamous	0	3 (1.3)	0	1 (1.0)
Larynx	50	50	49	50
Inflammation, Chronic Active	0	2 (1.5)	3 (1.0)	8** (1.0)
Metaplasia, Squamous	0	50** (1.0)	49** (2.0)	50** (3.4)
Necrosis	0	1 (2.0)	1 (1.0)	1 (1.0)
Trachea	50	50	47	50
Ulcer	0	0	0	1
Female				
Lung	50	50	50	50
Alveolar Epithelium, Hyperplasia	4 (1.5)	2 (2.0)	5 (1.2)	4 (2.0)
Bronchiole, Hyperplasia	7 (1.0)	4 (1.0)	22** (1.0)	41** (1.0)
Infiltration Cellular, Histiocyte	4 (1.0)	1 (1.0)	1 (1.0)	6 (1.5)
Perivascular, Inflammation, Chronic Active	4 (1.8)	3 (1.7)	0	11* (2.0)
Alveolar/bronchiolar Adenoma, Multiple	0	1	0	0
Alveolar/bronchiolar Adenoma (includes multiple)[g]	1	4	2	4

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Alveolar/bronchiolar Carcinoma[h]				
Overall rate[i]	4/50 (8%)	1/50 (2%)	4/50 (8%)	8/50 (16%)
Adjusted rate[j]	8.8%	2.2%	8.6%	18.2%
Terminal rate[k]	4/39 (10%)	1/37 (3%)	3/37 (8%)	6/33 (18%)
First incidence (days)	731 (T)	731 (T)	460	644
Poly-3 test[l]	P = 0.021	P = 0.176N	P = 0.627N	P = 0.163
Alveolar/bronchiolar Adenoma or Carcinoma[m]				
Overall rate	4/50 (8%)	5/50 (10%)	6/50 (12%)	12/50 (24%)
Adjusted rate	8.8%	10.9%	12.8%	27.2%
Terminal rate	4/39 (10%)	5/37 (14%)	4/37 (11%)	9/33 (27%)
First incidence (days)	731 (T)	731 (T)	460	644
Poly-3 test	P = 0.006	P = 0.505	P = 0.391	P = 0.021
Nose	50	49	50	50
Olfactory Epithelium, Accumulation, Hyaline Droplet	25 (1.8)	40** (1.9)	50** (2.6)	49** (3.0)
Olfactory Epithelium, Metaplasia, Respiratory	3 (1.0)	4 (1.3)	12* (1.6)	23** (1.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	34 (1.6)	48** (1.7)	50** (2.6)	50** (3.0)
Respiratory Epithelium, Metaplasia, Squamous	0	1 (1.0)	0	1 (1.0)
Larynx	49	49	50	50
Inflammation, Chronic Active	0	0	0	10** (1.2)
Metaplasia, Squamous	1 (1.0)	49** (1.1)	50** (2.1)	50** (3.5)
Necrosis	0	0	0	4 (1.5)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test.
**$P \leq 0.01$.
(T) = terminal kill.
[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.
[d]Historical control incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 39/300 (13.0% ± 4.2%), range 8%–20%; all routes: 145/950 (15.3% ± 6.2%), range 2%–26%.
[e]Historical control incidence for inhalation studies: 59/300 (19.7% ± 3.4%), range 16%–24%; all routes: 132/950 (13.9% ± 7.1%), range 4%–24%.
[f]Historical control incidence for inhalation studies: 90/300 (30.0% ± 5.5%), range 26%–40%; all routes: 263/950 (27.7% ± 5.7%), range 16%–40%.
[g]Historical control incidence for inhalation studies: 16/299 (5.4% ± 3.7%), range 2%-12%; all routes: 54/949 (5.7% ± 3.6%), range 0%–12%.
[h]Historical control incidence for inhalation studies: 13/299 (4.4% ± 4.3%), range 0%-10%; all routes: 38/949 (4.0% ± 3.6%), range 0%–14%.
[i]Number of animals with neoplasm per number of animals with lung examined microscopically.
[j]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[k]Observed incidence at terminal kill.
[l]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 differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.
[m]Historical incidence for inhalation studies: 28/299 (9.4% ± 4.8%), range 2%–16%; all routes: 90/949 (9.5% ± 4.8%), range 2%–22%.

Larynx: There were chemical-related increased incidences of chronic active inflammation, squamous metaplasia, and necrosis in both sexes (Table 18, Table C-4, and Table D-5). The incidences of squamous metaplasia in all exposed groups were significantly increased, and the severities increased with increasing exposure concentration. This lesion occurred in all exposed animals examined, but in only one chamber control mouse. There were significantly increased incidences of chronic active inflammation 100 mg/m^3 males and females. Epithelial necrosis occurred in a single male in each exposed group and in four 100 mg/m^3 females; while the incidences were low, this lesion was considered to be related to CIMSTAR 3800 exposure.

Squamous metaplasia was characterized by the replacement of the normal respiratory epithelium with three to 10 layers of squamous epithelium. This lesion was most commonly present at the base of the epiglottis, but at higher exposure concentrations it was also present on the lateral walls. Squamous metaplasia included squamous hyperplasia of the epithelium lining the arytenoid cartilages (three to eight layers of cells), which normally have one to three layers of squamous epithelium. In the 3-month study, epithelial dysplasia was present in association with squamous metaplasia, but there was no evidence of epithelial dysplasia in the 2-year study. Chronic active inflammation was characterized by the accumulation of lymphocytes with fewer macrophages, plasma cells, and neutrophils in the submucosa of the larynx with occasional small foci of subepithelial fibrosis. This lesion was limited to the base of the epiglottis. Epithelial necrosis was characterized by hypereosinophilia of the epithelial cells with nuclear pyknosis and karyorrhexis. While uncommon in the current study, this lesion was also considered to be related to CIMSTAR 3800 exposure.

Trachea: There was a single incidence of ulcer in 100 mg/m^3 males (Table 18 and Table C-4) that was characterized by loss of the epithelium from the surface of the trachea, exposing the underlying basement membrane. This is a very uncommon spontaneous lesion and is likely due to CIMSTAR 3800 exposure.

Genetic Toxicology

CIMSTAR 3800 (dose range tested, 1,000 to 10,000 µg/plate) was weakly mutagenic in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the absence of exogenous metabolic activation (S9); no mutagenic activity was observed in *Salmonella typhimurium* strains TA98 and TA100 tested over the same dose range, with or without S9, or in the *E. coli* strain with S9 (Table E-1).

In vivo, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were observed in peripheral blood samples from male or female F344/NTac rats or B6C3F1/N mice exposed to CIMSTAR 3800 via inhalation (25 to 400 mg/m^3) for 3 months (Table E-2 and Table E-3). In female rats, a significant trend was observed for micronucleated erythrocytes ($P = 0.003$). However, in rats, the appropriate cell population to evaluate for frequency of micronucleated cells in peripheral blood is the very young newly emerged reticulocyte population because the rat spleen is very efficient at quickly sequestering and destroying damaged erythrocytes. Thus, despite the very slight increase in micronucleated erythrocytes in 400 mg/m^3 female rats, the absence of supporting data in the female micronucleated reticulocyte data set and in the male rats suggests that the increase is not biologically significant.

In addition to the micronucleus endpoint, the percentage of reticulocytes among circulating red blood cells was calculated as a measure of bone marrow toxicity or perturbations in

erythropoiesis (Table E-2 and Table E-3). No significant alterations in the percentage of reticulocytes among red blood cells was seen with CIMSTAR 3800, suggesting an absence of treatment-related bone marrow toxicity. The very small and inconsistent changes in percentage of reticulocytes noted in both the male and female mice, in the absence of any observed hematologic effects, were not considered biologically significant.

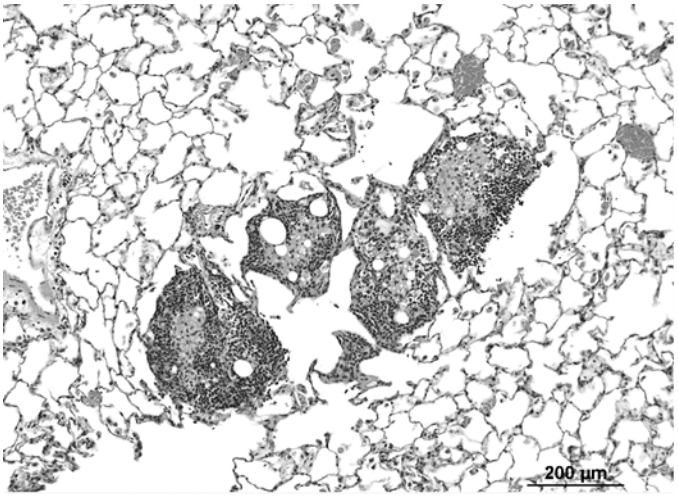


Figure 7. Lymphohistiocytic Inflammation in the Lung of a Male Wistar Han Rat Exposed to 100 mg/m^3 CIMSTAR 3800 by Whole Body Inhalation for Two Years (H&E)

Note the unusual inflammatory response characterized by clusters of large, foamy macrophages surrounded by numerous mononuclear inflammatory cells. The variably sized, clear vacuoles that appear to be both intracellular (within macrophages) and extracellular are suggestive of the presence of lipid material.

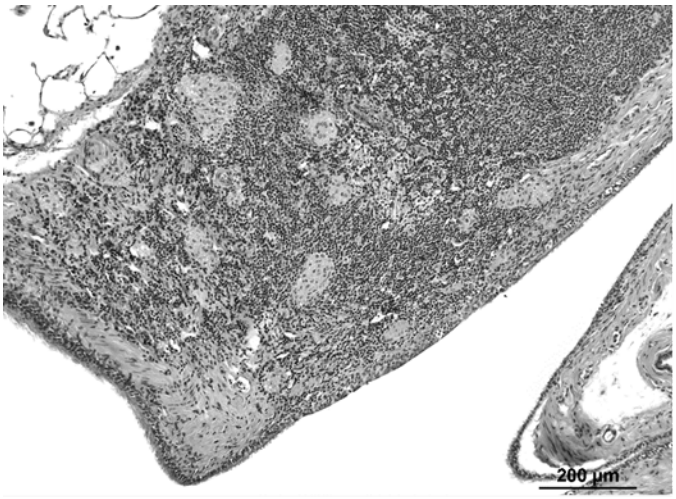


Figure 8. Lymphohistiocytic Hyperplasia of the Bronchus-associated Lymphoid Tissue in the Lung of a Male Wistar Han Rat Exposed to 100 mg/m^3 CIMSTAR 3800 by Whole Body Inhalation for Two Years (H&E)

Note the unusual hyperplastic lesion that is morphologically similar to the inflammation in the lung seen in these animals. It is characterized by clusters of large, foamy macrophages surrounded by numerous mononuclear inflammatory cells.

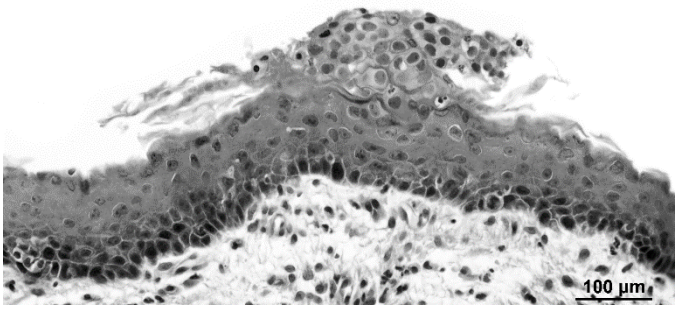


Figure 9. Dysplasia of the Epiglottis in the Larynx of a Male B6C3F1/N Mouse Exposed To 400 mg/m^3 CIMSTAR 3800 by Whole Body Inhalation for Three Months (H&E)

Note the unusual exophytic growth of epithelial cells above the stratum corneum. The cells also have increased nuclear to cytoplasmic ratios and variability in the staining of the nuclei and cytoplasm. The epithelium, which is normally a single layer of ciliated cuboidal to columnar cells, also exhibits squamous metaplasia.

Discussion

Occupational exposures to metalworking fluids occur primarily by inhalation and have been reported to cause a number of respiratory problems in workers. The upper respiratory tract was the primary target site in rodents exposed by whole body inhalation to CIMSTAR 3800 aerosols. In 3-month inhalation studies, exposure to CIMSTAR 3800 (25 to 400 mg/m^3) caused significant irritation of the nasal cavity of F344/NTac rats and B6C3F1/N mice. Hyaline droplet accumulation in the respiratory and olfactory epithelium of the nose was present in almost all exposed rats and mice, and goblet cell hyperplasia was present in the nasal cavity of almost all exposed rats. These lesions are commonly observed in the nasal cavity of rodents following inhalation of an irritant[104]. Rhinitis symptoms are common among machinists exposed to water-soluble metalworking fluids[33; 105-107].

The larynx was also a target of CIMSTAR 3800 toxicity in the 3-month studies. Squamous metaplasia of the respiratory epithelium lining the larynx was present in all exposed rats and was the most severe lesion observed in the 3-month studies. In addition, squamous epithelium hyperplasia and chronic active inflammation were present in the larynx of most rats and mice exposed to 100 mg/m^3 or greater. Minimal to mild laryngeal lesions are commonly observed in inhalation studies of irritant chemicals; however, in the 3-month CIMSTAR 3800 study, the marked severity of squamous metaplasia in the larynx of rats and mice and the presence of epithelial dysplasia in the epiglottis of some exposed mice were unusual. Squamous metaplasia is a common adaptive response to repeated injury to the epithelium and results in the replacement of injured epithelium with the more resistant squamous epithelium. Though marked squamous metaplasia of the larynx has only been reported in a few NTP inhalation studies, squamous metaplasia of the larynx, in general, is a common lesion in NTP inhalation studies. However, laryngeal neoplasia is extremely rare; therefore, laryngeal squamous metaplasia is not considered a preneoplastic lesion by NTP. Inhalation exposure to diethylamine[108] or triethylamine[109] vapors caused marked squamous metaplasia of the larynx, similar to lesions caused by CIMSTAR 3800. Notably, diethylamine and triethylamine are highly alkaline chemicals, like CIMSTAR 3800.

In the more distal respiratory tract, 3-month exposures to CIMSTAR 3800 caused minimal to mild bronchiole hyperplasia in the lungs of all exposed mice. Arteriole hypertrophy and perivascular chronic active inflammation were also observed in some mice at the higher concentrations. Effects on the lung were less in the rat and consisted of mild alveolar histiocytosis at the two highest exposure concentrations.

Exposure to 10, 30, or 100 mg/m^3 CIMSTAR 3800 for 2 years had no adverse effect on survival or body weights of Wistar Han rats or B6C3F1/N mice. As in the 3-month studies, nonneoplastic lesions were observed in the upper respiratory tract of exposed animals. Goblet cell hyperplasia, hyperplasia of olfactory submucosal glands, and hyaline droplet accumulation were present in the nasal cavity of most exposed rats and mice. Squamous metaplasia of the respiratory and olfactory epithelium of the nose was also present in mice; this lesion was not observed in the 3-month study.

Squamous metaplasia of the larynx was observed in almost all rats and mice exposed to CIMSTAR 3800 for 2 years. The severity of this lesion was exposure concentration-dependent and was moderate to marked in the 100 mg/m^3 group. Despite the severity and persistence of

squamous metaplasia, there were no neoplasms in the larynx of rats or mice. Squamous metaplasia of the larynx is not known to be preneoplastic in rodents, and there have been no incidences of adenoma or carcinoma of the larynx in rats or mice in NTP studies. Epidemiological studies have reported increased laryngeal cancer in workers exposed to straight oil metalworking fluids[59]; however, the evidence was significantly less for machinists exposed to soluble oils[58]. CIMSTAR 3800 is a semisynthetic oil and does not contain the carcinogens (e.g., polyaromatic hydrocarbons, diethanolamine) that were present in the straight oil metalworking fluids.

An unusual lymphohistiocytic inflammation was observed in the lungs of rats exposed to CIMSTAR 3800 for 2 years. The incidence and severity of lymphohistiocytic inflammation increased with increasing exposure concentration, and the lesion was present in the lungs of almost all male and female rats exposed to 100 mg/m^3. Lymphohistiocytic hyperplasia of the bronchus-associated lymphoid tissue also was observed in exposed rats suggesting that the lymphohistiocytic inflammation may have been mediated by an immunologic reaction. In the workplace, in-use CIMSTAR 3800 and other metalworking fluids can become contaminated by fungi and bacteria. These microbes and their by-products may cause immunologically mediated reactions such as hypersensitivity pneumonitis when inhaled. However, in the current study, rats were exposed to uncontaminated CIMSTAR 3800 indicating that chemical components were the cause of lymphohistiocytic inflammation. Formaldehyde-releasing biocides and alkanolamines present in metalworking fluids have been implicated as potential causes of hypersensitivity pneumonitis[13; 35], and CIMSTAR 3800 contains a triazine biocide as well as ethanolamine and triethanolamine. Repeated inhalation exposure to the highly alkaline CIMSTAR 3800 (pH 9) and accumulation of the oil components undoubtedly contributed to the upper respiratory tract lesions observed in rats and mice, although the contribution of the highly diluted chemical components of CIMSTAR 3800 cannot be discounted.

There is considerable evidence for increased risk of cancer at several sites in machinists exposed to metal-working fluids used before the mid-1970s. CIMSTAR 3800 and other newer semisynthetic formulations are presumed to be safer because a number of known and suspected carcinogens have been removed or reduced; however, the potential carcinogenicity of these newer formulations has not been evaluated.

There was some evidence of CIMSTAR 3800 carcinogenicity in the lungs of female mice exposed for 2 years. A significantly increased ($P = 0.021$) incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was observed in the lungs of female mice exposed to 100 mg/m^3. The incidence (24%) exceeded the historical control range (2% to 16%) for inhalation studies. The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in female mice exposed to lower concentrations were not significant; however, there were positive trends in the incidences of alveolar/bronchiolar carcinoma ($P = 0.021$), and alveolar/bronchiolar adenoma or carcinoma (combined) ($P = 0.006$). In addition, the incidences of bronchiole hyperplasia were significantly increased ($P < 0.001$) in female mice exposed to 30 or 100 mg/m^3. The severity of this minimal lesion did not increase with increasing exposure concentration. Bronchiole hyperplasia may contribute to the development of adenoma and carcinoma in the lung. This was considered only some evidence of carcinogenicity, rather than clear evidence of carcinogenicity, of lung neoplasms in female mice because the incidence in the 100 mg/m^3 group was only slightly greater than the historical control range and only the combined incidence of alveolar/bronchiolar adenoma or carcinoma in the 100 mg/m^3 group was significantly increased.

The incidences of bronchiole hyperplasia were also significantly increased in 30 and 100 mg/m^3 male mice, although there was no evidence of carcinogenicity in the lungs of male mice. There was also no evidence of carcinogenicity in the lungs of rats in the 2-year study. Epidemiological data do not show an association between lung cancer and soluble metalworking fluid exposure[58].

Follicular cell carcinoma of the thyroid gland was present in 6% of female mice exposed to 100 mg/m^3 CIMSTAR 3800 for 2 years. This incidence of follicular cell carcinoma was not statistically significant relative to concurrent chamber controls, and these neoplasms were not present in females exposed to lower concentrations or in exposed male mice. Follicular cell carcinomas have been observed in only two of 942 female historical control mice exposed by all routes. The incidence of follicular cell hyperplasia, a potential precursor to neoplasia, was increased in female mice in an exposure concentration-dependent manner up to 6% in the 100 mg/m^3 group. Because the neoplasms were malignant, are uncommon, and there were exposure concentration-related increased incidences of follicular cell hyperplasia, the follicular cell carcinomas were considered to be treatment related.

In female rats, three C-cell carcinomas of the thyroid gland occurred in the 100 mg/m^3 group, which exceeded the historical control range (0% to 2%) for all routes of exposure. There was also a positive trend in the incidence of C-cell adenoma or carcinoma (combined); however, the incidence in the 100 mg/m^3 group did not exceed the historical control range (6% to 14%) for all routes of exposure. Furthermore, the incidences of C-cell carcinoma and C-cell adenoma or carcinoma (combined) were not significantly increased in any exposed group when compared to the chamber control group. Therefore, the C-cell neoplasms in female rats were not considered to be exposure related.

An increased incidence of prostate gland adenoma or carcinoma (combined) was observed in male rats exposed to 100 mg/m^3 (6%); however, this incidence was not significantly different from that in the concurrent chamber controls. Prostate gland neoplasms were all adenomas except for one carcinoma in the 30 mg/m^3 group. No adenomas or carcinomas of the prostate gland have been reported in the previous two studies with Wistar Han rats (100 rats) and are rare in F344/N rats (3/698). Despite the rarity of these neoplasms and the exposure concentration-dependent increased incidences, it is unclear if this is a treatment-related effect because of the low incidences of neoplasms, the lack of statistical significance, and the lack of historical control data for inhalation studies in Wistar Han rats, which complicates the interpretation of data for rare neoplasms. Although there is epidemiological evidence of increased prostate gland cancer in metalworkers exposed to straight oils, there was no association in workers exposed to water-soluble formulations like CIMSTAR 3800[58].

A small number of uterine adenocarcinomas and a mixed malignant Müllerian tumor (MMMT) were observed in the original evaluation in female rats. In an effort by NTP to more thoroughly evaluate rat uteri, additional evaluations were conducted on the residual uterine tissue, which was sectioned longitudinally (i.e., parallel to the long axis), from this and other studies. In the residual evaluation in the current study, there were statistically significant increases in the incidences of uterine adenocarcinoma and adenocarcinoma or MMMT (combined) in the 30 and 100 mg/m^3 groups. However, when combined with the neoplasm incidences in the original evaluation, the incidences were not statistically significant. Additionally, in two residual uterine tissue evaluations in other Wistar Han rat studies, there were four adenocarcinomas in the control group from one study and eight adenocarcinomas in the control group from the other study[102; 103];

the incidence from the latter study exceeding the incidence of adenocarcinoma or MMMT (combined) in the 100 mg/m^3 group in the current study. These results suggest that background uterine adenocarcinomas are more prevalent in Wistar Han rats than originally expected. Therefore, it is unclear whether the incidences of uterine adenocarcinoma or MMMT (combined) in the current study are chemical related.

Skin contact is also a major route of occupational exposure to metalworking fluids. Although dermal application of metalworking fluids has been reported to cause skin papillomas and carcinomas in experimental animals[43-45], these studies were conducted with straight oil formulations before known or suspected carcinogens were removed. Similarly, substantial evidence exists for an increased risk of squamous cell skin cancer associated with exposure conditions of the mid-1970s and earlier[51-53]. In the mid-1980s many known and suspected carcinogens were removed or reduced in metalworking fluid formulations. New semisynthetic metalworking fluids such as CIMSTAR 3800 are considered to be safer.

Animals received significant dermal exposure to CIMSTAR 3800 during the 2-year whole body inhalation study due to deposition of the liquid aerosols on the fur and skin. Squamous cell papilloma or keratoacanthoma (combined) of the skin occurred in 8% of female rats exposed to 100 mg/m^3; however, this increased incidence was not statistically significant. Only one of these benign neoplasms was a keratoacanthoma. Squamous cell papilloma and keratoacanthoma of the skin were not observed in 150 female Wistar Han historical control rats from all routes of exposure. Despite the dermal exposure and a slight increased incidence in benign neoplasms in the 100 mg/m^3 group, it is unclear if this is a treatment-related effect because of the lack of statistical significance and the lack of historical control data from inhalation studies in the Wistar Han rat.

CIMSTAR 3800, like all metalworking fluids, is a complex mixture of chemicals. The chemical composition of CIMSTAR 3800 is proprietary information and its potentially toxic components have not been identified. The major component of CIMSTAR 3800 and most semisynthetic metalworking fluids is water. The CIMSTAR 3800 concentrate used in this study contained 60% water. In the workplace, this concentrate is diluted 1:10 to 1:20 with water prior to use, therefore workers are exposed to CIMSTAR 3800 aerosols composed of 90% to 95% water. Because it is technically difficult to generate and expose animals to liquid aerosols containing up to 90% water without saturating the chamber air, the aerosols in the current study were generated from undiluted concentrate and diluted with clean air to produce the desired concentrations. Thus, the CIMSTAR 3800 exposure concentrations used in the current study were considerably higher than those encountered in the workplace.

Although a complete chemical analysis of CIMSTAR 3800 was not performed, several major chemical groups were identified. In addition to water, CIMSTAR 3800 concentrate was found to contain approximately 25% (by weight) hexane extractable (water insoluble) compounds. The CIMSTAR 3800 concentrate also contained several alkanolamines including ethanolamine (4.7% to 5.7% by weight), triethanolamine (3.2% to 3.4% by weight) and 1-amino-2-propanol (1.4% to 1.6% by weight). Diethanolamine (a known carcinogen) was not detected. Alkanolamines are generally added to semisynthetic metal-working fluids to prevent corrosion and to maintain a high pH to facilitate rust removal[110].

There are no published genotoxicity data for CIMSTAR 3800 or other metalworking fluids, with the exception of the single epidemiological study that looked for an association between the incidence of DNA strand breaks and occupational exposure to *N*-nitrosodiethanolamine (NDELA), a known genotoxic and carcinogenic contaminant present in older formulations of metalworking fluids[64]. Although more modern (beginning mid-1980s) formulations of metalworking fluids do not contain the nitrosating agents that can result in the formation of NDELA, it is possible that other components of these complex mixtures have genotoxic potential, and indeed, CIMSTAR 3800 was shown to be a weak bacterial mutagen in assays presented in this Technical Report. Ethanolamine and triethanolamine, both present in CIMSTAR 3800, have been shown to be non-mutagenic in bacteria[111], while 1-amino-2-propanol was judged to be equivocal in a bacterial mutagenicity assay[112]. However, the small percentage of 1-amino-2-propanol in CIMSTAR 3800 and the pattern of activity (variable responses in *Salmonella typhimurium* strain TA1535 in the presence of S9 mix) indicate that it does not have a role in the weak positive response seen with CIMSTAR 3800 in the *Escherichia coli* tester strain in the absence of S9 only. NTP has evaluated the mutagenicity of several different metalworking fluids in bacteria and most were shown to be negative (NTP, unpublished data). Three types of metalworking fluids (two soluble oils and one semisynthetic) did demonstrate clear mutagenicity in the *E. coli* strain in the presence of S9 mix, and all three contained biocides that release formaldehyde, a known bacterial mutagen. CIMSTAR 3800 also contains a formaldehyde-releasing biocide, and therefore, formaldehyde may have been responsible for its mutagenic activity in bacteria. No indication of chromosomal damage, measured in an erythrocyte micronucleus assay, was seen in rats or mice from the current 3-month inhalation study.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity*[a] of CIMSTAR 3800 in male Wistar Han rats based on the incidences of prostate gland adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of CIMSTAR 3800 in female Wistar Han rats based on the incidences of squamous cell papilloma or keratoacanthoma (combined) of the skin and adenocarcinoma or mixed malignant Müllerian tumor (combined) of the uterus. There was *no evidence of carcinogenic activity* of CIMSTAR 3800 in male B6C3F1/N mice exposed to 10, 30, or 100 mg/m^3. There was *some evidence of carcinogenic activity* of CIMSTAR 3800 in female B6C3F1/N mice based on the incidences of follicular cell carcinoma of the thyroid gland and alveolar/bronchiolar adenoma or carcinoma (combined) of the lung.

Exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. A summary of the peer review panel comments and the public discussion on this Technical Report appears in Appendix K.

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Appendix A. Summary of Lesions in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	12	13	15
Natural deaths	–	4	3	2
Survivors				
Terminal kill	33	34	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(48)	(47)	(49)
Intestine large, colon	(50)	(49)	(47)	(49)
Intestine large, rectum	(48)	(47)	(47)	(48)
Intestine small, duodenum	(50)	(48)	(47)	(49)
Intestine small, ileum	(50)	(48)	(47)	(49)
Intestine small, jejunum	(50)	(48)	(47)	(49)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	–	1 (2%)	–	–
Hepatocellular carcinoma	–	1 (2%)	–	–
Mesentery	(7)	(7)	(1)	(2)
Liposarcoma	–	–	–	1 (50%)
Oral mucosa	(1)	(0)	(1)	(0)
Pancreas	(50)	(50)	(49)	(50)
Adenoma	1 (2%)	–	–	–
Carcinoma	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Sarcoma	–	–	–	1 (2%)
Schwannoma malignant	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)	–	–	–
Sarcoma	1 (2%)	–	–	–
Stomach, glandular	(50)	(50)	(49)	(50)
Sarcoma	1 (2%)	–	–	–
Tooth	(0)	(1)	(0)	(1)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	1 (2%)	–	2 (4%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Adenoma	2 (4%)	–	2 (4%)	1 (2%)
Carcinoma	–	1 (2%)	1 (2%)	1 (2%)
Bilateral, carcinoma	1 (2%)	–	–	–
Adrenal medulla	(50)	(48)	(49)	(50)
Pheochromocytoma benign	4 (8%)	–	1 (2%)	5 (10%)
Pheochromocytoma malignant	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	–	–	2 (4%)	2 (4%)
Carcinoma	–	–	1 (2%)	–
Parathyroid gland	(43)	(43)	(44)	(47)
Adenoma	–	1 (2%)	–	1 (2%)
Pituitary gland	(49)	(50)	(50)	(50)
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Pars distalis, adenoma	16 (33%)	18 (36%)	11 (22%)	17 (34%)
Pars distalis, carcinoma	–	–	1 (2%)	–
Pars intermedia, adenoma	2 (4%)	1 (2%)	2 (4%)	–
Thyroid gland	(50)	(50)	(49)	(50)
C-cell, adenoma	6 (12%)	5 (10%)	3 (6%)	5 (10%)
C-cell, adenoma, multiple	1 (2%)	–	–	–
C-cell, carcinoma	–	–	1 (2%)	1 (2%)
Follicular cell, adenoma	1 (2%)	–	–	–
Follicular cell, carcinoma	–	1 (2%)	1 (2%)	2 (4%)
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Genital System				
Coagulating gland	(0)	(0)	(0)	(1)
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate gland	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	2 (4%)
Adenoma, multiple	1 (2%)	–	1 (2%)	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Carcinoma	–	–	1 (2%)	–
Seminal vesicle	(50)	(50)	(48)	(50)
Adenoma	1 (2%)	–	–	–
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Bronchial associated lymphoid tissue	(0)	(1)	(5)	(19)
Lymph node	(12)	(5)	(7)	(5)
Lymph node, bronchial	(42)	(40)	(37)	(35)
Lymph node, mandibular	(46)	(46)	(44)	(45)
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	–
Lymph node, mediastinal	(46)	(45)	(50)	(49)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Hemangioma	–	–	1 (2%)	–
Hemangiosarcoma	3 (6%)	1 (2%)	–	2 (4%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Sarcoma	–	–	–	1 (2%)
Thymus	(40)	(43)	(44)	(46)
Thymoma benign	1 (3%)	–	2 (5%)	1 (2%)
Thymoma malignant	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(6)	(6)	(3)	(4)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	–	1 (2%)	–
Keratoacanthoma	1 (2%)	–	–	–
Squamous cell papilloma	–	–	1 (2%)	–
Sebaceous gland, adenoma	–	1 (2%)	–	–
Subcutaneous tissue, fibroma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, hemangiopericytoma	–	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma	–	–	–	1 (2%)
Subcutaneous tissue, lipoma	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, liposarcoma	–	–	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Subcutaneous tissue, neural crest tumor	–	1 (2%)	–	–
Subcutaneous tissue, schwannoma malignant	–	2 (4%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)	–	2 (4%)
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Skeletal muscle	(1)	(2)	(1)	(0)
Sarcoma	–	1 (50%)	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	–
Glioma	2 (4%)	1 (2%)	1 (2%)	–
Granular cell tumor benign	–	2 (4%)	–	1 (2%)
Granular cell tumor malignant	–	1 (2%)	1 (2%)	–
Meningioma malignant	1 (2%)	–	–	–
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Peripheral nerve	(5)	(4)	(6)	(6)
Spinal cord	(5)	(4)	(7)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	–	1 (2%)	–
Osteosarcoma, metastatic, bone	–	1 (2%)	–	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Trachea	(50)	(49)	(50)	(50)
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(48)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Sarcoma, metastatic, salivary glands	–	–	–	1 (2%)
Zymbal's gland	(3)	(2)	(1)	(0)
Carcinoma	2 (67%)	2 (100%)	–	–
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Lipoma	–	–	–	1 (2%)
Renal tubule, adenoma	–	1 (2%)	–	–
Transitional epithelium, papilloma	1 (2%)	–	–	–
Urinary bladder	(50)	(50)	(48)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	–	2 (4%)	1 (2%)	1 (2%)
Mesothelioma malignant	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	37	36	30	39
Total primary neoplasms	62	51	45	60
Total animals with benign neoplasms	32	27	24	31
Total benign neoplasms	45	34	33	42
Total animals with malignant neoplasms	13	14	9	16
Total malignant neoplasms	15	15	11	18
Total animals with metastatic neoplasms	–	4	–	2
Total metastatic neoplasms	–	4	–	9
Total animals with malignant neoplasms of uncertain primary site	–	1	–	–
Total animals with uncertain neoplasms – benign or malignant	2	2	1	–
Total uncertain neoplasms	2	2	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 A-2. Statistical Analysis of Primary Neoplasms in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	4/50 (8%)	0/48 (0%)	1/49 (2%)	5/50 (10%)
Adjusted rate[b]	9.1%	0.0%	2.2%	11.5%
Terminal rate[c]	2/33 (6%)	0/32 (0%)	1/34 (3%)	4/33 (12%)
First incidence (days)	612	–[e]	729 (T)	725
Poly-3 test[d]	P = 0.118	P = 0.070N	P = 0.172N	P = 0.492
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	4/50 (8%)	1/48 (2%)	1/49 (2%)	5/50 (10%)
Adjusted rate	9.1%	2.4%	2.2%	11.5%
Terminal rate	2/33 (6%)	1/32 (3%)	1/34 (3%)	4/33 (12%)
First incidence (days)	612	729 (T)	729 (T)	725
Poly-3 test	P = 0.177	P = 0.201N	P = 0.172N	P = 0.492
Brain: Benign or Malignant Granular Cell Tumor				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	0.0%	7.0%	2.2%	2.3%
Terminal rate	0/33 (0%)	2/34 (6%)	0/34 (0%)	1/33 (3%)
First incidence (days)	–	704	684	729 (T)
Poly-3 test	P = 0.593N	P = 0.116	P = 0.511	P = 0.500
Mesenteric Lymph Node: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Adjusted rate	6.7%	2.3%	0.0%	4.6%
Terminal rate	1/33 (3%)	0/34 (0%)	0/34 (0%)	2/33 (6%)
First incidence (days)	345	704	–	729 (T)
Poly-3 test	P = 0.642N	P = 0.321N	P = 0.115N	P = 0.515N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/49 (6%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	6.5%	4.6%
Terminal rate	0/33 (0%)	0/34 (0%)	1/34 (3%)	2/33 (6%)
First incidence (days)	–	–	704	729 (T)
Poly-3 test	P = 0.175	–[f]	P = 0.129	P = 0.236
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	16/49 (33%)	18/50 (36%)	11/50 (22%)	17/50 (34%)
Adjusted rate	35.2%	40.2%	23.3%	37.5%
Terminal rate	8/32 (25%)	13/34 (38%)	6/34 (18%)	10/33 (30%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
First incidence (days)	520	505	620	504
Poly-3 test	P = 0.494	P = 0.395	P = 0.150N	P = 0.496
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	16/49 (33%)	18/50 (36%)	12/50 (24%)	17/50 (34%)
Adjusted rate	35.2%	40.2%	25.4%	37.5%
Terminal rate	8/32 (25%)	13/34 (38%)	6/34 (18%)	10/33 (30%)
First incidence (days)	520	505	620	504
Poly-3 test	P = 0.501	P = 0.395	P = 0.211N	P = 0.496
Prostate Gland: Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.3%	2.3%	2.2%	6.9%
Terminal rate	1/33 (3%)	1/34 (3%)	1/34 (3%)	3/33 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P = 0.157	P = 0.757	P = 0.749N	P = 0.304
Prostate Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.3%	2.3%	4.3%	6.9%
Terminal rate	1/33 (3%)	1/34 (3%)	1/34 (3%)	3/33 (9%)
First incidence (days)	729 (T)	729 (T)	684	729 (T)
Poly-3 test	P = 0.187	P = 0.757	P = 0.520	P = 0.304
Thymus: Benign Thymoma				
Overall rate	1/40 (3%)	0/43 (0%)	2/44 (5%)	1/46 (2%)
Adjusted rate	2.8%	0.0%	4.9%	2.5%
Terminal rate	1/27 (4%)	0/27 (0%)	1/29 (3%)	1/30 (3%)
First incidence (days)	729 (T)	–	722	729 (T)
Poly-3 test	P = 0.613	P = 0.498N	P = 0.545	P = 0.734N
Thymus: Benign or Malignant Thymoma				
Overall rate	1/40 (3%)	0/43 (0%)	2/44 (5%)	2/46 (4%)
Adjusted rate	2.8%	0.0%	4.9%	4.9%
Terminal rate	1/27 (4%)	0/27 (0%)	1/29 (3%)	1/30 (3%)
First incidence (days)	729 (T)	–	722	457
Poly-3 test	P = 0.329	P = 0.498N	P = 0.545	P = 0.548
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/50 (14%)	5/50 (10%)	3/49 (6%)	5/50 (10%)
Adjusted rate	16.0%	11.6%	6.6%	11.5%

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Terminal rate	6/33 (18%)	4/34 (12%)	2/34 (6%)	4/33 (12%)
First incidence (days)	704	717	704	725
Poly-3 test	P = 0.438N	P = 0.390N	P = 0.143N	P = 0.381N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/50 (14%)	5/50 (10%)	4/49 (8%)	6/50 (12%)
Adjusted rate	16.0%	11.6%	8.9%	13.8%
Terminal rate	6/33 (18%)	4/34 (12%)	3/34 (9%)	5/33 (15%)
First incidence (days)	704	717	704	725
Poly-3 test	P = 0.579N	P = 0.390N	P = 0.241N	P = 0.503N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
Adjusted rate	6.7%	2.3%	0.0%	9.2%
Terminal rate	1/33 (3%)	0/34 (0%)	0/34 (0%)	4/33 (12%)
First incidence (days)	345	704	–	729 (T)
Poly-3 test	P = 0.188	P = 0.321N	P = 0.115N	P = 0.482
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	6.7%	2.3%	2.2%	9.2%
Terminal rate	1/33 (3%)	0/34 (0%)	0/34 (0%)	4/33 (12%)
First incidence (days)	345	704	722	729 (T)
Poly-3 test	P = 0.213	P = 0.321N	P = 0.297N	P = 0.482
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	27/50 (54%)	24/50 (48%)	31/50 (62%)
Adjusted rate	67.6%	59.4%	50.7%	67.8%
Terminal rate	21/33 (64%)	20/34 (59%)	15/34 (44%)	22/33 (67%)
First incidence (days)	520	505	620	504
Poly-3 test	P = 0.351	P = 0.269N	P = 0.068N	P = 0.577
All Organs: Malignant Neoplasms				
Overall rate	13/50 (26%)	15/50 (30%)	9/50 (18%)	16/50 (32%)
Adjusted rate	28.3%	31.5%	18.7%	34.6%
Terminal rate	7/33 (21%)	7/34 (21%)	4/34 (12%)	8/33 (24%)
First incidence (days)	345	286	309	457
Poly-3 test	P = 0.284	P = 0.455	P = 0.197N	P = 0.333

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
All Organs: Benign or Malignant Neoplasms				
Overall rate	37/50 (74%)	36/50 (72%)	30/50 (60%)	39/50 (78%)
Adjusted rate	75.3%	72.8%	61.2%	81.0%
Terminal rate	23/33 (70%)	22/34 (65%)	17/34 (50%)	24/33 (73%)
First incidence (days)	345	286	309	457
Poly-3 test	P = 0.201	P = 0.480N	P = 0.098N	P = 0.332

(T) = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, brain, pancreatic islets, pituitary gland, prostate gland, thymus, 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 differential mortality in animals that do not reach terminal kill. 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 A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	17	12	13	15
Natural deaths	–	4	3	2
Survivors				
Terminal kill	33	34	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(48)	(47)	(49)
Inflammation, acute	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Intestine large, colon	(50)	(49)	(47)	(49)
Inflammation, acute	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Intestine large, rectum	(48)	(47)	(47)	(48)
Intestine small, duodenum	(50)	(48)	(47)	(49)
Intestine small, ileum	(50)	(48)	(47)	(49)
Intestine small, jejunum	(50)	(48)	(47)	(49)
Diverticulum	–	1 (2%)	–	–
Inflammation, chronic	–	–	–	1 (2%)
Lymphatic, angiectasis	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	7 (14%)	8 (16%)	7 (14%)
Basophilic focus	11 (22%)	20 (40%)	18 (36%)	18 (36%)
Clear cell focus	29 (58%)	26 (52%)	17 (34%)	26 (52%)
Congestion	–	1 (2%)	–	–
Eosinophilic focus	–	–	1 (2%)	–
Fatty change	–	–	–	1 (2%)
Inflammation, granulomatous	–	1 (2%)	–	–
Mixed cell focus	3 (6%)	–	–	1 (2%)
Necrosis	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Bile duct, cyst	1 (2%)	1 (2%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Bile duct, dilatation	3 (6%)	2 (4%)	2 (4%)	4 (8%)
Bile duct, hyperplasia	1 (2%)	2 (4%)	–	–
Bile duct, inflammation, chronic active	1 (2%)	–	–	–
Mesentery	(7)	(7)	(1)	(2)
Fat, necrosis	6 (86%)	7 (100%)	1 (100%)	–
Oral mucosa	(1)	(0)	(1)	(0)
Developmental malformation	1 (100%)	–	1 (100%)	–
Pancreas	(50)	(50)	(49)	(50)
Atrophy	1 (2%)	4 (8%)	4 (8%)	5 (10%)
Basophilic focus	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Inflammation, acute	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	1 (2%)	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	–	–	–
Ulcer	–	1 (2%)	–	2 (4%)
Stomach, glandular	(50)	(50)	(49)	(50)
Necrosis	–	–	1 (2%)	–
Tooth	(0)	(1)	(0)	(1)
Dysplasia	–	–	–	1 (100%)
Inflammation, chronic active	–	1 (100%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	28 (56%)	40 (80%)	32 (64%)	33 (66%)
Necrosis	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Accessory adrenal cortical nodule	1 (2%)	–	–	–
Degeneration, cystic	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia	26 (52%)	25 (50%)	28 (57%)	26 (52%)
Hypertrophy	20 (40%)	21 (42%)	14 (29%)	16 (32%)
Adrenal medulla	(50)	(48)	(49)	(50)
Angiectasis	–	–	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hyperplasia	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Parathyroid gland	(43)	(43)	(44)	(47)
Hyperplasia	1 (2%)	–	1 (2%)	1 (2%)
Pituitary gland	(49)	(50)	(50)	(50)
Cyst	1 (2%)	–	–	–
Pars distalis, hyperplasia	14 (29%)	21 (42%)	13 (26%)	16 (32%)
Pars intermedia, hyperplasia	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Thyroid gland	(50)	(50)	(49)	(50)
C-cell, hyperplasia	34 (68%)	30 (60%)	30 (61%)	28 (56%)
Follicular cell, hyperplasia	5 (10%)	1 (2%)	5 (10%)	–
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Genital System				
Coagulating gland	(0)	(0)	(0)	(1)
Inflammation, chronic active	–	–	–	1 (100%)
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	2 (4%)	–	–	–
Preputial gland	(50)	(50)	(50)	(50)
Inflammation, chronic active	3 (6%)	5 (10%)	3 (6%)	3 (6%)
Duct, ectasia	–	–	–	1 (2%)
Prostate gland	(50)	(50)	(50)	(50)
Hyperplasia	12 (24%)	17 (34%)	12 (24%)	11 (22%)
Inflammation, chronic active	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Seminal vesicle	(50)	(50)	(48)	(50)
Hyperplasia	1 (2%)	–	–	1 (2%)
Inflammation, chronic active	–	–	2 (4%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	15 (30%)	8 (16%)	5 (10%)	5 (10%)
Edema	16 (32%)	11 (22%)	18 (36%)	11 (22%)
Necrosis	1 (2%)	–	–	–
Artery, inflammation, chronic active	–	2 (4%)	–	–
Interstitial cell, hyperplasia	–	–	–	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lymph node	(12)	(5)	(7)	(5)
Axillary, ectasia	–	–	–	1 (20%)
Axillary, infiltration cellular, plasma cell	1 (8%)	–	–	–
Iliac, ectasia	2 (17%)	–	1 (14%)	1 (20%)
Iliac, inflammation, chronic active	–	–	1 (14%)	–
Lumbar, ectasia	2 (17%)	2 (40%)	2 (29%)	–
Lumbar, hemorrhage	1 (8%)	–	–	–
Lumbar, infiltration cellular, plasma cell	5 (42%)	1 (20%)	2 (29%)	–
Pancreatic, ectasia	1 (8%)	–	–	–
Pancreatic, inflammation, chronic active	1 (8%)	–	–	–
Renal, ectasia	–	2 (40%)	–	2 (40%)
Lymph node, bronchial	(42)	(40)	(37)	(35)
Ectasia	–	–	–	1 (3%)
Hyperplasia, lymphohistiocytic	–	–	10 (27%)	28 (80%)
Lymph node, mandibular	(46)	(46)	(44)	(45)
Ectasia	1 (2%)	2 (4%)	–	–
Hyperplasia, lymphoid	–	1 (2%)	–	1 (2%)
Infiltration, cellular, plasma cell	–	1 (2%)	1 (2%)	–
Lymph node, mediastinal	(46)	(45)	(50)	(49)
Ectasia	1 (2%)	–	–	–
Hyperplasia, lymphohistiocytic	–	–	4 (8%)	29 (59%)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Ectasia	2 (4%)	–	–	–
Hemorrhage	–	–	–	1 (2%)
Infiltration cellular, plasma cell	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	–	4 (8%)	2 (4%)
Inflammation, granulomatous	–	1 (2%)	–	–
Necrosis	–	–	1 (2%)	–
Thymus	(40)	(43)	(44)	(46)
Atrophy	1 (3%)	–	–	–
Cyst	–	–	1 (2%)	–
Integumentary System				
Mammary gland	(6)	(6)	(3)	(4)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Galactocele	1 (17%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Angiectasis	–	–	–	1 (2%)
Cyst epithelial inclusion	1 (2%)	1 (2%)	–	1 (2%)
Inflammation, chronic active	31 (62%)	23 (46%)	30 (60%)	22 (44%)
Thrombosis	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)	–	–	–
Inflammation, chronic active	–	–	1 (2%)	–
Skeletal muscle	(1)	(2)	(1)	(0)
Necrosis	–	1 (50%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	–	–	–	1 (2%)
Hemorrhage	–	–	1 (2%)	–
Peripheral nerve	(5)	(4)	(6)	(6)
Spinal cord	(5)	(4)	(7)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	–	–	1 (2%)	–
Inflammation, acute	–	1 (2%)	–	–
Inflammation, chronic	–	–	–	1 (2%)
Inflammation, chronic active	–	2 (4%)	2 (4%)	–
Metaplasia, squamous	1 (2%)	47 (94%)	50 (100%)	50 (100%)
Epiglottis, fibrosis	–	–	–	1 (2%)
Epiglottis, ulcer	–	–	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	–	–	–
Hemorrhage	1 (2%)	–	–	–
Inflammation, lymphohistiocytic	6 (12%)	14 (28%)	41 (82%)	47 (94%)
Inflammation, acute	–	2 (4%)	–	–
Metaplasia, squamous	–	1 (2%)	–	–
Alveolar epithelium, hyperplasia	4 (8%)	6 (12%)	11 (22%)	13 (26%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic	–	1 (2%)	5 (10%)	19 (38%)
Nose	(50)	(50)	(50)	(50)
Foreign body	–	–	1 (2%)	1 (2%)
Inflammation, acute	–	1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic active	4 (8%)	1 (2%)	1 (2%)	–
Glands, olfactory epithelium, hyperplasia	1 (2%)	39 (78%)	47 (94%)	50 (100%)
Goblet cell, hyperplasia	–	20 (40%)	25 (50%)	34 (68%)
Olfactory epithelium, accumulation, hyaline droplet	19 (38%)	50 (100%)	50 (100%)	50 (100%)
Respiratory epithelium, accumulation, hyaline droplet	–	17 (34%)	25 (50%)	29 (58%)
Trachea	(50)	(49)	(50)	(50)
Inflammation, acute	–	1 (2%)	–	–
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(48)	(50)
Cataract	2 (4%)	3 (6%)	1 (2%)	–
Cornea, inflammation, chronic active	–	1 (2%)	–	1 (2%)
Retina, atrophy	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	–	–
Hyperplasia	17 (34%)	7 (14%)	12 (24%)	6 (12%)
Hypertrophy	2 (4%)	3 (6%)	–	–
Zymbal’s gland	(3)	(2)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Cyst	4 (8%)	1 (2%)	3 (6%)	–
Hydronephrosis	–	1 (2%)	2 (4%)	–
Infarct	–	–	–	1 (2%)
Inflammation, suppurative	–	3 (6%)	2 (4%)	4 (8%)
Nephropathy	43 (86%)	38 (76%)	38 (78%)	38 (76%)
Renal tubule, hyperplasia	–	–	–	3 (6%)
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	–
Urinary bladder	(50)	(50)	(48)	(49)
Calculus gross observation	–	1 (2%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Inflammation, chronic active	–	1 (2%)	–	1 (2%)
Transitional epithelium, hyperplasia	–	1 (2%)	–	1 (2%)

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

Appendix B. Summary of Lesions in Female Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Wistar Han Rats in the Two year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	13	16	14	19
Natural deaths	2	–	–	1
Survivors				
Died last week of study	1	1	–	1
Terminal kill	34	32	36	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(49)
Intestine large, colon	(49)	(49)	(50)	(49)
Intestine large, rectum	(46)	(50)	(47)	(46)
Intestine small, duodenum	(49)	(50)	(50)	(49)
Intestine small, ileum	(49)	(50)	(50)	(49)
Intestine small, jejunum	(49)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	–	2 (4%)	1 (2%)	–
Mesentery	(7)	(8)	(8)	(5)
Oral mucosa	(0)	(0)	(0)	(1)
Pancreas	(49)	(50)	(50)	(50)
Carcinoma, metastatic, uterus	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Stomach, glandular	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Tongue	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Chemodectoma benign	–	–	1 (2%)	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hemangioma	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Adrenal medulla	(49)	(50)	(49)	(49)
Pheochromocytoma benign	1 (2%)	–	1 (2%)	–
Pheochromocytoma malignant	1 (2%)	1 (2%)	–	–
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Parathyroid gland	(47)	(44)	(40)	(42)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	35 (70%)	33 (66%)	36 (72%)	30 (60%)
Pars intermedia, adenoma	1 (2%)	–	–	2 (4%)
Thyroid gland	(50)	(50)	(50)	(49)
C-cell, adenoma	3 (6%)	2 (4%)	4 (8%)	4 (8%)
C-cell, carcinoma	–	–	–	3 (6%)
Follicular cell, adenoma	1 (2%)	–	3 (6%)	1 (2%)
Follicular cell, carcinoma	1 (2%)	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(46)	(50)	(49)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign	1 (2%)	–	1 (2%)	–
Granulosa cell tumor malignant	–	2 (4%)	–	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Fibroma	–	–	1 (2%)	–
Leiomyoma	–	–	1 (2%)	–
Polyp stromal	4 (8%)	6 (12%)	3 (6%)	3 (6%)
Polyp stromal, multiple	1 (2%)	–	–	1 (2%)
Sarcoma stromal	2 (4%)	–	–	–
Sarcoma stromal, multiple	–	–	–	1 (2%)
Endometrium, adenocarcinoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Endometrium, malignant mixed Müllerian tumor	–	–	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Vagina	(0)	(1)	(0)	(1)
Polyp	–	–	–	1 (100%)
Squamous cell carcinoma	–	1 (100%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Bronchial associated lymphoid tissue	(0)	(0)	(0)	(5)
Lymph node	(2)	(2)	(1)	(0)
Lymph node, bronchial	(38)	(35)	(32)	(35)
Lymph node, mandibular	(49)	(47)	(43)	(49)
Lymph node, mediastinal	(49)	(46)	(45)	(47)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(48)	(45)	(45)
Thymoma benign	5 (10%)	4 (8%)	7 (16%)	3 (7%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma	3 (6%)	5 (10%)	5 (10%)	3 (6%)
Carcinoma, multiple	1 (2%)	–	4 (8%)	1 (2%)
Fibroadenoma	6 (12%)	8 (16%)	9 (18%)	8 (16%)
Fibroadenoma, multiple	3 (6%)	2 (4%)	4 (8%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma	–	–	–	1 (2%)
Squamous cell papilloma	–	–	–	3 (6%)
Subcutaneous tissue, lipoma	–	–	–	1 (2%)
Subcutaneous tissue, liposarcoma	1 (2%)	–	–	–
Subcutaneous tissue, sarcoma	–	2 (4%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	2 (4%)	1 (2%)	–	1 (2%)
Granular cell tumor malignant	–	–	–	2 (4%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Oligodendroglioma malignant	–	–	–	1 (2%)
Peripheral nerve	(2)	(9)	(5)	(6)
Spinal cord	(2)	(9)	(5)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	–	–	1 (2%)
Squamous cell carcinoma	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Harderian gland	(49)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)	–	–	–
Transitional epithelium, papilloma	–	1 (2%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	2 (4%)	–	–	–
Mesothelioma malignant	–	1 (2%)	–	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	43	40	44	43
Total primary neoplasms	85	78	87	79
Total animals with benign neoplasms	42	39	43	40
Total benign neoplasms	70	62	76	64
Total animals with malignant neoplasms	11	15	11	13
Total malignant neoplasms	15	16	11	15
Total animals with metastatic neoplasms	–	–	–	1
Total metastatic neoplasms	–	–	–	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 B-2. Statistical Analysis of Primary Neoplasms in Female Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Mammary Gland: Fibroadenoma				
Overall rate[a]	9/50 (18%)	10/50 (20%)	13/50 (26%)	10/50 (20%)
Adjusted rate[b]	19.8%	22.2%	27.6%	21.4%
Terminal rate[c]	6/35 (17%)	5/32 (16%)	10/36 (28%)	4/29 (14%)
First incidence (days)	619	627	642	633
Poly-3 test[d]	P = 0.563	P = 0.490	P = 0.261	P = 0.527
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	11/50 (22%)	11/50 (22%)	13/50 (26%)	10/50 (20%)
Adjusted rate	24.0%	24.4%	27.6%	21.4%
Terminal rate	7/35 (20%)	6/32 (19%)	10/36 (28%)	4/29 (14%)
First incidence (days)	619	627	642	633
Poly-3 test	P = 0.399N	P = 0.579	P = 0.438	P = 0.479N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	9/50 (18%)	4/50 (8%)
Adjusted rate	8.9%	11.4%	19.2%	8.7%
Terminal rate	3/35 (9%)	3/32 (9%)	7/36 (19%)	1/29 (3%)
First incidence (days)	698	683	673	639
Poly-3 test	P = 0.437N	P = 0.485	P = 0.130	P = 0.629N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	6/50 (12%)	10/50 (20%)	5/50 (10%)
Adjusted rate	13.2%	13.6%	21.3%	10.8%
Terminal rate	4/35 (11%)	4/32 (13%)	8/36 (22%)	2/29 (7%)
First incidence (days)	645	683	673	639
Poly-3 test	P = 0.368N	P = 0.599	P = 0.226	P = 0.487N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	13/50 (26%)	16/50 (32%)	21/50 (42%)	14/50 (28%)
Adjusted rate	28.3%	35.2%	44.1%	29.5%
Terminal rate	9/35 (26%)	9/32 (28%)	16/36 (44%)	5/29 (17%)
First incidence (days)	619	627	642	633
Poly-3 test	P = 0.417N	P = 0.314	P = 0.082	P = 0.541
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	35/50 (70%)	33/50 (66%)	36/50 (72%)	30/50 (60%)
Adjusted rate	72.7%	67.4%	73.2%	62.4%
Terminal rate	24/35 (69%)	17/32 (53%)	25/36 (69%)	15/29 (52%)
First incidence (days)	499	530	563	586

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Poly-3 test	P = 0.186N	P = 0.365N	P = 0.568	P = 0.192N
Skin: Squamous Cell Papilloma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.5%
Terminal rate	0/35 (0%)	0/32 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	–[e]	–	–	673
Poly-3 test	P = 0.008	–[f]	–	P = 0.122
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	8.7%
Terminal rate	0/35 (0%)	0/32 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	–	–	–	673
Poly-3 test	P = 0.002	–	–	P = 0.063
Thymus: Benign Thymoma				
Overall rate	5/48 (10%)	4/48 (8%)	7/45 (16%)	3/45 (7%)
Adjusted rate	11.4%	9.6%	16.8%	7.3%
Terminal rate	3/34 (9%)	3/30 (10%)	5/31 (16%)	1/24 (4%)
First incidence (days)	610	729	708	639
Poly-3 test	P = 0.338N	P = 0.532N	P = 0.341	P = 0.393N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	4/49 (8%)
Adjusted rate	6.6%	4.5%	8.6%	8.8%
Terminal rate	1/35 (3%)	0/32 (0%)	4/36 (11%)	3/29 (10%)
First incidence (days)	619	568	731 (T)	670
Poly-3 test	P = 0.357	P = 0.509N	P = 0.512	P = 0.497
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/49 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.6%
Terminal rate	0/35 (0%)	0/32 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	–	–	–	691
Poly-3 test	P = 0.008	–	–	P = 0.119
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	7/49 (14%)
Adjusted rate	6.6%	4.5%	8.6%	15.4%
Terminal rate	1/35 (3%)	0/32 (0%)	4/36 (11%)	5/29 (17%)
First incidence (days)	619	568	731 (T)	670
Poly-3 test	P = 0.050	P = 0.509N	P = 0.512	P = 0.156

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Adjusted rate	2.2%	0.0%	6.4%	2.2%
Terminal rate	1/35 (3%)	0/32 (0%)	2/36 (6%)	1/29 (3%)
First incidence (days)	731 (T)	–	642	731 (T)
Poly-3 test	P = 0.599	P = 0.506N	P = 0.320	P = 0.760N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	1/49 (2%)
Adjusted rate	4.5%	2.3%	6.4%	2.2%
Terminal rate	2/35 (6%)	1/32 (3%)	2/36 (6%)	1/29 (3%)
First incidence (days)	731 (T)	731 (T)	642	731 (T)
Poly-3 test	P = 0.455N	P = 0.510N	P = 0.519	P = 0.499N
Uterus: Stromal Polyp (Original Evaluation)				
Overall rate	5/50 (10%)	6/50 (12%)	3/50 (6%)	4/50 (8%)
Adjusted rate	11.1%	13.6%	6.4%	8.8%
Terminal rate	4/35 (11%)	5/32 (16%)	2/36 (6%)	2/29 (7%)
First incidence (days)	709	633	646	725
Poly-3 test	P = 0.374N	P = 0.485	P = 0.335N	P = 0.491N
Uterus: Stromal Polyp (Residual Evaluation)				
Overall rate	6/50 (12%)	6/50 (12%)	7/50 (14%)	1/50 (2%)
Adjusted rate	13.2%	13.7%	14.8%	2.2%
Terminal rate	4/35 (11%)	6/32 (19%)	5/36 (14%)	1/29 (3%)
First incidence (days)	610	731 (T)	563	731 (T)
Poly-3 test	P = 0.034N	P = 0.593	P = 0.531	P = 0.055N
Uterus: Stromal Polyp (Original and Residual Evaluations)				
Overall rate	10/50 (20%)	10/50 (20%)	7/50 (14%)	5/50 (10%)
Adjusted rate	21.9%	22.7%	14.8%	10.9%
Terminal rate	7/35 (20%)	9/32 (28%)	5/36 (14%)	3/29 (10%)
First incidence (days)	610	633	563	725
Poly-3 test	P = 0.080N	P = 0.567	P = 0.267N	P = 0.127N
Uterus: Stromal Polyp or Stromal Sarcoma (Original Evaluation)				
Overall rate	6/50 (12%)	6/50 (12%)	3/50 (6%)	5/50 (10%)
Adjusted rate	13.2%	13.6%	6.4%	10.9%
Terminal rate	4/35 (11%)	5/32 (16%)	2/36 (6%)	2/29 (7%)
First incidence (days)	586	633	646	715
Poly-3 test	P = 0.452N	P = 0.598	P = 0.228N	P = 0.496N

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Uterus: Stromal Polyp or Stromal Sarcoma (Residual Evaluation)				
Overall rate	7/50 (14%)	6/50 (12%)	7/50 (14%)	1/50 (2%)
Adjusted rate	15.2%	13.7%	14.8%	2.2%
Terminal rate	4/35 (11%)	6/32 (19%)	5/36 (14%)	1/29 (3%)
First incidence (days)	586	731 (T)	563	731 (T)
Poly-3 test	P = 0.023N	P = 0.539N	P = 0.590N	P = 0.031N
Uterus: Stromal Polyp or Stromal Sarcoma (Original and Residual Evaluations)				
Overall rate	11/50 (22%)	10/50 (20%)	7/50 (14%)	6/50 (12%)
Adjusted rate	23.9%	22.7%	14.8%	13.1%
Terminal rate	7/35 (20%)	9/32 (28%)	5/36 (14%)	3/29 (10%)
First incidence (days)	586	633	563	715
Poly-3 test	P = 0.111N	P = 0.546N	P = 0.197N	P = 0.143N
Uterus: Adenocarcinoma (Residual Evaluation)				
Overall rate	0/50 (0%)	4/50 (8%)	5/50 (10%)	5/50 (10%)
Adjusted rate	0.0%	9.2%	10.7%	11.0%
Terminal rate	0/35 (0%)	4/32 (13%)	3/36 (8%)	4/29 (14%)
First incidence (days)	–	731 (T)	646	725
Poly-3 test	P = 0.126	P = 0.057	P = 0.034	P = 0.032
Uterus: Adenocarcinoma (Original and Residual Evaluations)				
Overall rate	1/50 (2%)	4/50 (8%)	5/50 (10%)	5/50 (10%)
Adjusted rate	2.2%	9.2%	10.7%	11.0%
Terminal rate	1/35 (3%)	4/32 (13%)	3/36 (8%)	4/29 (14%)
First incidence (days)	731 (T)	731 (T)	646	725
Poly-3 test	P = 0.195	P = 0.170	P = 0.111	P = 0.105
Uterus: Adenocarcinoma or Mixed Malignant Müllerian Tumor (Original Evaluation)				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	2.3%	2.1%	6.6%
Terminal rate	1/35 (3%)	1/32 (3%)	0/36 (0%)	3/29 (10%)
First incidence (days)	731 (T)	731 (T)	646	731 (T)
Poly-3 test	P = 0.163	P = 0.755	P = 0.752N	P = 0.311
Uterus: Adenocarcinoma or Mixed Malignant Müllerian Tumor (Residual Evaluation)				
Overall rate	0/50 (0%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	0.0%	9.2%	10.7%	13.1%
Terminal rate	0/35 (0%)	4/32 (13%)	3/36 (8%)	5/29 (17%)
First incidence (days)	–	731 (T)	646	725
Poly-3 test	P = 0.059	P = 0.057	P = 0.034	P = 0.016

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Uterus: Adenocarcinoma or Mixed Malignant Müllerian Tumor (Original and Residual Evaluations)				
Overall rate	1/50 (2%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	2.2%	9.2%	10.7%	13.1%
Terminal rate	1/35 (3%)	4/32 (13%)	3/36 (8%)	5/29 (17%)
First incidence (days)	731 (T)	731 (T)	646	725
Poly-3 test	P = 0.102	P = 0.170	P = 0.111	P = 0.058
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	39/50 (78%)	43/50 (86%)	40/50 (80%)
Adjusted rate	87.1%	79.1%	87.2%	82.1%
Terminal rate	30/35 (86%)	22/32 (69%)	31/36 (86%)	23/29 (79%)
First incidence (days)	499	530	563	586
Poly-3 test	P = 0.451N	P = 0.215N	P = 0.618	P = 0.338N
All Organs: Malignant Neoplasms				
Overall rate	11/50 (22%)	15/50 (30%)	11/50 (22%)	13/50 (26%)
Adjusted rate	24.0%	32.5%	23.3%	27.5%
Terminal rate	7/35 (20%)	8/32 (25%)	8/36 (22%)	6/29 (21%)
First incidence (days)	586	481	646	523
Poly-3 test	P = 0.547	P = 0.251	P = 0.564N	P = 0.442
All Organs: Benign or Malignant Neoplasms				
Overall rate	43/50 (86%)	40/50 (80%)	44/50 (88%)	43/50 (86%)
Adjusted rate	88.3%	80.0%	88.8%	86.6%
Terminal rate	30/35 (86%)	22/32 (69%)	31/36 (86%)	23/29 (79%)
First incidence (days)	499	481	563	523
Poly-3 test	P = 0.463	P = 0.197N	P = 0.595	P = 0.524N

(T) = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for pituitary gland, thymus, 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 differential mortality in animals that do not reach terminal kill. 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 B-3. Summary of the Incidence of Nonneoplastic Lesions in Female Wistar Han Rats in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	13	16	14	19
Natural deaths	2	–	–	1
Survivors				
Died last week of study	1	1	–	1
Terminal kill	34	32	36	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)	–	–	–
Necrosis	1 (2%)	–	–	–
Intestine large, cecum	(49)	(50)	(50)	(49)
Intestine large, colon	(49)	(49)	(50)	(49)
Intestine large, rectum	(46)	(50)	(47)	(46)
Intestine small, duodenum	(49)	(50)	(50)	(49)
Muscularis, hypertrophy, focal	–	–	1 (2%)	–
Intestine small, ileum	(49)	(50)	(50)	(49)
Intestine small, jejunum	(49)	(50)	(50)	(49)
Diverticulum	1 (2%)	–	–	–
Inflammation, acute	1 (2%)	–	–	–
Inflammation, chronic	–	–	1 (2%)	–
Necrosis	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	5 (10%)	5 (10%)	1 (2%)
Basophilic focus	34 (68%)	33 (66%)	35 (70%)	32 (64%)
Clear cell focus	14 (28%)	6 (12%)	17 (34%)	14 (28%)
Degeneration, cystic	–	–	–	1 (2%)
Eosinophilic focus	1 (2%)	–	1 (2%)	1 (2%)
Fatty change	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hepatodiaphragmatic nodule	1 (2%)	–	1 (2%)	–
Mixed cell focus	2 (4%)	–	–	–
Necrosis	1 (2%)	1 (2%)	1 (2%)	–
Bile duct, cyst	1 (2%)	1 (2%)	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Bile duct, cyst, multiple	–	4 (8%)	1 (2%)	3 (6%)
Bile duct, dilatation	1 (2%)	–	2 (4%)	3 (6%)
Bile duct, hyperplasia	5 (10%)	2 (4%)	3 (6%)	6 (12%)
Bile duct, inflammation, chronic active	1 (2%)	–	–	–
Mesentery	(7)	(8)	(8)	(5)
Inflammation, chronic active	–	–	–	1 (20%)
Fat, necrosis	7 (100%)	8 (100%)	8 (100%)	4 (80%)
Oral mucosa	(0)	(0)	(0)	(1)
Developmental malformation	–	–	–	1 (100%)
Inflammation, chronic active	–	–	–	1 (100%)
Pancreas	(49)	(50)	(50)	(50)
Atrophy	6 (12%)	4 (8%)	2 (4%)	5 (10%)
Basophilic focus	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	1 (2%)	–	–	–
Inflammation, acute	1 (2%)	–	2 (4%)	–
Inflammation, chronic active	–	1 (2%)	1 (2%)	–
Ulcer	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)	–	–	–
Inflammation, chronic active	–	–	–	1 (2%)
Mineralization	1 (2%)	–	1 (2%)	–
Necrosis	2 (4%)	–	–	–
Tongue	(1)	(0)	(0)	(0)
Inflammation, granulomatous	1 (100%)	–	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	16 (32%)	17 (34%)	16 (32%)	14 (28%)
Mineralization	–	–	–	1 (2%)
Endocardium, hyperplasia	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	–	–	–
Angiectasis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Degeneration, cystic	26 (52%)	23 (46%)	29 (58%)	35 (70%)
Hyperplasia	17 (34%)	20 (40%)	27 (54%)	22 (44%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hypertrophy	14 (28%)	20 (40%)	24 (48%)	21 (42%)
Metaplasia, osseous	–	1 (2%)	–	–
Necrosis	1 (2%)	1 (2%)	–	–
Adrenal medulla	(49)	(50)	(49)	(49)
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	–
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	1 (2%)
Parathyroid gland	(47)	(44)	(40)	(42)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, hyperplasia	8 (16%)	11 (22%)	10 (20%)	9 (18%)
Pars intermedia, hyperplasia	1 (2%)	–	–	1 (2%)
Thyroid gland	(50)	(50)	(50)	(49)
C-cell, hyperplasia	37 (74%)	44 (88%)	43 (86%)	35 (71%)
Follicular cell, hyperplasia	2 (4%)	–	1 (2%)	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(46)	(50)	(49)
Inflammation, chronic	–	–	1 (2%)	–
Inflammation, chronic active	6 (12%)	4 (9%)	1 (2%)	1 (2%)
Ovary	(50)	(50)	(50)	(50)
Cyst	11 (22%)	9 (18%)	5 (10%)	11 (22%)
Interstitial cell, hyperplasia	–	–	–	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	–	–	1 (2%)	–
Cervix, cyst, squamous	–	–	1 (2%)	–
Cervix, hypertrophy	2 (4%)	–	1 (2%)	1 (2%)
Endometrium, hyperplasia, cystic	11 (22%)	4 (8%)	9 (18%)	5 (10%)
Vagina	(0)	(1)	(0)	(1)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(2)	(2)	(1)	(0)
Lumbar, ectasia	–	1 (50%)	–	–
Lumbar, hemorrhage	–	1 (50%)	–	–
Lumbar, hyperplasia, lymphoid	–	–	1 (100%)	–
Lumbar, pigmentation, hemosiderin	1 (50%)	–	–	–
Renal, ectasia	1 (50%)	–	–	–
Renal, pigmentation, hemosiderin	1 (50%)	–	–	–
Lymph node, bronchial	(38)	(35)	(32)	(35)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hyperplasia, lymphohistiocytic	–	–	7 (22%)	30 (86%)
Lymph node, mandibular	(49)	(47)	(43)	(49)
Ectasia	–	1 (2%)	–	2 (4%)
Lymph node, mediastinal	(49)	(46)	(45)	(47)
Hemorrhage	–	–	1 (2%)	–
Hyperplasia, lymphohistiocytic	–	–	4 (9%)	23 (49%)
Hyperplasia, lymphoid	–	–	–	1 (2%)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Ectasia	1 (2%)	1 (2%)	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Thymus	(48)	(48)	(45)	(45)
Cyst	–	–	1 (2%)	–
Hyperplasia, tubular	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)	–	–	1 (2%)
Inflammation, chronic active	–	–	–	2 (4%)
Skin	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Inflammation, chronic active	2 (4%)	4 (8%)	5 (10%)	4 (8%)
Subcutaneous tissue, hemorrhage	–	1 (2%)	–	–
Subcutaneous tissue, necrosis	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Callus	1 (2%)	–	–	–
Skeletal muscle	(0)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, hyperplasia	1 (2%)	–	–	1 (2%)
Peripheral nerve	(2)	(9)	(5)	(6)
Spinal cord	(2)	(9)	(5)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Inflammation, acute	–	–	–	4 (8%)
Metaplasia, squamous	1 (2%)	50 (100%)	50 (100%)	50 (100%)
Lung	(50)	(50)	(50)	(50)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Inflammation, lymphohistiocytic	3 (6%)	20 (40%)	42 (84%)	50 (100%)
Alveolar epithelium, hyperplasia	4 (8%)	3 (6%)	1 (2%)	7 (14%)
Bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic	–	–	–	5 (10%)
Nose	(50)	(50)	(50)	(50)
Inflammation, acute	–	–	–	1 (2%)
Inflammation, chronic active	2 (4%)	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Glands, olfactory epithelium, hyperplasia	1 (2%)	32 (64%)	48 (96%)	49 (98%)
Goblet cell, hyperplasia	–	25 (50%)	34 (68%)	42 (84%)
Olfactory epithelium, accumulation, hyaline droplet	16 (32%)	50 (100%)	50 (100%)	50 (100%)
Respiratory epithelium, accumulation, hyaline droplet	1 (2%)	24 (48%)	31 (62%)	34 (68%)
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Cataract	–	–	1 (2%)	–
Cornea, inflammation, chronic active	–	–	–	1 (2%)
Retina, atrophy	3 (6%)	4 (8%)	4 (8%)	2 (4%)
Harderian gland	(49)	(50)	(50)	(50)
Hyperplasia	3 (6%)	3 (6%)	5 (10%)	4 (8%)
Zymbal’s gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	–	1 (2%)	2 (4%)	–
Hydronephrosis	–	1 (2%)	–	1 (2%)
Inflammation, suppurative	1 (2%)	2 (4%)	–	–
Mineralization	–	1 (2%)	–	1 (2%)
Nephropathy	17 (34%)	16 (32%)	19 (38%)	18 (36%)
Renal tubule, hyperplasia	–	–	–	1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	3 (6%)	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)

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

Appendix C. Summary of Lesions in Male Mice in the Two-year Inhalation Study of CIMSTAR 3800

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	12	7	4
Natural deaths	6	7	7	6
Survivors				
Terminal kill	39	31	36	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Squamous cell papilloma	–	–	–	1 (2%)
Gallbladder	(44)	(44)	(44)	(44)
Intestine large, cecum	(50)	(50)	(49)	(49)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Serosa, carcinoma, metastatic, kidney	–	–	1 (2%)	–
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(49)	(48)	(48)	(47)
Adenoma	1 (2%)	–	–	1 (2%)
Carcinoma	–	–	1 (2%)	–
Hemangiosarcoma	–	–	1 (2%)	–
Intestine small, ileum	(50)	(50)	(49)	(48)
Hemangioma	–	–	1 (2%)	–
Intestine small, jejunum	(50)	(48)	(49)	(48)
Adenoma	–	1 (2%)	1 (2%)	–
Carcinoma	–	1 (2%)	–	–
Liver	(50)	(50)	(50)	(49)
Hemangiosarcoma, multiple	–	1 (2%)	–	–
Hepatoblastoma	–	1 (2%)	1 (2%)	–
Hepatocellular adenoma	10 (20%)	18 (36%)	15 (30%)	14 (29%)
Hepatocellular adenoma, multiple	14 (28%)	6 (12%)	10 (20%)	16 (33%)
Hepatocellular carcinoma	9 (18%)	15 (30%)	7 (14%)	13 (27%)
Hepatocellular carcinoma, multiple	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Hepatocholangiocarcinoma	–	–	2 (4%)	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Sarcoma	–	–	1 (2%)	–
Mesentery	(4)	(5)	(3)	(4)
Hemangioma	1 (25%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	1 (25%)	–	–	–
Sarcoma, metastatic, liver	–	–	1 (33%)	–
Pancreas	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	–	–	1 (2%)	–
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Sarcoma, metastatic, liver	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	–	–
Tooth	(50)	(47)	(49)	(50)
Cardiovascular System				
Blood vessel	(45)	(49)	(48)	(48)
Aorta, carcinoma, metastatic, kidney	–	–	1 (2%)	–
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	–	–	1 (2%)	–
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Pericardium, alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	1 (2%)	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	2 (4%)	–	–	4 (8%)
Sarcoma, metastatic, liver	–	–	1 (2%)	–
Bilateral, subcapsular, adenoma	1 (2%)	–	–	–
Extra adrenal tissue, carcinoma, metastatic, kidney	–	–	1 (2%)	–
Subcapsular, adenoma	7 (14%)	4 (8%)	5 (10%)	6 (12%)
Subcapsular, adenoma, multiple	1 (2%)	–	–	–
Adrenal medulla	(48)	(50)	(50)	(50)
Pheochromocytoma benign	–	–	1 (2%)	–
Bilateral, pheochromocytoma benign	1 (2%)	–	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Parathyroid gland	(34)	(28)	(27)	(21)
Pituitary gland	(50)	(50)	(50)	(48)
Thyroid gland	(50)	(50)	(49)	(50)
General Body System				
Tissue NOS	(0)	(2)	(0)	(1)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (50%)	–	1 (100%)
Mediastinum, hepatocellular carcinoma, metastatic, liver	–	1 (50%)	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(0)	(0)	(1)
Preputial gland	(50)	(49)	(48)	(49)
Prostate gland	(50)	(49)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, skin	–	–	–	1 (2%)
Lymph node	(4)	(3)	(1)	(1)
Renal, sarcoma, metastatic, liver	–	–	1 (100%)	–
Lymph node, bronchial	(26)	(31)	(26)	(18)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (3%)	1 (4%)	–
Lymph node, mandibular	(31)	(36)	(34)	(33)
Lymph node, mediastinal	(39)	(39)	(32)	(35)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)	1 (3%)	–	1 (3%)
Carcinoma, metastatic, stomach, glandular	1 (3%)	–	–	–
Lymph node, mesenteric	(49)	(50)	(48)	(49)
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Hemangiosarcoma	–	1 (2%)	–	–
Spleen	(50)	(50)	(49)	(49)
Hemangiosarcoma	–	2 (4%)	–	–
Thymus	(41)	(38)	(39)	(41)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(1)	(0)	(1)	(0)
Skin	(50)	(49)	(50)	(50)
Carcinoma, metastatic, kidney	–	–	1 (2%)	–
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Melanoma benign	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, fibroma	–	–	1 (2%)	–
Subcutaneous tissue, fibrous histiocytoma	–	–	1 (2%)	–
Subcutaneous tissue, hemangiosarcoma	–	–	–	1 (2%)
Subcutaneous tissue, mast cell tumor malignant	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Maxilla, carcinoma, metastatic, Harderian gland	–	1 (2%)	–	–
Skeletal muscle	(1)	(3)	(2)	(3)
Carcinoma, metastatic, kidney	–	–	1 (50%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(1)	(2)	(1)	(2)
Spinal cord	(1)	(3)	(1)	(2)
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	2 (4%)	8 (16%)	9 (18%)
Alveolar/bronchiolar carcinoma	7 (14%)	7 (14%)	8 (16%)	5 (10%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	1 (2%)	–	5 (10%)
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Carcinoma, metastatic, kidney	–	–	1 (2%)	–
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	5 (10%)	9 (18%)	1 (2%)	3 (6%)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Mast cell tumor malignant, metastatic, uncertain primary site	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Pleura	(1)	(0)	(0)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)	–	–	–
Trachea	(50)	(50)	(47)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(49)	(50)
Adenoma	7 (14%)	3 (6%)	4 (8%)	2 (4%)
Carcinoma	4 (8%)	5 (10%)	1 (2%)	1 (2%)
Bilateral, adenoma	–	–	–	1 (2%)
Bilateral, carcinoma	1 (2%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, stomach, glandular	1 (2%)	–	–	–
Renal tubule, adenoma	–	–	1 (2%)	1 (2%)
Renal tubule, carcinoma	–	–	1 (2%)	–
Ureter	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)	–	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	1 (2%)	–
Lymphoma malignant	3 (6%)	5 (10%)	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	46	40	44
Total primary neoplasms	83	80	75	86
Total animals with benign neoplasms	39	33	33	38
Total benign neoplasms	53	39	48	57
Total animals with malignant neoplasms	26	31	17	22
Total malignant neoplasms	30	41	27	29
Total animals with metastatic neoplasms	8	11	4	6
Total metastatic neoplasms	17	15	16	7
Total animals with malignant neoplasms of uncertain primary site	–	–	–	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 C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Adrenal Cortex: Adenoma				
Overall rate[a]	9/49 (18%)	4/50 (8%)	5/50 (10%)	10/50 (20%)
Adjusted rate[b]	19.5%	9.2%	11.3%	21.3%
Terminal rate[c]	8/39 (21%)	3/31 (10%)	4/36 (11%)	9/40 (23%)
First incidence (days)	669	591	698	623
Poly-3 test[d]	P = 0.212	P = 0.136N	P = 0.212N	P = 0.517
Harderian Gland: Adenoma				
Overall rate	7/50 (14%)	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	15.0%	7.0%	9.0%	6.4%
Terminal rate	7/39 (18%)	3/31 (10%)	3/36 (8%)	2/40 (5%)
First incidence (days)	729 (T)	729 (T)	715	607
Poly-3 test	P = 0.210N	P = 0.191N	P = 0.290N	P = 0.156N
Harderian Gland: Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	1/50 (2%)	1/50 (2%)
Adjusted rate	10.7%	11.4%	2.3%	2.2%
Terminal rate	4/39 (10%)	2/31 (7%)	1/36 (3%)	1/40 (3%)
First incidence (days)	709	632	729 (T)	729 (T)
Poly-3 test	P = 0.053N	P = 0.588	P = 0.114N	P = 0.103N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	12/50 (24%)	8/50 (16%)	5/50 (10%)	4/50 (8%)
Adjusted rate	25.7%	18.3%	11.3%	8.5%
Terminal rate	11/39 (28%)	5/31 (16%)	4/36 (11%)	3/40 (8%)
First incidence (days)	709	632	715	607
Poly-3 test	P = 0.027N	P = 0.276N	P = 0.065N	P = 0.025N
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	24/50 (48%)	25/50 (50%)	30/49 (61%)
Adjusted rate	49.5%	51.7%	55.0%	62.1%
Terminal rate	20/39 (51%)	14/31 (45%)	20/36 (56%)	24/50 (63%)
First incidence (days)	499	547	636	344
Poly-3 test	P = 0.110	P = 0.497	P = 0.370	P = 0.145
Liver: Hepatocellular Carcinoma				
Overall rate	11/50 (22%)	17/50 (34%)	8/50 (16%)	15/49 (31%)
Adjusted rate	23.0%	36.7%	17.5%	31.5%
Terminal rate	5/39 (13%)	7/31 (23%)	4/36 (11%)	9/40 (23%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
First incidence (days)	565	547	438	572
Poly-3 test	P = 0.362	P = 0.107	P = 0.344N	P = 0.239
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	32/50 (64%)	32/50 (64%)	31/50 (62%)	35/49 (71%)
Adjusted rate	65.0%	67.0%	66.4%	71.6%
Terminal rate	23/39 (59%)	18/31 (58%)	23/36 (64%)	27/40 (68%)
First incidence (days)	499	547	438	344
Poly-3 test	P = 0.281	P = 0.503	P = 0.529	P = 0.316
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	11/50 (22%)	18/50 (36%)	8/50 (16%)	15/49 (31%)
Adjusted rate	23.0%	38.9%	17.5%	31.5%
Terminal rate	5/39 (3%)	8/31 (26%)	4/36 (11%)	9/40 (23%)
First incidence (days)	565	547	438	572
Poly-3 test	P = 0.403	P = 0.071	P = 0.344N	P = 0.239
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	32/50 (64%)	33/50 (66%)	31/50 (62%)	35/49 (71%)
Adjusted rate	65.0%	69.1%	66.4%	71.6%
Terminal rate	23/39 (59%)	19/31 (61%)	23/36 (64%)	27/40 (68%)
First incidence (days)	499	547	438	344
Poly-3 test	P = 0.315	P = 0.416	P = 0.529	P = 0.316
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	2/50 (4%)	8/50 (16%)	9/50 (18%)
Adjusted rate	10.7%	4.6%	17.8	19.0%
Terminal rate	5/39 (13%)	2/31 (7%)	6/36 (17%)	7/40 (18%)
First incidence (days)	729 (T)	729 (T)	573	590
Poly-3 test	P = 0.068	P = 0.249N	P = 0.250	P = 0.200
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	8/50 (16%)	8/50 (16%)	8/50 (16%)	10/50 (20%)
Adjusted rate	16.8%	18.0%	17.2%	20.9%
Terminal rate	6/39 (15%)	4/31 (13%)	3/36 (8%)	6/40 (15%)
First incidence (days)	541	547	534	590
Poly-3 test	P = 0.354	P = 0.552	P = 0.590	P = 0.403
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	9/50 (18%)	14/50 (28%)	17/50 (34%)
Adjusted rate	27.4%	20.2%	30.1%	35.3%

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Terminal rate	11/39 (28%)	5/31 (16%)	8/36 (22%)	12/40 (30%)
First incidence (days)	541	547	534	590
Poly-3 test	P = 0.120	P = 0.288N	P = 0.476	P = 0.270
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.1%	9.0%	2.3%	2.2%
Terminal rate	0/39 (0%)	1/31 (3%)	1/36 (3%)	1/40 (3%)
First incidence (days)	559	547	729 (T)	729 (T)
Poly-3 test	P = 0.347N	P = 0.162	P = 0.746	P = 0.757
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.2%	9.0%	4.5%	2.2%
Terminal rate	1/39 (3%)	1/31 (3%)	2/36 (6%)	1/40 (3%)
First incidence (days)	559	547	729 (T)	729 (T)
Poly-3 test	P = 0.241N	P = 0.311	P = 0.671	P = 0.506N
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	5/50 (10%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.4%	11.4%	2.3%	2.2%
Terminal rate	2/39 (5%)	3/31 (10%)	1/36 (3%)	1/40 (3%)
First incidence (days)	724	628	729 (T)	729 (T)
Poly-3 test	P = 0.125N	P = 0.321	P = 0.324N	P = 0.307N
All Organs: Benign Neoplasms				
Overall rate	39/50 (78%)	33/50 (66%)	33/50 (66%)	38/50 (76%)
Adjusted rate	79.7%	69.8%	70.7%	76.8%
Terminal rate	32/39 (82%)	21/31 (68%)	25/36 (69%)	31/40 (78%)
First incidence (days)	499	547	534	344
Poly-3 test	P = 0.478	P = 0.182N	P = 0.211N	P = 0.458N
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	31/50 (62%)	17/50 (34%)	23/50 (46%)
Adjusted rate	52.7%	64.7%	35.6%	46.9%
Terminal rate	16/39 (41%)	16/31 (52%)	9/36 (25%)	15/40 (38%)
First incidence (days)	541	547	438	572
Poly-3 test	P = 0.170N	P = 0.159	P = 0.066N	P = 0.355N

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	46/50 (92%)	40/50 (80%)	44/50 (88%)
Adjusted rate	92.0%	93.5%	82.3%	88.1%
Terminal rate	35/39 (90%)	28/31 (90%)	28/36 (78%)	35/40 (88%)
First incidence (days)	499	547	438	344
Poly-3 test	P = 0.306N	P = 0.542	P = 0.122N	P = 0.379N

T = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, and lung; 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 differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.

Table C-3. Historical Incidence of Alveolar/Bronchiolar Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July 2003)	6/50	8/50	13/50
CIMSTAR 3800 (May 2008)	5/50	8/50	13/50
Cobalt metal (May 2006)	7/50	11/50	16/50
Diethylamine (August 2003)	4/50	12/50	15/50
Tetralin (June 2003)	10/50	11/50	20/50
Vinylidene chloride (June 2005)	7/50	9/50	13/50
Total (%)	39/300 (13.0%)	59/300 (19.7%)	90/300 (30.0%)
Mean ± standard deviation	13.0% ± 4.2%	19.7% ± 3.4%	30.0% ± 5.5%
Range	8%–20%	16%–24%	26%–40%
Overall Historical Incidence: All Routes			
Total (%)	145/950 (15.3%)	132/950 (13.9%)	263/950 (27.7%)
Mean ± standard deviation	15.3% ± 6.2%	13.9% ± 7.1%	27.7% ± 5.7%
Range	2%–26%	4%–24%	16%–40%

[a]Data as of June 2013.

Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	12	7	4
Natural deaths	6	7	7	6
Survivors				
Terminal kill	39	31	36	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(44)	(44)	(44)	(44)
Cytoplasmic alteration	–	–	–	1 (2%)
Inflammation, suppurative	1 (2%)	–	–	1 (2%)
Intestine large, cecum	(50)	(50)	(49)	(49)
Hemorrhage	–	–	1 (2%)	–
Intestine large, colon	(50)	(50)	(50)	(50)
Ulcer	–	1 (2%)	–	–
Intestine large, rectum	(50)	(50)	(50)	(49)
Cyst	–	1 (2%)	–	–
Fibrosis	–	1 (2%)	–	–
Intestine small, duodenum	(49)	(48)	(48)	(47)
Artery, infiltration cellular, lymphocyte	1 (2%)	–	–	–
Intestine small, ileum	(50)	(50)	(49)	(48)
Hyperplasia, lymphoid	–	–	–	1 (2%)
Inflammation, chronic active	–	–	2 (4%)	2 (4%)
Epithelium, hyperplasia	1 (2%)	–	–	–
Lymphoid follicle, hyperplasia	1 (2%)	–	–	–
Peyer’s patch, hyperplasia, lymphoid	1 (2%)	–	–	–
Intestine small, jejunum	(50)	(48)	(49)	(48)
Inflammation, chronic active	–	–	1 (2%)	–
Serosa, inflammation, granulomatous	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(49)
Angiectasis	–	1 (2%)	–	–
Basophilic focus	1 (2%)	3 (6%)	–	4 (8%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Clear cell focus	10 (20%)	9 (18%)	3 (6%)	6 (12%)
Congestion	–	1 (2%)	1 (2%)	–
Cyst	–	1 (2%)	1 (2%)	–
Eosinophilic focus	14 (28%)	3 (6%)	20 (40%)	17 (35%)
Fatty change	24 (48%)	22 (44%)	24 (48%)	25 (51%)
Hepatodiaphragmatic nodule	1 (2%)	–	1 (2%)	–
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	–
Infiltration cellular, lymphocyte	–	–	1 (2%)	–
Inflammation, chronic	1 (2%)	–	–	–
Inflammation, chronic active	–	–	–	1 (2%)
Mixed cell focus	6 (12%)	4 (8%)	11 (22%)	5 (10%)
Necrosis	5 (10%)	7 (14%)	6 (12%)	7 (14%)
Tension lipidosis	3 (6%)	2 (4%)	5 (10%)	7 (14%)
Mesentery	(4)	(5)	(3)	(4)
Inflammation, chronic active	–	1 (20%)	1 (33%)	–
Arteriole, inflammation, chronic active	–	1 (20%)	–	–
Artery, inflammation, chronic active	–	–	–	1 (25%)
Fat, necrosis	1 (25%)	2 (40%)	2 (67%)	3 (75%)
Pancreas	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	–	–	–
Lipomatosis	–	1 (2%)	–	–
Necrosis	–	–	–	1 (2%)
Acinus, hypertrophy	1 (2%)	1 (2%)	–	–
Artery, inflammation, chronic active	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst, squamous	–	1 (2%)	–	–
Hyperplasia, squamous	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	–
Ulcer	–	–	1 (2%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	–	–	2 (4%)
Ulcer	–	–	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Arteriole, serosa, inflammation, chronic active	–	1 (2%)	–	–
Glands, mineralization	–	–	2 (4%)	1 (2%)
Tooth	(50)	(47)	(49)	(50)
Dysplasia	43 (86%)	35 (74%)	41 (84%)	40 (80%)
Inflammation, chronic active	3 (6%)	1 (2%)	6 (12%)	5 (10%)
Cardiovascular System				
Blood vessel	(45)	(49)	(48)	(48)
Inflammation, chronic active	1 (2%)	–	–	–
Aorta, inflammation, chronic active	–	1 (2%)	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, chronic active	–	–	–	1 (2%)
Thrombosis	1 (2%)	–	–	2 (4%)
Artery, inflammation, chronic active	–	1 (2%)	–	1 (2%)
Valve, fibrosis	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Hyperplasia	24 (49%)	23 (46%)	26 (52%)	24 (48%)
Capsule, inflammation, chronic active	1 (2%)	–	–	–
Adrenal medulla	(48)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	–	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(34)	(28)	(27)	(21)
Cyst	1 (3%)	–	–	–
Pituitary gland	(50)	(50)	(50)	(48)
Angiectasis	–	–	–	1 (2%)
Pars distalis, cyst	1 (2%)	–	–	1 (2%)
Pars distalis, hyperplasia	5 (10%)	8 (16%)	7 (14%)	2 (4%)
Pars intermedia, hypertrophy	–	1 (2%)	–	–
Thyroid gland	(50)	(50)	(49)	(50)
Inflammation, chronic active	–	–	1 (2%)	–
Follicular cell, hyperplasia	–	–	1 (2%)	1 (2%)
General Body System				

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Tissue NOS	(0)	(2)	(0)	(1)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	–	2 (4%)
Spermatocele	–	1 (2%)	–	–
Penis	(0)	(0)	(0)	(1)
Congestion	–	–	–	1 (100%)
Preputial gland	(50)	(49)	(48)	(49)
Ectasia	8 (16%)	4 (8%)	7 (15%)	7 (14%)
Inflammation, chronic active	9 (18%)	4 (8%)	11 (23%)	4 (8%)
Prostate gland	(50)	(49)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	–	–	1 (2%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Angiectasis	–	–	–	1 (2%)
Nuclear alteration, focal	–	1 (2%)	–	–
Germinal epithelium, atrophy	1 (2%)	4 (8%)	–	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	–
Hyperplasia, granulocytes	–	–	–	1 (2%)
Lymph node	(4)	(3)	(1)	(1)
Lumbar, infiltration cellular, mixed cell	1 (25%)	–	–	–
Lumbar, inflammation, pyogranulomatous	–	–	–	1 (100%)
Lumbar, lymphoid follicle, hyperplasia	1 (25%)	–	–	–
Lymphoid follicle, renal, hyperplasia	1 (25%)	–	–	–
Pancreatic, inflammation, pyogranulomatous	–	–	–	1 (100%)
Renal, inflammation, pyogranulomatous	–	–	–	1 (100%)
Lymph node, bronchial	(26)	(31)	(26)	(18)
Infiltration cellular, polymorphonuclear	1 (4%)	–	–	–
Lymph node, mandibular	(31)	(36)	(34)	(33)
Hyperplasia, lymphoid	–	2 (6%)	1 (3%)	–
Infiltration, cellular, plasma cell	–	1 (3%)	–	–
Inflammation, pyogranulomatous	–	–	–	1 (3%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lymph node, mediastinal	(39)	(39)	(32)	(35)
Hematopoietic cell proliferation	–	–	1 (3%)	–
Hyperplasia, plasma cell	–	–	1 (3%)	–
Infiltration cellular, mixed cell	–	–	1 (3%)	–
Infiltration, pyogranulomatous	–	–	–	1 (3%)
Lymphoid follicle, hyperplasia	1 (3%)	–	–	–
Lymph node, mesenteric	(49)	(50)	(48)	(49)
Fibrosis	1 (2%)	–	–	–
Hemorrhage	–	1 (2%)	1 (2%)	–
Hyperplasia, plasma cell	–	–	1 (2%)	–
Infiltration cellular, histiocyte	–	–	1 (2%)	–
Inflammation, pyogranulomatous	–	–	–	1 (2%)
Lymphoid follicle, hyperplasia	1 (2%)	–	–	–
Spleen	(50)	(50)	(49)	(49)
Congestion	–	–	1 (2%)	–
Fibrosis	–	1 (2%)	–	–
Hematopoietic cell proliferation	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Lymphoid follicle, hyperplasia	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Thymus	(41)	(38)	(39)	(41)
Atrophy	–	–	1 (3%)	1 (2%)
Integumentary System				
Mammary gland	(1)	(0)	(1)	(0)
Skin	(50)	(49)	(50)	(50)
Abscess	–	–	–	1 (2%)
Angiectasis	–	–	1 (2%)	–
Inflammation, chronic active	2 (4%)	4 (8%)	1 (2%)	3 (6%)
Ulcer	1 (2%)	4 (8%)	2 (4%)	–
Epidermis, hyperplasia	–	–	–	1 (2%)
Sebaceous gland, hyperplasia	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	7 (14%)	7 (14%)	10 (20%)	7 (14%)
Cranium, fracture	–	1 (2%)	–	–
Cranium, inflammation, chronic active	3 (6%)	2 (4%)	3 (6%)	5 (10%)
Joint, vertebra, fracture	–	1 (2%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Maxilla, necrosis	–	1 (2%)	–	–
Skeletal muscle	(1)	(3)	(2)	(3)
Infiltration cellular, lymphocyte	–	1 (33%)	–	–
Infiltration cellular, mixed cell	–	–	–	1 (33%)
Inflammation, chronic active	–	–	–	1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	–	1 (2%)	–
Thrombosis	–	–	1 (2%)	–
Arteriole, inflammation, chronic active	–	1 (2%)	–	–
Ventricle, dilatation	–	–	1 (2%)	–
Venule, infiltration cellular, lymphoid	–	1 (2%)	–	–
Peripheral nerve	(1)	(2)	(1)	(2)
Spinal cord	(1)	(3)	(1)	(2)
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Hyperplasia	–	–	1 (2%)	–
Inflammation, suppurative	–	1 (2%)	–	1 (2%)
Inflammation, chronic active	–	2 (4%)	3 (6%)	8 (16%)
Metaplasia, squamous	–	50 (100%)	49 (100%)	50 (100%)
Necrosis	–	1 (2%)	1 (2%)	1 (2%)
Lung	(50)	(50)	(50)	(50)
Congestion	–	–	1 (2%)	–
Erythrophagocytosis	–	1 (2%)	–	–
Hemorrhage	–	–	2 (4%)	1 (2%)
Infiltration cellular, histiocyte	5 (10%)	5 (10%)	1 (2%)	9 (18%)
Inflammation, acute	–	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	4 (8%)	4 (8%)	6 (12%)	7 (14%)
Bronchiole, hyperplasia	11 (22%)	11 (22%)	32 (64%)	44 (88%)
Interstitium, inflammation, chronic active	1 (2%)	–	1 (2%)	1 (2%)
Perivascular, inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation, chronic active	6 (12%)	8 (16%)	4 (8%)	12 (24%)
Necrosis	2 (4%)	2 (4%)	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Nasopharyngeal duct, necrosis	–	–	–	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	4 (8%)	31 (62%)	43 (86%)	49 (98%)
Olfactory epithelium, atrophy	1 (2%)	–	–	4 (8%)
Olfactory epithelium, metaplasia, respiratory	7 (14%)	15 (30%)	25 (50%)	37 (74%)
Olfactory epithelium, necrosis	–	1 (2%)	–	–
Respiratory epithelium, accumulation, hyaline droplet	7 (14%)	36 (72%)	50 (100%)	50 (100%)
Respiratory epithelium, hyperplasia	38 (76%)	44 (88%)	36 (72%)	41 (82%)
Respiratory epithelium, metaplasia, squamous	–	3 (6%)	–	1 (2%)
Turbinate, atrophy	–	–	–	1 (2%)
Turbinate, degeneration	1 (2%)	1 (2%)	–	–
Pleura	(1)	(0)	(0)	(0)
Trachea	(50)	(50)	(47)	(50)
Ulcer	–	–	–	1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	–	–
Cornea, hyperplasia, squamous	–	–	–	1 (2%)
Cornea, inflammation, chronic active	–	4 (8%)	2 (4%)	2 (4%)
Cornea, vacuolization cytoplasmic	2 (4%)	–	–	–
Lens, degeneration	1 (2%)	1 (2%)	–	1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Cyst	–	1 (2%)	–	–
Hyperplasia	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Congestion	1 (2%)	–	–	–
Cyst	4 (8%)	2 (4%)	3 (6%)	5 (10%)
Dilatation	1 (2%)	–	–	–
Infarct	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	–	–	–
Metaplasia, osseous	1 (2%)	1 (2%)	–	–
Nephropathy	48 (96%)	48 (96%)	45 (90%)	48 (96%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Artery, hemorrhage	1 (2%)	–	–	–
Artery, inflammation, chronic active	1 (2%)	3 (6%)	–	–
Capsule, fibrosis	–	1 (2%)	–	–
Capsule, hemorrhage	–	1 (2%)	–	–
Papilla, necrosis	–	1 (2%)	–	–
Pelvis, inflammation, suppurative	–	1 (2%)	–	–
Ureter	(0)	(0)	(0)	(1)
Inflammation, chronic active	–	–	–	1 (100%)
Urinary bladder	(50)	(50)	(50)	(49)

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

Appendix D. Summary of Lesions in Female Mice in the Two-year Inhalation Study of CIMSTAR 3800

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	7	8	10	11
Natural deaths	4	5	3	5
Survivors				
Terminal kill	39	37	37	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(46)	(49)	(41)	(41)
Leiomyosarcoma, metastatic, intestine small, duodenum	–	–	–	1 (2%)
Intestine large, cecum	(50)	(50)	(49)	(50)
Leiomyoma	–	–	–	1 (2%)
Intestine large, colon	(49)	(50)	(49)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(47)	(50)	(50)	(49)
Adenoma	1 (2%)	–	1 (2%)	–
Leiomyosarcoma	–	–	–	1 (2%)
Intestine small, ileum	(49)	(50)	(49)	(49)
Carcinoma	–	1 (2%)	–	–
Intestine small, jejunum	(48)	(50)	(48)	(48)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Hepatoblastoma	1 (2%)	–	–	–
Hepatocellular adenoma	10 (20%)	11 (22%)	9 (18%)	7 (14%)
Hepatocellular adenoma, multiple	4 (8%)	3 (6%)	5 (10%)	3 (6%)
Hepatocellular carcinoma	10 (20%)	8 (16%)	2 (4%)	1 (2%)
Hepatocellular carcinoma, multiple	–	1 (2%)	–	–
Hepatocholangiocarcinoma	–	–	–	1 (2%)
Leiomyosarcoma, metastatic, uterus	–	–	1 (2%)	–
Mesentery	(16)	(23)	(21)	(15)
Hemangioma	–	2 (9%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hemangiosarcoma	–	–	1 (5%)	–
Fat, leiomyosarcoma, metastatic, intestine small, duodenum	–	–	–	1 (7%)
Pancreas	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, intestine small, duodenum	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(1)	(0)	(2)	(4)
Cardiovascular System				
Blood vessel	(46)	(44)	(49)	(44)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	–	–	1 (2%)	–
Subcapsular, adenoma	–	2 (4%)	1 (2%)	–
Adrenal medulla	(49)	(50)	(49)	(50)
Pheochromocytoma benign	1 (2%)	2 (4%)	1 (2%)	–
Pheochromocytoma malignant	–	–	1 (2%)	–
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(26)	(29)	(22)	(25)
Pituitary gland	(50)	(50)	(49)	(50)
Meningioma malignant, metastatic, brain	–	–	–	1 (2%)
Pars distalis, adenoma	5 (10%)	4 (8%)	6 (12%)	9 (18%)
Pars intermedia, adenoma	–	–	2 (4%)	–
Thyroid gland	(50)	(48)	(50)	(50)
C-cell, adenoma	–	–	–	1 (2%)
Follicular cell, carcinoma	–	–	–	3 (6%)
General Body System				
Tissue NOS	(3)	(2)	(0)	(0)
Mediastinum, carcinoma	–	1 (50%)	–	–
Genital System				
Clitoral gland	(44)	(45)	(43)	(44)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Ovary	(50)	(50)	(48)	(50)
Cystadenocarcinoma	–	–	1 (2%)	–
Cystadenoma	2 (4%)	1 (2%)	–	–
Granulosa cell tumor benign	1 (2%)	–	–	–
Leiomyosarcoma, metastatic, uterus	–	–	1 (2%)	–
Luteoma	1 (2%)	1 (2%)	–	–
Uterus	(50)	(50)	(50)	(50)
Carcinoma	–	–	1 (2%)	–
Hemangiosarcoma	1 (2%)	1 (2%)	–	–
Leiomyoma	1 (2%)	–	–	–
Leiomyosarcoma	–	–	1 (2%)	–
Polyp stromal	–	3 (6%)	2 (4%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)	–	–	–
Lymph node	(11)	(12)	(16)	(10)
Renal, hemangiosarcoma	–	–	–	1 (10%)
Lymph node, bronchial	(26)	(27)	(28)	(27)
Carcinoma, metastatic, uncertain primary site	–	–	–	1 (4%)
Lymph node, mandibular	(41)	(42)	(43)	(35)
Hemangiosarcoma	–	–	–	1 (3%)
Lymph node, mediastinal	(43)	(46)	(40)	(47)
Lymph node, mesenteric	(48)	(50)	(49)	(49)
Spleen	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)	1 (2%)	–	–
Thymus	(44)	(46)	(45)	(45)
Thymoma benign	–	–	1 (2%)	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	–	–	–	1 (2%)
Carcinoma	2 (4%)	–	2 (4%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Lip, squamous cell papilloma	1 (2%)	–	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Subcutaneous tissue, fibrosarcoma	–	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, schwannoma malignant	–	1 (2%)	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Femur, sarcoma	1 (2%)	–	–	–
Vertebra, rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Skeletal muscle	(3)	(3)	(1)	(4)
Leiomyosarcoma, metastatic, intestine small, duodenum	–	–	–	1 (25%)
Rhabdomyosarcoma	–	–	–	1 (25%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meningioma malignant	–	–	–	1 (2%)
Peripheral nerve	(2)	(3)	(1)	(3)
Rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (33%)
Spinal cord	(3)	(3)	(1)	(3)
Respiratory System				
Larynx	(49)	(49)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	3 (6%)	2 (4%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	–	–
Alveolar/bronchiolar carcinoma	4 (8%)	1 (2%)	4 (8%)	8 (16%)
Carcinoma, metastatic, mammary gland	–	–	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	4 (8%)	1 (2%)	–
Schwannoma malignant, metastatic, skin	–	–	1 (2%)	–
Nose	(50)	(49)	(50)	(50)
Carcinoma, metastatic, Harderian gland	–	–	1 (2%)	–
Trachea	(49)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland	–	–	1 (2%)	–
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	6 (12%)	3 (6%)	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Carcinoma	–	1 (2%)	1 (2%)	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Ureter	(0)	(0)	(1)	(0)
Urethra	(0)	(1)	(0)	(0)
Sarcoma	–	1 (100%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	18 (36%)	18 (36%)	17 (34%)	20 (40%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	42	43	40
Total primary neoplasms	74	77	69	73
Total animals with benign neoplasms	26	30	28	23
Total benign neoplasms	31	40	35	29
Total animals with malignant neoplasms	30	31	28	34
Total malignant neoplasms	43	37	34	44
Total animals with metastatic neoplasms	2	4	4	4
Total metastatic neoplasms	2	4	8	10
Total animals with malignant neoplasms of uncertain primary site	–	–	–	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 D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Inhalation Study of CIMSTAR 3800

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Harderian Gland: Adenoma				
Overall rate[a]	2/50 (4%)	6/50 (12%)	3/50 (6%)	1/50 (2%)
Adjusted rate[b]	4.4%	13.1%	6.5%	2.3%
Terminal rate[c]	2/39 (5%)	5/37 (14%)	3/37 (8%)	0/33 (0%)
First incidence (days)	731 (T)	677	731 (T)	644
Poly-3 test[d]	P = 0.160N	P = 0.137	P = 0.506	P = 0.511N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	7/50 (14%)	4/50 (8%)	3/50 (6%)
Adjusted rate	4.4%	15.3%	8.7%	6.8%
Terminal rate	2/39 (5%)	6/37 (16%)	3/37 (8%)	2/33 (6%)
First incidence (days)	731 (T)	677	644	644
Poly-3 test	P = 0.428N	P = 0.082	P = 0.347	P = 0.486
Liver: Hepatocellular Adenoma				
Overall rate	14/50 (28%)	14/50 (28%)	14/50 (28%)	10/50 (20%)
Adjusted rate	29.8%	29.7%	29.8%	22.7%
Terminal rate	11/39 (28%)	10/37 (27%)	10/37 (27%)	7/33 (21%)
First incidence (days)	527	583	576	669
Poly-3 test	P = 0.239N	P = 0.582N	P = 0.588N	P = 0.297N
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	9/50 (18%)	2/50 (4%)	1/50 (2%)
Adjusted rate	21.9%	19.2%	4.3%	2.3%
Terminal rate	9/39 (23%)	5/37 (14%)	1/37 (3%)	1/33 (3%)
First incidence (days)	605	552	644	731 (T)
Poly-3 test	P = 0.002N	P = 0.473N	P = 0.012N	P = 0.005N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	20/50 (40%)[e]	19/50 (38%)	15/50 (30%)	10/50 (20%)
Adjusted rate	42.6%	39.6%	32.0%	22.7%
Terminal rate	17/39 (44%)	12/37 (32%)	11/37 (30%)	7/33 (21%)
First incidence (days)	527	552	576	669
Poly-3 test	P = 0.024N	P = 0.463N	P = 0.195N	P = 0.034N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	8.8%	4.3%	9.2%
Terminal rate	1/39 (3%)	4/37 (11%)	1/37 (3%)	3/33 (9%)
First incidence (days)	731 (T)	731 (T)	677	712
Poly-3 test	P = 0.245	P = 0.181	P = 0.506	P = 0.168
Lung: Alveolar/bronchiolar Carcinoma				

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Overall rate	4/50 (8%)	1/50 (2%)	4/50 (8%)	8/50 (16%)
Adjusted rate	8.8%	2.2%	8.6%	18.2%
Terminal rate	4/39 (10%)	1/37 (3%)	3/37 (8%)	6/33 (18%)
First incidence (days)	731 (T)	731 (T)	460	644
Poly-3 test	P = 0.021	P = 0.176N	P = 0.627N	P = 0.163
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	6/50 (12%)	12/50 (24%)
Adjusted rate	8.8%	10.9%	12.8%	27.2%
Terminal rate	4/39 (10%)	5/37 (14%)	4/37 (11%)	9/33 (27%)
First incidence (days)	731 (T)	731 (T)	460	644
Poly-3 test	P = 0.006	P = 0.505	P = 0.391	P = 0.021
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/50 (10%)	4/50 (8%)	6/49 (12%)	9/50 (18%)
Adjusted rate	11.0%	8.8%	13.2%	20.3%
Terminal rate	5/39 (13%)	4/37 (11%)	5/37 (14%)	5/33 (15%)
First incidence (days)	731 (T)	731 (T)	681	634
Poly-3 test	P = 0.076	P = 0.495N	P = 0.502	P = 0.180
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rate	0/50 (0%)	0/48 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.9%
Terminal rate	0/39 (0%)	0/36 (0%)	0/37 (0%)	2/33 (6%)
First incidence (days)	–[f]	–	–	712
Poly-3 test	P = 0.008	–[g]	–	P = 0.112
Uterus: Stromal Polyp				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	6.6%	4.3%	0.0%
Terminal rate	0/39 (0%)	3/37 (8%)	1/37 (3%)	0/33 (0%)
First incidence (days)	–	731 (T)	576	–
Poly-3 test	P = 0.293N	P = 0.120	P = 0.243	–
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.6%	4.4%	2.2%	6.9%
Terminal rate	3/39 (8%)	2/37 (5%)	0/37 (0%)	2/33 (6%)
First incidence (days)	731 (T)	731 (T)	576	699
Poly-3 test	P = 0.483	P = 0.496N	P = 0.297N	P = 0.646
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.6%	8.8%	2.2%	6.9%
Terminal rate	3/39 (8%)	4/37 (11%)	0/37 (0%)	2/33 (6%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
First incidence (days)	731 (T)	731 (T)	576	699
Poly-3 test	P = 0.576N	P = 0.505	P = 0.297N	P = 0.646
All Organs: Malignant Lymphoma				
Overall rate	18/50 (36%)	18/50 (36%)	17/50 (34%)	20/50 (40%)
Adjusted rate	39.7%	39.1%	35.8%	43.7%
Terminal rate	17/39 (44%)	16/37 (43%)	12/37 (32%)	13/33 (39%)
First incidence (days)	705	655	460	536
Poly-3 test	P = 0.348	P = 0.562N	P = 0.433N	P = 0.429
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	30/50 (60%)	28/50 (56%)	23/50 (46%)
Adjusted rate	54.1%	63.6%	59.1%	51.0%
Terminal rate	20/39 (51%)	26/37 (70%)	22/37 (60%)	15/33 (46%)
First incidence (days)	345	583	576	634
Poly-3 test	P = 0.256N	P = 0.228	P = 0.384	P = 0.465N
All Organs: Malignant Neoplasms				
Overall rate	30/50 (60%)	31/50 (62%)	28/50 (56%)	34/50 (68%)
Adjusted rate	64.0%	63.9%	57.0%	72.4%
Terminal rate	26/39 (67%)	22/37 (60%)	19/37 (51%)	22/33 (67%)
First incidence (days)	527	508	460	536
Poly-3 test	P = 0.175	P = 0.579N	P = 0.310N	P = 0.254
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	42/50 (84%)	43/50 (86%)	40/50 (80%)
Adjusted rate	84.5%	85.4%	86.8%	84.9%
Terminal rate	34/39 (87%)	31/37 (84%)	31/37 (84%)	27/33 (82%)
First incidence (days)	345	508	460	536
Poly-3 test	P = 0.569N	P = 0.567	P = 0.486	P = 0.592

(T) = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]A single incidence of hepatoblastoma occurred in an animal that also had a hepatocellular adenoma and a hepatocellular carcinoma.
[f]Not applicable; no neoplasms in animal group.
[g]Value of statistic cannot be computed.

Table D-3. Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July 2003)	0/49	0/49	0/49
CIMSTAR 3800 (May 2008)	0/50	0/50	0/50
Cobalt metal (May 2006)	1/50	0/50	1/50
Diethylamine (August 2003)	0/50	0/50	0/50
Tetralin (June 2003)	0/50	1/50	1/50
Vinylidene chloride (June 2005)	0/50	0/50	0/50
Total (%)	1/299 (0.3%)	1/299 (0.3%)	2/299 (0.7%)
Mean ± standard deviation	0.3% ± 0.8%	0.3% ± 0.8%	0.7% ± 1.0%
Range	0%–2%	0%–2%	0%–2%
Overall Historical Incidence: All Routes			
Total (%)	2/942 (0.2%)	2/942 (0.2%)	4/942 (0.4%)
Mean ± standard deviation	0.2% ± 0.6%	0.2% ± 0.6%	0.4% ± 0.8%
Range	0%–2%	0%–2%	0%–2%

[a]Data as of June 2013.

Table D-4. Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July 2003)	1/50	0/50	1/50
CIMSTAR 3800 (May 2008)	1/50	4/50	4/50
Cobalt metal (May 2006)	3/49	5/49	8/49
Diethylamine (August 2003)	2/50	3/50	5/50
Tetralin (June 2003)	6/50	0/50	6/50
Vinylidene chloride (June 2005)	3/50	1/50	4/50
Total (%)	16/299 (5.4%)	13/299 (4.4%)	28/299 (9.4%)
Mean ± standard deviation	5.4% ± 3.7%	4.4% ± 4.3%	9.4% ± 4.8%
Range	2%–12%	0%–10%	2%–16%
Overall Historical Incidence: All Routes			
Total (%)	54/949 (5.7%)	38/949 (4.0%)	90/949 (9.5%)
Mean ± standard deviation	5.7% ± 3.6%	4.0% ± 3.6%	9.5% ± 4.8%
Range	0%–12%	0%–14%	2%–22%

[a]Data as of June 2013.

Table D-5. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	7	8	10	11
Natural deaths	4	5	3	5
Survivors				
Terminal kill	39	37	37	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(46)	(49)	(41)	(41)
Epithelium, hyperplasia	–	1 (2%)	–	–
Intestine large, cecum	(50)	(50)	(49)	(50)
Hyperplasia	–	1 (2%)	–	–
Inflammation, chronic active	–	–	1 (2%)	–
Intestine large, colon	(49)	(50)	(49)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation, chronic active	–	–	1 (2%)	1 (2%)
Intestine small, duodenum	(47)	(50)	(50)	(49)
Intestine small, ileum	(49)	(50)	(49)	(49)
Inflammation, chronic active	–	–	1 (2%)	–
Intestine small, jejunum	(48)	(50)	(48)	(48)
Liver	(50)	(50)	(50)	(50)
Amyloid deposition	–	–	–	1 (2%)
Angiectasis	–	2 (4%)	1 (2%)	2 (4%)
Basophilic focus	6 (12%)	5 (10%)	3 (6%)	4 (8%)
Clear cell focus	1 (2%)	–	–	–
Cyst	–	1 (2%)	–	1 (2%)
Degeneration	–	1 (2%)	–	–
Eosinophilic focus	6 (12%)	1 (2%)	5 (10%)	13 (26%)
Fatty change	3 (6%)	2 (4%)	1 (2%)	5 (10%)
Hyperplasia	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	–	1 (2%)	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Infiltration cellular, mixed cell	–	1 (2%)	–	–
Inflammation, chronic active	–	–	1 (2%)	–
Mixed cell focus	2 (4%)	4 (8%)	1 (2%)	–
Necrosis	2 (4%)	–	2 (4%)	4 (8%)
Tension lipidosis	6 (12%)	5 (10%)	5 (10%)	6 (12%)
Vacuolization cytoplasmic	1 (2%)	–	–	–
Centrilobular, degeneration, acute	–	1 (2%)	–	–
Centrilobular, hypertrophy	–	–	1 (2%)	–
Centrilobular, necrosis	–	1 (2%)	–	–
Mesentery	(16)	(23)	(21)	(15)
Angiectasis	1 (6%)	–	–	–
Cyst	1 (6%)	–	–	–
Fibrosis	–	–	1 (5%)	–
Inflammation, suppurative	–	–	1 (5%)	–
Inflammation, chronic active	1 (6%)	1 (4%)	1 (5%)	–
Necrosis	–	1 (4%)	1 (5%)	–
Arteriole, inflammation, chronic active	–	–	1 (5%)	–
Fat, cyst	–	–	1 (5%)	–
Fat, necrosis	11 (69%)	17 (74%)	7 (33%)	8 (53%)
Vein, inflammation, chronic active	–	–	–	1 (7%)
Pancreas	(50)	(50)	(50)	(50)
Cyst	–	1 (2%)	–	–
Fibrosis	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	–	–	1 (2%)
Lipomatosis	–	1 (2%)	–	1 (2%)
Acinus, atrophy	1 (2%)	–	1 (2%)	2 (4%)
Acinus, hyperplasia	–	–	–	1 (2%)
Acinus, hypertrophy	–	–	1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	–	–	–
Hyperplasia, squamous	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic active	–	–	–	1 (2%)
Ulcer	–	1 (2%)	1 (2%)	1 (2%)
Arteriole, inflammation, chronic active	–	–	1 (2%)	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Stomach, glandular	(50)	(50)	(50)	(50)
Degeneration, hyaline	1 (2%)	–	–	–
Fibrosis	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	–	–	–
Ulcer	–	–	–	1 (2%)
Arteriole, inflammation, chronic active	–	–	1 (2%)	–
Glands, mineralization	–	–	2 (4%)	1 (2%)
Tooth	(1)	(0)	(2)	(4)
Dysplasia	1 (100%)	–	2 (100%)	3 (75%)
Inflammation, chronic active	–	–	–	2 (50%)
Cardiovascular System				
Blood vessel	(46)	(44)	(49)	(44)
Inflammation, chronic active	–	–	1 (2%)	–
Aorta, embolus bacterial	1 (2%)	–	–	1 (2%)
Aorta, inflammation, suppurative	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)	3 (6%)	8 (16%)	5 (10%)
Congestion	–	1 (2%)	–	1 (2%)
Mineralization	–	–	1 (2%)	–
Necrosis	1 (2%)	1 (2%)	–	1 (2%)
Thrombosis	–	1 (2%)	–	–
Artery, inflammation, chronic active	1 (2%)	–	–	–
Capillary, hyperplasia	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Atrophy	–	–	1 (2%)	1 (2%)
Degeneration, cystic	–	1 (2%)	–	–
Hyperplasia	10 (20%)	5 (10%)	10 (20%)	6 (12%)
Vacuolization cytoplasmic	2 (4%)	2 (4%)	–	2 (4%)
Adrenal medulla	(49)	(50)	(49)	(50)
Hyperplasia	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Parathyroid gland	(26)	(29)	(22)	(25)
Cyst	–	1 (3%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hyperplasia	1 (4%)	–	–	–
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis	2 (4%)	1 (2%)	–	4 (8%)
Pars distalis, cyst	–	1 (2%)	–	1 (2%)
Pars distalis, hyperplasia	17 (34%)	21 (42%)	19 (39%)	24 (48%)
Pars intermedia, pars nervosa, cyst	–	–	–	1 (2%)
Thyroid gland	(50)	(48)	(50)	(50)
Inflammation, chronic active	–	1 (2%)	2 (4%)	2 (4%)
C-cell, hyperplasia	1 (2%)	1 (2%)	–	–
Follicle, cyst	–	1 (2%)	–	–
Follicular cell, hyperplasia	1 (2%)	1 (2%)	2 (4%)	3 (6%)
General Body System				
Tissue NOS	(3)	(2)	(0)	(0)
Mediastinum, infiltration cellular, lymphocyte	1 (33%)	–	–	–
Mediastinum, thrombosis	1 (33%)	–	–	–
Genital System				
Clitoral gland	(44)	(45)	(43)	(44)
Hyperplasia, basal cell	–	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	–
Ovary	(50)	(50)	(48)	(50)
Angiectasis	3 (6%)	2 (4%)	–	3 (6%)
Cyst	14 (28%)	12 (24%)	12 (25%)	16 (32%)
Hemorrhage	12 (24%)	16 (32%)	7 (15%)	15 (30%)
Infiltration cellular, lymphocyte	–	1 (2%)	1 (2%)	–
Inflammation, chronic active	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Mineralization	1 (2%)	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Thrombosis	–	–	–	1 (2%)
Corpus luteum, hyperplasia	3 (6%)	1 (2%)	–	–
Uterus	(50)	(50)	(50)	(50)
Angiectasis	5 (10%)	3 (6%)	1 (2%)	1 (2%)
Atrophy	1 (2%)	–	–	–
Decidual reaction	1 (2%)	–	–	–
Dilatation	6 (12%)	5 (10%)	8 (16%)	6 (12%)
Hemorrhage	–	–	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Inflammation, suppurative	1 (2%)	–	–	–
Inflammation, chronic active	2 (4%)	2 (4%)	3 (6%)	4 (8%)
Thrombosis	1 (2%)	–	–	–
Endometrium, hyperplasia, cystic	28 (56%)	29 (58%)	33 (66%)	34 (68%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(11)	(12)	(16)	(10)
Ectasia	–	–	–	1 (10%)
Hemorrhage	1 (9%)	–	–	–
Iliac, angiectasis	–	1 (8%)	–	–
Iliac, ectasia	1 (9%)	–	–	–
Iliac, hyperplasia, lymphoid	1 (9%)	1 (8%)	1 (6%)	–
Iliac, hyperplasia, plasma cell	–	–	–	1 (10%)
Lumbar, ectasia	1 (9%)	1 (8%)	2 (13%)	–
Lumbar, hyperplasia, lymphoid	–	1 (8%)	–	1 (10%)
Lumbar, infiltration cellular, histiocyte	–	1 (8%)	–	–
Lumbar, infiltration cellular, mixed cell	–	–	1 (6%)	–
Pancreatic, inflammation, chronic active	1 (9%)	–	–	–
Renal, angiectasis	–	1 (8%)	–	–
Renal, ectasia	1 (9%)	–	1 (6%)	–
Renal, hyperplasia, lymphoid	–	2 (17%)	–	–
Renal, necrosis	–	–	1 (6%)	–
Lymph node, bronchial	(26)	(27)	(28)	(27)
Infiltration cellular, histiocyte	–	–	–	1 (4%)
Necrosis	–	–	1 (4%)	–
Lymph node, mandibular	(41)	(42)	(43)	(35)
Necrosis	–	–	1 (2%)	–
Lymph node, mediastinal	(43)	(46)	(40)	(47)
Hematopoietic cell proliferation	–	1 (2%)	–	–
Hyperplasia, lymphoid	–	1 (2%)	–	2 (4%)
Infiltration cellular, mixed cell	–	–	–	1 (2%)
Lymph node, mesenteric	(48)	(50)	(49)	(49)
Angiectasis	–	1 (2%)	2 (4%)	1 (2%)
Ectasia	–	–	2 (4%)	–
Hematopoietic cell proliferation	–	1 (2%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hemorrhage	–	2 (4%)	–	–
Hyperplasia, lymphoid	–	–	1 (2%)	–
Infiltration cellular, histiocyte	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(49)
Hematopoietic cell proliferation	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, mast cell	–	–	–	1 (2%)
Lymphoid follicle, hyperplasia	–	–	1 (2%)	–
Thymus	(44)	(46)	(45)	(45)
Angiectasis	–	–	1 (2%)	–
Atrophy	2 (5%)	–	–	1 (2%)
Hyperplasia, lymphoid	1 (2%)	–	–	–
Necrosis	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	–	–	1 (2%)
Skin	(50)	(50)	(50)	(50)
Infiltration cellular, mast cell	–	–	–	1 (2%)
Inflammation, chronic active	–	–	1 (2%)	2 (4%)
Ulcer	1 (2%)	–	1 (2%)	3 (6%)
Epidermis, hyperplasia	1 (2%)	–	–	–
Subcutaneous tissue, fibrosis	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	23 (46%)	25 (50%)	27 (54%)	23 (46%)
Cartilage, femur, metaphysis, hyperplasia	1 (2%)	–	–	–
Cranium, inflammation, chronic active	1 (2%)	–	2 (4%)	1 (2%)
Femur, fracture	1 (2%)	–	–	–
Femur, tibia, arthrosis	–	–	–	1 (2%)
Maxilla, hyperostosis	–	–	1 (2%)	–
Pelvis, degeneration	–	–	–	1 (2%)
Vertebra, fracture	–	–	–	1 (2%)
Skeletal muscle	(3)	(3)	(1)	(4)
Degeneration	–	–	–	1 (25%)
Infiltration cellular, lymphocyte	3 (100%)	1 (33%)	–	–

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Infiltration cellular, polymorphonuclear	–	–	–	1 (25%)
Arteriole, inflammation, chronic active	–	–	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Meninges, inflammation, chronic active	–	–	–	1 (2%)
Peripheral nerve	(2)	(3)	(1)	(3)
Degeneration	–	1 (33%)	–	1 (33%)
Infiltration cellular, mixed cell	1 (50%)	–	–	–
Infiltration cellular, polymorphonuclear	–	1 (33%)	–	–
Spinal cord	(3)	(3)	(1)	(3)
Infiltration cellular, lymphocyte	–	1 (33%)	–	–
Respiratory System				
Larynx	(49)	(49)	(50)	(50)
Inflammation, chronic active	–	–	–	10 (20%)
Metaplasia, squamous	1 (2%)	49 (100%)	50 (100%)	50 (100%)
Necrosis	–	–	–	4 (8%)
Lung	(50)	(50)	(50)	(50)
Congestion	–	–	–	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, histiocyte	4 (8%)	1 (2%)	1 (2%)	6 (12%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–
Infiltration cellular, mixed cell	–	–	–	1 (2%)
Metaplasia, osseous	–	1 (2%)	–	–
Thrombosis	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)	5 (10%)	4 (8%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	–	–	–
Bronchiole, hyperplasia	7 (14%)	4 (8%)	22 (44%)	41 (82%)
Interstitium, inflammation, chronic active	1 (2%)	–	–	1 (2%)
Perivascular, inflammation, chronic active	4 (8%)	3 (6%)	–	11 (22%)
Nose	(50)	(49)	(50)	(50)
Inflammation, chronic active	9 (18%)	–	3 (6%)	5 (10%)
Necrosis	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	25 (50%)	40 (82%)	50 (100%)	49 (98%)
Olfactory epithelium, atrophy	1 (2%)	1 (2%)	–	1 (2%)

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Olfactory epithelium, metaplasia, respiratory	3 (6%)	4 (8%)	12 (24%)	23 (46%)
Respiratory epithelium, accumulation, hyaline droplet	34 (68%)	48 (98%)	50 (100%)	50 (100%)
Respiratory epithelium, hyperplasia	39 (78%)	48 (98%)	39 (78%)	39 (78%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	–	1 (2%)
Respiratory epithelium, necrosis	–	1 (2%)	–	–
Trachea	(49)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	–	–	2 (4%)	–
Cornea, inflammation, chronic active	–	1 (2%)	–	1 (2%)
Lens, degeneration	2 (4%)	–	2 (4%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	1 (2%)	–
Hyperplasia	2 (4%)	1 (2%)	6 (12%)	–
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	1 (2%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)	1 (2%)	–	–
Infarct	1 (2%)	4 (8%)	4 (8%)	1 (2%)
Inflammation, suppurative	–	–	–	1 (2%)
Metaplasia, osseous	–	1 (2%)	2 (4%)	1 (2%)
Necrosis	1 (2%)	–	–	–
Nephropathy	47 (94%)	46 (92%)	49 (98%)	47 (94%)
Glomerulus, amyloid deposition	1 (2%)	–	–	1 (2%)
Ureter	(0)	(0)	(1)	(0)
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	1 (2%)	–
Arteriole, inflammation, chronic active	–	–	1 (2%)	–
Artery, serosa, inflammation, chronic active	–	1 (2%)	–	–

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

Appendix E. Genetic Toxicology

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Tables

E.1. Bacterial Mutagenicity Test Protocol

Testing was performed as reported by Zeiger et al.[113] with slight modifications. CIMSTAR 3800 was sent to the laboratory as a coded aliquot. It was incubated with the Salmonella typhimurium tester strains TA98 and TA100 and the Escherichia coli strain WP2 uvrA/pKM101 either in buffer or 10% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with l-histidine and d-biotin (or tryptophan for the E. coli strain) was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of CIMSTAR 3800. The high dose was limited by assay design to 10,000 µg/plate. All trials were repeated.

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.

E.2. Peripheral Blood Micronucleus Test Protocol

At the termination of the 3-month studies, blood samples were collected from male and female rats and mice, placed in EDTA-coated tubes, fixed in ultracold methanol and frozen at −80°C until analysis. Thawed blood samples were analyzed for frequency of micronucleated reticulocytes (polychromatic erythrocytes, PCEs) and mature erythrocytes (normochromatic erythrocytes, NCEs) using a flow cytometer[114]; both the mature erythrocyte population and the immature reticulocyte population can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte population can be targeted using this technique, rat blood samples can be analyzed for damage in the bone marrow that occurred within the past 24 to 48 hours, before the rat spleen appreciably alters the percentage of micronucleated reticulocytes in circulation[115]. In mice, both the reticulocyte and mature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. These achieve steady state in the peripheral blood of mice following 4 weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs are analyzed per sample for frequency of micronucleated cells, and the percent PCEs is calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques[116], it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise

comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$.

In the micronucleus test (for each data set: PCEs, NCEs, percentage of PCEs), a positive result is preferably based on the presence of both a significant trend as well as at least one significantly elevated dose group compared with the corresponding control group. The presence of either a significant trend or a single significant dose group generally results in an equivocal call. The absence of both a trend and a significant dose group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

E.3. 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 samples of a chemical were tested in the same assay, and different results were obtained among these samples 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.

E.4. Results

CIMSTAR 3800 (dose range tested, 1,000 to 10,000 µg/plate) was weakly mutagenic in E. coli strain WP2 uvrA/pKM101 in the absence of exogenous metabolic activation (S9); no mutagenic activity was observed in S. typhimurium strains TA98 and TA100 tested over the same dose range, with or without S9, or in the E. coli strain with S9 (Table E-1).

In vivo, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were observed in peripheral blood samples from male or female F344/NTac rats or B6C3F1/N mice exposed to CIMSTAR 3800 via inhalation (25 to 400 mg/m^3) for 3 months (Table E-2 and

Table E-3). In female rats, a significant trend was observed for micronucleated erythrocytes (P = 0.003). However, in rats, the appropriate cell population to evaluate for frequency of micronucleated cells in peripheral blood is the very young newly emerged reticulocyte population because the rat spleen is very efficient at quickly sequestering and destroying damaged erythrocytes. Thus, despite the very slight increase in micronucleated erythrocytes in 400 mg/m^3 female rats, the absence of supporting data in the female micronucleated reticulocyte data set and in the male rats suggests that the increase is not biologically significant.

In addition to the micronucleus endpoint, the percentage of reticulocytes among circulating red blood cells was calculated as a measure of bone marrow toxicity or perturbations in erythropoiesis (Table E-2 and Table E-3). No significant alterations in the percentage of reticulocytes among red blood cells was seen with CIMSTAR 3800, suggesting an absence of treatment-related bone marrow toxicity. The very small and inconsistent changes in percentage of reticulocytes noted in both the male and female mice, in the absence of any observed hematologic effects, were not considered biologically significant.

Table E-1. Mutagenicity of CIMSTAR 3800 in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA100					
	0	92 ± 1	96 ± 5	80 ± 3	126 ± 2
	1,000	110 ± 5	101 ± 12	97 ± 10	127 ± 9
	2,500	105 ± 5	79 ± 8	119 ± 15	132 ± 2
	5,000	74 ± 7	73 ± 6	107 ± 10	140 ± 7
	7,500	63 ± 6	73 ± 7	113 ± 4	136 ± 6
	10,000	54 ± 4	32 ± 8	103 ± 1	151 ± 7
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		376 ± 7	745 ± 56	853 ± 37	776 ± 53
TA98					
	0	27 ± 4	34 ± 1	35 ± 2	30 ± 0
	1,000	22 ± 1	36 ± 3	35 ± 3	35 ± 2
	2,500	21 ± 1	31 ± 5	31 ± 3	36 ± 1
	5,000	33 ± 4	30 ± 3	35 ± 1	41 ± 2
	7,500	22 ± 4	27 ± 4	30 ± 3	43 ± 5
	10,000	4 ± 0	11 ± 2	29 ± 2	39 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control		574 ± 17	740 ± 46	652 ± 29	776 ± 56
***Escherichia coli* WP2 uvrA/pKM101 (analogous to TA102)**					
	0	164 ± 5	216 ± 11	200 ± 1	275 ± 5
	1,000	205 ± 6	280 ± 12	218 ± 7	247 ± 14
	2,500	240 ± 28	352 ± 12	250 ± 27	314 ± 39

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
	5,000	269 ± 9	358 ± 12	262 ± 20	310 ± 7
	7,500	283 ± 14	342 ± 22	235 ± 27	346 ± 10
	10,000	232 ± 15	371 ± 10	287 ± 9	316 ± 9
Trial summary		Weakly Positive	Weakly Positive	Equivocal	Negative
Positive control		701 ± 80	950 ± 26	777 ± 19	875 ± 51

[a]Study was performed at SITEK Research Laboratories. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-o-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of F344/NTac Rats Following Treatment with CIMSTAR 3800 by Inhalation for Three Months[a]

	Dose (mg/m^3)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[d]	PCEs[b](%)	P Value[c]
Male								
Air[e]	0	6	0.32 ± 0.06		0.08 ± 0.03		1.829 ± 0.12	
CIMSTAR 3800	25	5	0.39 ± 0.05	0.4618	0.14 ± 0.03	0.7029	1.788 ± 0.14	1.0000
	50	5	0.42 ± 0.03	0.5440	0.12 ± 0.01	0.7029	1.973 ± 0.11	0.6911
	100	5	0.28 ± 0.05	0.5801	0.03 ± 0.00	1.0000	1.888 ± 0.14	0.7333
	200	5	0.21 ± 0.05	0.5982	0.04 ± 0.02	1.0000	2.130 ± 0.05	0.3089
	400	5	0.32 ± 0.07	0.6106	0.05 ± 0.01	1.0000	1.993 ± 0.19	0.3145
			P = 0.849[f]		P = 0.993[g]		P = 0.243[f]	
Female								
Air	0	5	0.30 ± 0.07		0.06 ± 0.02		0.931 ± 0.07	
CIMSTAR 3800	25	5	0.32 ± 0.10	0.7841	0.04 ± 0.01	0.9517	0.932 ± 0.06	0.9759
	50	5	0.28 ± 0.06	0.8602	0.02 ± 0.00	0.9792	0.994 ± 0.05	0.8457
	100	5	0.19 ± 0.03	0.8866	0.02 ± 0.01	0.9857	0.944 ± 0.09	0.8879
	200	5	0.17 ± 0.03	0.8985	0.06 ± 0.01	0.5357	1.019 ± 0.04	0.4927
	400	5	0.12 ± 0.04	0.9080	0.08 ± 0.01	0.1019	1.123 ± 0.11	0.1381
			P = 0.995[f]		P = 0.003[f]		P = 0.049[f]	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[114]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by Williams' test.
[d]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by Dunn's (males) or Williams' (females) test.
[e]Chamber control.
[f]Dose-related trend; significant at $P \leq 0.025$ by linear regression.
[g]Dose-related trend; significant at $P \leq 0.025$ by Jonckheere's test.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with CIMSTAR 3800 by Inhalation for Three Months[a]

	Dose (mg/m^3)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b](%)	P Value[d]
Male								
Air[e]	0	5	2.42 ± 0.15		1.31 ± 0.04		1.904 ± 0.09	
CIMSTAR 3800	25	6	1.89 ± 0.21	0.9222	1.38 ± 0.05	0.5601	3.133 ± 1.28	1.0000
	50	5	2.07 ± 0.16	0.9589	1.33 ± 0.04	0.6490	1.952 ± 0.07	1.0000
	100	5	2.10 ± 0.04	0.9697	1.27 ± 0.03	0.6825	1.548 ± 0.08	0.0745
	200	5	2.16 ± 0.16	0.9568	1.25 ± 0.02	0.6997	1.627 ± 0.04	0.2371
	400	5	2.19 ± 0.11	0.9455	1.26 ± 0.02	0.7101	1.617 ± 0.06	0.3264
			P = 0.385[f]		P = 0.981[f]		P = 0.003[g]	
Female								
Air	0	5	1.74 ± 0.11		0.98 ± 0.04		1.365 ± 0.08	
CIMSTAR 3800	25	5	1.63 ± 0.15	0.6130	0.91 ± 0.03	0.8893	1.834 ± 0.10	0.1029
	50	5	1.69 ± 0.15	0.6898	0.91 ± 0.02	0.9415	1.593 ± 0.16	0.1221
	100	5	1.70 ± 0.16	0.7032	0.91 ± 0.03	0.9561	1.422 ± 0.11	0.1298
	200	5	1.81 ± 0.15	0.6251	0.95 ± 0.02	0.9631	1.609 ± 0.10	0.1171
	400	5	1.68 ± 0.09	0.6372	0.90 ± 0.01	0.9677	1.758 ± 0.05	0.0132
			P = 0.452[f]		P = 0.766[f]		P = 0.145[f]	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[114]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by Williams' test.
[d]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by Dunn's (males) or Williams' (females) test.
[e]Chamber control.
[f]Dose-related trend; significant at $P \leq 0.025$ by linear regression.
[g]Dose-related trend; significant at $P \leq 0.025$ by Jonckheere's test.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for F344/NTac Rats in the Three-month Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	48.2 ± 0.2	47.3 ± 0.3	47.6 ± 0.4	47.4 ± 0.3	47.5 ± 0.3	48.1 ± 0.4
Packed cell volume (%)	46.7 ± 0.3	45.7 ± 0.4	46.3 ± 0.5	46.0 ± 0.4	45.9 ± 0.3	46.7 ± 0.3
Hemoglobin (g/dL)	15.3 ± 0.1	15.0 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.2 ± 0.1
Erythrocytes (10^6/μL)	9.49 ± 0.08	9.29 ± 0.04	9.38 ± 0.10	9.34 ± 0.09	9.34 ± 0.05	9.40 ± 0.06
Reticulocytes (10^3/μL)	248 ± 9	291 ± 7*	259 ± 11	242 ± 12	258 ± 7	258 ± 11
Nucleated erythrocytes (10^3/μL)	7.96 ± 0.30	7.48 ± 0.34	8.53 ± 0.43	8.01 ± 0.39	8.05 ± 0.19	8.75 ± 0.23
Nucleated erythrocytes/100 leukocytes	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.0 ± 0.0
Mean cell volume (fL)	49.2 ± 0.3	49.3 ± 0.3	49.3 ± 0.2	49.3 ± 0.2	49.2 ± 0.2	49.7 ± 0.3
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.2 ± 0.0	16.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.8 ± 0.1	32.6 ± 0.1	32.8 ± 0.1	32.8 ± 0.1	32.5 ± 0.1
Platelets (10^3/μL)	766 ± 11	745 ± 25	749 ± 18	727 ± 15	779 ± 17	814 ± 11
Leukocytes (10^3/μL)	7.96 ± 0.30	7.47 ± 0.34	8.52 ± 0.43	8.00 ± 0.38	8.03 ± 0.19	8.75 ± 0.23
Segmented neutrophils (10^3/μL)	1.68 ± 0.10	1.83 ± 0.10	1.77 ± 0.12	1.73 ± 0.15	1.91 ± 0.13	2.19 ± 0.14*
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)	5.89 ± 0.27	5.32 ± 0.31	6.43 ± 0.36	5.90 ± 0.38	5.85 ± 0.26	6.24 ± 0.26
Monocytes (10^3/μL)	0.25 ± 0.07	0.23 ± 0.05	0.17 ± 0.06	0.26 ± 0.08	0.14 ± 0.05	0.19 ± 0.06
Basophils (10^3/μL)	0.010 ± 0.004	0.004 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.005 ± 0.002	0.004 ± 0.002
Eosinophils (10^3/μL)	0.13 ± 0.01	0.09 ± 0.01	0.15 ± 0.03	0.11 ± 0.02	0.12 ± 0.02	0.12 ± 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)	15.5 ± 0.4	14.1 ± 0.4	13.7 ± 0.5*	15.0 ± 0.4	14.3 ± 0.3	14.1 ± 0.4
Creatinine (mg/dL)	0.39 ± 0.02	0.44 ± 0.02	0.39 ± 0.02	0.40 ± 0.03	0.38 ± 0.01	0.44 ± 0.02
Serum glucose (mg/dL)	132 ± 4	123 ± 3	123 ± 3	127 ± 3	120 ± 2*	116 ± 3**
Total protein (g/dL)	7.7 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.7 ± 0.1	7.7 ± 0.1
Albumin (g/dL)	4.9 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.9 ± 0.0	4.9 ± 0.1
Globulin (g/dL)	2.8 ± 0.0	2.8 ± 0.0	2.8 ± 0.1	2.8 ± 0.0	2.8 ± 0.0	2.8 ± 0.1
Albumin/ globulin ratio	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.7 ± 0.0
Alanine aminotransferase (IU/L)	110 ± 7	90 ± 3*	86 ± 3**	94 ± 7*	85 ± 6**	87 ± 8**
Alkaline phosphatase (IU/L)	272 ± 6	263 ± 4	253 ± 6	265 ± 8	266 ± 6	261 ± 8
Creatine kinase (IU/L)	292 ± 27	200 ± 18	247 ± 52	426 ± 113	234 ± 50	243 ± 24
Sorbitol dehydrogenase (IU/L)	27 ± 2	20 ± 1**	19 ± 1**	19 ± 2**	15 ± 1**	15 ± 1**
Bile acids (μmol/L)	5.6 ± 0.6	4.8 ± 0.3	5.5 ± 0.3	4.2 ± 0.2	4.6 ± 0.2	3.9 ± 0.2**

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Female						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	47.4 ± 0.4	47.4 ± 0.3	46.8 ± 0.4	47.0 ± 0.4	47.3 ± 0.4	46.6 ± 0.3
Packed cell volume (%)	47.1 ± 0.4	47.1 ± 0.4	46.5 ± 0.4	46.3 ± 0.4	46.8 ± 0.5	46.4 ± 0.3
Hemoglobin (g/dL)	15.5 ± 0.1	15.5 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.4 ± 0.1	15.1 ± 0.1
Erythrocytes (10^6/μL)	8.81 ± 0.06	8.79 ± 0.07	8.74 ± 0.08	8.74 ± 0.06	8.75 ± 0.08	8.66 ± 0.06
Reticulocytes (10^3/μL)	203 ± 11	216 ± 11	221 ± 12	217 ± 12	228 ± 11	236 ± 4
Nucleated erythrocytes (10^3/μL)	8.87 ± 0.50	9.64 ± 0.76	8.14 ± 0.45	9.69 ± 0.47	9.48 ± 0.37	9.29 ± 0.55
Nucleated erythrocytes/100 leukocytes	0.1 ± 0.1	0.8 ± 0.2*	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.2
Mean cell volume (fL)	53.5 ± 0.2	53.6 ± 0.3	53.2 ± 0.2	52.9 ± 0.2	53.4 ± 0.3	53.6 ± 0.3
Mean cell hemoglobin (pg)	17.6 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.4 ± 0.1	17.5 ± 0.1	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.9 ± 0.1	32.8 ± 0.1	32.9 ± 0.1	32.8 ± 0.1	32.8 ± 0.1	32.7 ± 0.1
Platelets (10^3/μL)	738 ± 12	758 ± 15	750 ± 13	742 ± 15	779 ± 12	786 ± 22
Leukocytes (10^3/μL)	8.86 ± 0.50	9.57 ± 0.76	8.12 ± 0.45	9.67 ± 0.47	9.47 ± 0.37	9.24 ± 0.56
Segmented neutrophils (10^3/μL)	1.73 ± 0.10	2.00 ± 0.18	1.68 ± 0.09	1.90 ± 0.11	1.67 ± 0.11	1.46 ± 0.10
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)	6.77 ± 0.45	7.30 ± 0.62	6.17 ± 0.39	7.54 ± 0.43	7.50 ± 0.28	7.46 ± 0.47
Monocytes (10^3/μL)	0.24 ± 0.08	0.13 ± 0.02	0.16 ± 0.06	0.11 ± 0.02	0.16 ± 0.06	0.19 ± 0.05
Basophils (10^3/μL)	0.005 ± 0.003	0.001 ± 0.001	0.001 ± 0.001	0.003 ± 0.002	0.001 ± 0.001	0.001 ± 0.001
Eosinophils (10^3/μL)	0.12 ± 0.02	0.15 ± 0.02	0.11 ± 0.01	0.12 ± 0.03	0.14 ± 0.03	0.12 ± 0.03
Clinical Chemistry						
Urea nitrogen (mg/dL)	15.0 ± 0.4	14.3 ± 0.3	15.1 ± 0.5	16.1 ± 0.3	15.9 ± 0.3	14.6 ± 0.5
Creatinine (mg/dL)	0.44 ± 0.02	0.46 ± 0.02	0.44 ± 0.02	0.41 ± 0.02	0.41 ± 0.02	0.42 ± 0.02
Serum glucose (mg/dL)	129 ± 4	128 ± 3	132 ± 5	125 ± 5	128 ± 3	130 ± 5
Total protein (g/dL)	7.8 ± 0.1	7.9 ± 0.1	7.8 ± 0.1	7.6 ± 0.1	7.7 ± 0.1	7.7 ± 0.1
Albumin (g/dL)	5.2 ± 0.1	5.2 ± 0.0	5.2 ± 0.1	5.0 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
Globulin (g/dL)	2.7 ± 0.0	2.7 ± 0.0	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.0	2.5 ± 0.1
Albumin/globulin ratio	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.0*
Alanine aminotransferase (IU/L)	68 ± 4	82 ± 5	67 ± 5	62 ± 3	84 ± 4	77 ± 3
Alkaline phosphatase (IU/L)	238 ± 7	217 ± 9	241 ± 6	234 ± 6	224 ± 7	230 ± 8
Creatine kinase (IU/L)	226 ± 20	157 ± 6	180 ± 23	193 ± 19	216 ± 20	204 ± 21
Sorbitol dehydrogenase (IU/L)	20 ± 1	19 ± 1	17 ± 1	15 ± 1**	15 ± 0**	14 ± 1**
Bile acids (μmol/L)	8.1 ± 0.7	6.6 ± 0.4	6.5 ± 0.5	6.6 ± 1.4*	6.2 ± 0.5	6.0 ± 0.4

*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]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Table F-2. Hematology Data for Mice in the Three-month Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
n	10	8	10	10	9	7
Hematocrit (%)	49.7 ± 0.7	50.2 ± 0.9	50.0 ± 0.7	50.6 ± 0.4	51.2 ± 0.5	48.4 ± 0.8
Packed cell volume (%)	50.6 ± 0.6	50.9 ± 0.7	51.2 ± 0.7	51.1 ± 0.5	51.8 ± 0.8	49.7 ± 0.9
Hemoglobin (g/dL)	15.7 ± 0.2	15.7 ± 0.3	15.9 ± 0.2	16.0 ± 0.1	16.0 ± 0.3	15.4 ± 0.3
Erythrocytes (10^6/µL)	10.39 ± 0.10	10.19 ± 0.23	10.41 ± 0.15	10.49 ± 0.11	10.52 ± 0.20	10.31 ± 0.17
Reticulocytes (10^3/µL)	242 ± 14	266 ± 16	253 ± 22	261 ± 15	258 ± 18	274 ± 8
Nucleated erythrocytes (10^3/µL)	2.03 ± 0.35	2.27 ± 0.35	2.17 ± 0.24	2.42 ± 0.50	2.39 ± 0.33	1.77 ± 0.23
Nucleated erythrocytes/ 100 leukocytes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Mean cell volume (fL)	48.7 ± 0.2	50.0 ± 0.7	49.2 ± 0.3	48.7 ± 0.2	49.3 ± 0.5	48.2 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.4 ± 0.2	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.0 ± 0.1	30.8 ± 0.2	31.1 ± 0.1	31.3 ± 0.1	30.9 ± 0.2	31.0 ± 0.2
Platelets (10^3/µL)	848 ± 17	819 ± 57	814 ± 16	844 ± 15	867 ± 17	815 ± 11
Leukocytes (10^3/µL)	2.03 ± 0.35	2.27 ± 0.35	2.17 ± 0.24	2.42 ± 0.50	2.39 ± 0.33	1.77 ± 0.23
Segmented neutrophils (10^3/µL)	0.38 ± 0.10	0.25 ± 0.04	0.26 ± 0.04	0.34 ± 0.09	0.24 ± 0.03	0.21 ± 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)	1.56 ± 0.26	1.94 ± 0.31	1.85 ± 0.21	2.02 ± 0.41	2.07 ± 0.31	1.49 ± 0.24
Monocytes (10^3/µL)	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02
Basophils (10^3/µL)	0.008 ± 0.002	0.016 ± 0.012	0.010 ± 0.005	0.007 ± 0.002	0.008 ± 0.002	0.004 ± 0.002
Eosinophils (10^3/µL)	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.02 ± 0.01
Female						
n	10	10	10	10	10	10
Hematocrit (%)	51.2 ± 0.2	51.9 ± 0.4	51.9 ± 0.4	50.7 ± 0.4	51.0 ± 0.4	49.9 ± 0.4
Packed cell volume (%)	52.1 ± 0.3	52.7 ± 0.4	52.3 ± 0.4	51.6 ± 0.4	51.4 ± 0.4	50.6 ± 0.4*
Hemoglobin (g/dL)	16.4 ± 0.1	16.6 ± 0.1	16.5 ± 0.1	16.2 ± 0.1	16.3 ± 0.1	15.9 ± 0.1**
Erythrocytes (10^6/µL)	10.50 ± 0.09	10.65 ± 0.07	10.57 ± 0.09	10.42 ± 0.08	10.55 ± 0.08	10.34 ± 0.06
Reticulocytes (10^3/µL)	270 ± 8	291 ± 13	285 ± 9	278 ± 9	288 ± 9	276 ± 16
Nucleated erythrocytes (10^3/µL)	2.78 ± 0.22	3.00 ± 0.34	3.04 ± 0.39	2.43 ± 0.28	3.15 ± 0.69	2.52 ± 0.24
Nucleated erythrocytes/ 100 leukocytes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Mean cell volume (fL)	49.7 ± 0.3	49.5 ± 0.2	49.5 ± 0.2	49.6 ± 0.2	48.8 ± 0.2**	49.0 ± 0.2*
Mean cell hemoglobin (pg)	15.6 ± 0.1	15.6 ± 0.0	15.7 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.4 ± 0.0

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Mean cell hemoglobin concentration (g/dL)	31.4 ± 0.1	31.5 ± 0.1	31.7 ± 0.1	31.3 ± 0.2	31.7 ± 0.2	31.5 ± 0.2
Platelets (10^3/µL)	776 ± 20	796 ± 12	788 ± 13	792 ± 18	773 ± 15	754 ± 15
Leukocytes (10^3/µL)	2.78 ± 0.22	2.98 ± 0.34	3.04 ± 0.39	2.43 ± 0.28	3.15 ± 0.69	2.52 ± 0.24
Segmented neutrophils (10^3/µL)	0.36 ± 0.05	0.30 ± 0.04	0.28 ± 0.04	0.28 ± 0.05	0.21 ± 0.02	0.24 ± 0.03
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)	2.34 ± 0.19	2.55 ± 0.30	2.68 ± 0.37	2.10 ± 0.23	2.89 ± 0.66	2.22 ± 0.21
Monocytes (10^3/µL)	0.04 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.019 ± 0.007	0.025 ± 0.009	0.013 ± 0.003	0.008 ± 0.002	0.008 ± 0.003	0.010 ± 0.002
Eosinophils (10^3/µL)	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.01

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

Appendix G. Organ Weights and Organ-Weight-To-Body-Weight Ratios

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/NTac Rats in the Three-month Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	349 ± 6	353 ± 9	354 ± 7	354 ± 7	352 ± 5	350 ± 6
Heart						
Absolute	0.99 ± 0.02	0.98 ± 0.03	1.00 ± 0.02	0.99 ± 0.02	0.97 ± 0.01	0.98 ± 0.02
Relative	2.83 ± 0.03	2.78 ± 0.03	2.81 ± 0.03	2.80 ± 0.02	2.76 ± 0.03	2.81 ± 0.02
R. Kidney						
Absolute	1.07 ± 0.02	1.09 ± 0.03	1.09 ± 0.03	1.13 ± 0.03	1.07 ± 0.01	1.10 ± 0.03
Relative	3.07 ± 0.03	3.09 ± 0.04	3.08 ± 0.04	3.18 ± 0.05	3.04 ± 0.03	3.14 ± 0.04
Liver						
Absolute	11.55 ± 0.31	11.54 ± 0.39	11.71 ± 0.33	12.23 ± 0.40	11.68 ± 0.20	11.82 ± 0.29
Relative	33.08 ± 0.41	32.61 ± 0.39	32.99 ± 0.41	34.44 ± 0.57	33.21 ± 0.24	33.73 ± 0.51
Lung						
Absolute	1.89 ± 0.06	1.93 ± 0.08	2.06 ± 0.09	1.95 ± 0.08	1.87 ± 0.04	1.94 ± 0.05
Relative	5.42 ± 0.19	5.46 ± 0.16	5.79 ± 0.20	5.49 ± 0.15	5.32 ± 0.08	5.53 ± 0.10
Spleen						
Absolute	0.641 ± 0.012	0.670 ± 0.018	0.658 ± 0.012	0.690 ± 0.015	0.652 ± 0.012	0.672 ± 0.017
Relative	1.84 ± 0.03	1.90 ± 0.02	1.86 ± 0.01	1.95 ± 0.03*	1.85 ± 0.02	1.92 ± 0.03
R. Testis						
Absolute	1.414 ± 0.023	1.444 ± 0.027	1.440 ± 0.021	1.424 ± 0.026	1.417 ± 0.017	1.411 ± 0.024
Relative	4.059 ± 0.061	4.103 ± 0.095	4.070 ± 0.069	4.023 ± 0.061	4.035 ± 0.058	4.030 ± 0.048
Thymus						
Absolute	0.327 ± 0.006	0.337 ± 0.015	0.349 ± 0.011	0.325 ± 0.015	0.347 ± 0.016	0.341 ± 0.014
Relative	0.938 ± 0.016	0.954 ± 0.039	0.985 ± 0.026	0.918 ± 0.037	0.989 ± 0.047	0.974 ± 0.039
Female						
Necropsy body wt	188 ± 5	190 ± 5	195 ± 5	189 ± 5	185 ± 5	184 ± 4
Heart						
Absolute	0.62 ± 0.02	0.62 ± 0.02	0.64 ± 0.01	0.62 ± 0.02	0.61 ± 0.01	0.61 ± 0.01
Relative	3.28 ± 0.05	3.26 ± 0.04	3.29 ± 0.05	3.30 ± 0.05	3.32 ± 0.05	3.34 ± 0.05
R. Kidney						
Absolute	0.61 ± 0.02	0.63 ± 0.02	0.65 ± 0.01	0.62 ± 0.02	0.62 ± 0.01	0.62 ± 0.02
Relative	3.25 ± 0.07	3.33 ± 0.05	3.33 ± 0.06	3.29 ± 0.06	3.34 ± 0.07	3.35 ± 0.04

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Liver						
Absolute	6.03 ± 0.10	5.97 ± 0.20	6.25 ± 0.14	5.98 ± 0.19	6.13 ± 0.20	6.03 ± 0.16
Relative	32.20 ± 0.72	31.39 ± 0.41	32.11 ± 0.64	31.63 ± 0.65	33.11 ± 0.41	32.81 ± 0.71
Lung						
Absolute	1.21 ± 0.05	1.23 ± 0.05	1.26 ± 0.04	1.21 ± 0.04	1.16 ± 0.04	1.18 ± 0.03
Relative	6.39 ± 0.11	6.47 ± 0.14	6.49 ± 0.16	6.41 ± 0.09	6.24 ± 0.09	6.41 ± 0.09
Spleen						
Absolute	0.424 ± 0.010	0.453 ± 0.021	0.445 ± 0.012	0.431 ± 0.010	0.451 ± 0.009	0.450 ± 0.010
Relative	2.27 ± 0.07	2.38 ± 0.06	2.29 ± 0.06	2.28 ± 0.02	2.45 ± 0.05	2.45 ± 0.06
Thymus						
Absolute	0.238 ± 0.009	0.246 ± 0.007	0.280 ± 0.015*	0.256 ± 0.010	0.248 ± 0.008	0.250 ± 0.009
Relative	1.268 ± 0.048	1.300 ± 0.040	1.430 ± 0.058	1.357 ± 0.044	1.347 ± 0.047	1.360 ± 0.031

*Significantly different ($P \leq 0.05$) from the chamber control group by 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 G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of CIMSTAR 3800[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	39.5 ± 0.9	38.1 ± 1.3	38.6 ± 0.7	37.4 ± 0.6	37.8 ± 0.6	32.9 ± 0.9**
Heart						
Absolute	0.17 ± 0.01	0.18 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Relative	4.29 ± 0.11	4.73 ± 0.16	4.33 ± 0.18	4.62 ± 0.20	4.48 ± 0.17	4.86 ± 0.14
R. Kidney						
Absolute	0.32 ± 0.01	0.34 ± 0.00	0.32 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
Relative	8.21 ± 0.16	8.88 ± 0.31	8.44 ± 0.31	8.84 ± 0.22	8.19 ± 0.24	9.05 ± 0.20
Liver						
Absolute	1.70 ± 0.06	1.69 ± 0.05	1.61 ± 0.04	1.67 ± 0.06	1.59 ± 0.05	1.52 ± 0.07*
Relative	43.08 ± 0.95	44.55 ± 1.03	41.67 ± 0.58	44.69 ± 1.38	42.11 ± 1.15	46.22 ± 1.54
Lung						
Absolute	0.21 ± 0.01	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.01*	0.24 ± 0.01*	0.23 ± 0.01*
Relative	5.23 ± 0.14	6.28 ± 0.47	5.62 ± 0.10	6.28 ± 0.21**	6.27 ± 0.16**	7.04 ± 0.21**
Spleen						
Absolute	0.077 ± 0.003	0.086 ± 0.007	0.074 ± 0.002	0.075 ± 0.002	0.072 ± 0.003	0.065 ± 0.002*
Relative	1.95 ± 0.05	2.28 ± 0.21	1.92 ± 0.05	2.02 ± 0.08	1.92 ± 0.10	1.97 ± 0.05
R. Testis						
Absolute	0.115 ± 0.001	0.112 ± 0.002	0.108 ± 0.009	0.112 ± 0.003	0.114 ± 0.002	0.110 ± 0.003
Relative	2.921 ± 0.050	2.958 ± 0.089	2.808 ± 0.248	3.001 ± 0.056	3.010 ± 0.061	3.360 ± 0.076*
Thymus						
Absolute	0.054 ± 0.002	0.047 ± 0.002	0.051 ± 0.003	0.044 ± 0.002	0.052 ± 0.002	0.039 ± 0.003**
Relative	1.362 ± 0.049	1.241 ± 0.051	1.316 ± 0.068	1.185 ± 0.059	1.378 ± 0.064	1.169 ± 0.073
Female						
Necropsy body wt	33.0 ± 1.2	31.6 ± 0.7	31.8 ± 0.8	31.9 ± 0.7	30.5 ± 0.8*	28.1 ± 0.5**
Heart						
Absolute	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00
Relative	4.38 ± 0.18	4.67 ± 0.14	4.71 ± 0.14	4.46 ± 0.13	4.54 ± 0.12	4.84 ± 0.08
R. Kidney						
Absolute	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.00	0.21 ± 0.01	0.20 ± 0.01
Relative	6.45 ± 0.21	6.32 ± 0.25	6.56 ± 0.23	6.50 ± 0.12	6.83 ± 0.19	7.15 ± 0.23

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Liver						
Absolute	1.43 ± 0.05	1.38 ± 0.04	1.46 ± 0.05	1.39 ± 0.04	1.37 ± 0.04	1.29 ± 0.04
Relative	43.26 ± 0.74	43.84 ± 0.83	45.82 ± 1.12	43.51 ± 1.00	44.99 ± 0.87	45.80 ± 1.00
Lung						
Absolute	0.21 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.23 ± 0.00
Relative	6.46 ± 0.18	7.36 ± 0.50	6.83 ± 0.18	6.88 ± 0.16	7.59 ± 0.24**	8.26 ± 0.15**
Spleen						
Absolute	0.097 ± 0.003	0.095 ± 0.002	0.101 ± 0.004	0.091 ± 0.003	0.094 ± 0.004	0.082 ± 0.004**
Relative	2.97 ± 0.13	3.02 ± 0.09	3.18 ± 0.11	2.85 ± 0.06	3.09 ± 0.12	2.91 ± 0.12
Thymus						
Absolute	0.050 ± 0.004	0.053 ± 0.003	0.051 ± 0.002	0.053 ± 0.003	0.050 ± 0.002	0.043 ± 0.003
Relative	1.514 ± 0.094	1.689 ± 0.101	1.622 ± 0.068	1.672 ± 0.076	1.653 ± 0.060	1.523 ± 0.096

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[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).

Appendix H. Chemical Characterization and Generation of Chamber Concentrations

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H.1. Procurement and Characterization of CIMSTAR 3800

CIMSTAR 3800 was obtained from Milacron (Cincinnati, OH) in three lots (60224BBN, 71205BN, and 90317JN). Lot 60224BBN was used during the 3-month studies, and lots 71205BN and 90317JN were used during the 2-year studies. Characterization and stability analyses of the test material were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the CIMSTAR 3800 studies are on file at the National Institute of Environmental Health Sciences.

Spectra of lots 60224BBN, 71205BN, and 90317JN of the test material, a yellow-orange liquid, were obtained by the analytical chemistry laboratory using Fourier transform infrared (FTIR) spectroscopy. FTIR spectroscopy was used to determine a fingerprint which was used to estimate the relative presence of various functional groups and create a reference for future comparison for the same lot or different lots. In general, the spectra were consistent with the presence of organic amines and spectra of the three lots were similar to each other; a representative FTIR spectrum is presented in Figure H-1.

Analyses for all lots performed by the analytical chemistry laboratory included Karl Fischer titration for water content; the determination of pH, specific gravity and refractive index; elemental analysis for carbon, hydrogen, nitrogen and sulfur using a C, H, N, S analyzer; elemental and metal analysis using inductively coupled plasma/optical emission spectrometry (ICP/OES); chloride, nitrate, and nitrite analysis by ion chromatography (IC); and iodide by ion specific electrode (ISE) titration (Fisher Accumet Research AR25 pH/mV/Ion Meter, Fisher Scientific, Pittsburgh, PA). The study laboratory determined the initial identification of general organic components using gas chromatography (GC) with flame ionization detection (FID) by system A and the identification of the major components using GC with mass spectrometry (MS) by system B (lot 60224BBN) (Table H-1). The identification and quantitation of alkanolamines were determined using high performance liquid chromatography (HPLC) with MS detection by systems A (lot 60224BBN) or B (lots 71205BN, and 90317JN) (Table H-2). The quantitation of methanol was determined using GC/FID by a system similar to system A (lot 60224BBN) or GC/MS by system C (lots 71205BN and 90317JN) (Table H-1). The assessment of fatty acid methyl esters was determined using GC/FID by system D. The total amount of hexane extractable material was determined using United States Environmental Protection Agency Method #1664[117], and the amounts of bacteria and fungi were determined using a Sani-Check BF test kit (Biosan Laboratories, Warren, MI); samples were diluted to 10% with sterile water and applied to paddles coated on one side with media for the determination of bacteria and on the other side for the determination of fungi, then incubated for 7 days at 25° to 30°C.

I) For ICP/OES (Perkin-Elmer, Waltham, MA) analysis, samples were weighed into clean, acid-rinsed volumetric flasks, and analyzed. In ICP analysis, high frequency energy transferred by inductive coupling to a flow of inert gas containing the sample as an aerosol causes vaporization, exciting the free atoms to emit light; the intensity of this light is related to the concentration of the emitting atoms.

II) For IC analysis, chloride, nitrate, and nitrite were extracted from samples using ethyl ether at a high pH, excess base was neutralized with a strong cation exchanger type acid from Dowex DR-2-30, and analyzed using aqueous buffers to gradually elute ions of

interest separated in an ion exchange column with conductivity detection (Dionex, Thermo Fisher Scientific, Inc., Waltham, MA).

For lot 60224BBN, the water content was 60%, pH was 9.1, specific gravity was 1.025 at 60°F, and refractive index was 1.400. Metal analysis by ICP/OES indicated boron at 0.8% with trace amounts of phosphorous, tin, and thallium; elemental analysis indicated 29.8% carbon, 10.5% hydrogen, 1.4% nitrogen, and 0.09% sulfur; IC results indicated that chloride, nitrate, and nitrite were less than 0.01%; and ISE titration indicated 26 ppm iodide. GC/FID analysis for alkanolamines indicated six separate peaks and a cluster of peaks. Using standard addition and GC/MS, the peaks were identified as methanol (0.3% by weight), ethanolamine, oxazolidine compound (tentative identification, no standard available), 1-amino-2-propanol, 5-methyloxazolidine (tentative identification, no standard available), triethanolamine, and the cluster of peaks as mineral oils (mostly fatty acid methyl esters and aliphatics). Quantitation of the major alkanolamimes using HPLC/MS indicated 4.7% ethanolamine, 1.8% 1-amino-2-propanol, and 3.4% triethanolamine by weight, representing approximately 10% of the test material mass. Quantitation of the mineral oils using GC/FID indicated 0.24% methyl palmitate, 0.71% methyl stearate, 2.6% methyl oleate, and 1.68% methyl linoleate. The total amount of hexane extractable materials was determined to be approximately 26% by weight. The amount of bacteria was less than 100 colony forming units (CFU)/mL and the amount of fungi was less than 10 CFU/mL.

For lot 71205BN, the water content was 60%, pH was 9.0, specific gravity was 1.025, and refractive index was 1.400. Metal analysis by ICP/OES indicated boron at 0.6% with trace amounts of calcium and tin; elemental analysis indicated 26.9% carbon, 10.0% hydrogen, 1.4% nitrogen, and 0.20% sulfur; IC results indicated that chloride, nitrate, and nitrite were less than 0.01%; and ISE titration indicated less than 5 ppm iodide. GC/FID analysis for alkanolamines indicated six peaks plus a cluster of peaks. GC/MS indicated 0.3% methanol by weight. Quantitation of the major alkanolamimes using HPLC/MS indicated 5.7% ethanolamine, 1.4% 1-amino-2-propanol, and 3.3% triethanolamine by weight, representing approximately 10% of the test material mass. Quantitation of the mineral oils using GC/FID indicated 0.21% methyl palmitate, 0.77% methyl stearate, 2.26% methyl oleate, and 1.54% methyl linoleate by weight. The total amount of hexane extractable materials was approximately 25% by weight. The amount of bacteria was less than 1,000 CFU/mL and the amount of fungi was less than 100 CFU/mL.

For lot 90317JN, the water content was 60%, pH was 9.0, specific gravity was 1.026, and refractive index was 1.408. ICP/OES indicated boron at 0.9%; elemental analysis indicated 28.4% carbon, 10.4% hydrogen, 2.3% nitrogen, and 0.10% sulfur; IC results indicated that chloride, nitrate, and nitrite were less than 0.01%; and ISE titration indicated less than 0.05% iodide. GC/MS indicated 0.35% methanol by weight. Quantitation of the major alkanolamimes using HPLC/MS indicated 5.4% ethanolamine, 1.6% 1-amino-2-propanol, and 3.2% triethanolamine, representing approximately 10% of the test material mass. Quantitation of the mineral oils using GC/FID indicated 0.20% methyl palmitate, 0.78% methyl stearate, 2.66% methyl oleate, and 1.77% methyl linoleate. The total amount of hexane extractable materials was approximately 26% by weight. The amount of bacteria was less than 1,000 CFU/mL and the amount of fungi was less than 100 CFU/mL.

The test material was determined to be primarily composed of water, alkanolamines, and oil. Boron was present at approximately 0.6%. The material was basic with a pH of approximately

9.0. In general, the FTIR spectra were consistent with the presence of organic amines, and contained less than 0.01% of water soluble nitrates, nitrites, or chlorides. The mass balance percentages based on elemental and compound contributions resulted in 96% and 96% (lot 60224BBN) and 92% and 96% (lot 71205BN) coverage, respectively. There were no significant differences between the three lots. Taken together, these data indicate that all three lots of the test material were CIMSTAR 3800 with the expected composition of organic amines (Table H-3).

Periodic reanalyses of lots 71205BN and 90317JN were performed by the analytical chemistry laboratory and the study laboratory at least every 6 months during the 2-year studies. For each lot, FTIR spectra were obtained and compared to reference spectra of the same lot and other lots; determinations were made for the pH, specific gravity, and refractive index; determination and quantitation of alkanolamines was performed using HPLC/MS by system B (Table H-2); assessment of mineral oils (fatty acid methyl esters) was performed using GC/FID by system D (Table H-1); and the amounts of bacteria and fungi were determined using a Sani-Check BF test kit. To ensure stability, the bulk test material was stored at approximately 63°F, protected from light, in metal drums, and no degradation of the bulk test material was detected.

H.2. Aerosol Generation and Exposure System

The generation and delivery system used in the 3-month and 2-year studies consisted of two generator assemblies configured together so that the output from each assembly was directed to a common distribution line (Figure H-2). Each assembly contained up to three multi-jet nebulizers, of which two were operational and the third was a backup. The bottom of the generator assembly contained the liquid reservoir, the sides of which were surrounded by a band heater that was maintained at a temperature sufficient to keep the liquid test material at room temperature during the aerosolization process. CIMSTAR 3800 was continuously pumped to the liquid reservoir from the chemical cabinet reservoir by metering pumps during the aerosol generation process to ensure a fresh supply of test material and pumping rates that exceeded the rates at which aerosol was removed from the generator assemblies. A constant volume of test material was maintained in each assembly by a siphon tube inserted near the top of the assembly and connected to a vacuum source; excess liquid was removed to a waste container. Ports in the generator assembly introduced heated compressed air to drive the nebulizers and heated dilution air to transport aerosol to the distribution line.

Each nebulizer assembly consisted of a multi-jet thimble nebulizer, a liquid uptake tube, and a compressed air supply port. High velocity compressed air created a vacuum in the liquid uptake tube that drew test material from the liquid reservoir into the multi-jet nebulizer streams where shear force broke the resultant liquid filaments into droplets. Large droplets were impacted on the impaction plate of the nebulizer or the generator assembly walls and were returned to the liquid reservoir. Smaller droplets were drawn into the heated dilution air and transported to the common distribution line made of bonded stainless steel, grounded to prevent electrostatic charge buildup. The common distribution line was divided into two branches to supply aerosol to exposure chambers located on both sides of the exposure room; each branch line was insulated and terminated in a filter protecting the flowmeter controlling the line via the house vacuum supply. A second distribution line flow control system used during nonexposure periods consisted of a HEPA filter protecting the airvac pump that created a vacuum in the distribution lines that exceeded the pressures in the chambers, creating a minimal backflow from each

chamber inlet tee ensuring that material did not migrate into the chambers during off exposure periods.

During exposures, at each chamber position, aerosol was removed from the distribution line and injected into a tee fitting where it was directed either to the inlet of the exposure chamber where it was mixed with conditioned air or to siphon flow exhaust. Conditioned air was defined as the mix of air derived from each exposure chamber's wet and dry air duct supplies. The temperature of the resultant mixture of air was adjusted by passage over a temperature controlled radiator after treatment with Purafil, charcoal, and HEPA filters. Target dewpoint temperature of the wet duct was 60°F and 40°F for the dry duct. Air for the ducts was obtained from the building air supply and was either passed over chillers to lower the dewpoint (dry duct) or injected with steam to raise it (wet duct). The amount of aerosol removed from the distribution line was controlled by a control orifice and siphon flow rotameter. Minor adjustments to chamber concentrations were performed by changing the amount of aerosol drawn off through a HEPA-filter protected siphon flow rotameter.

H.3. Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table H-4and Table H-5. The concentration of boron in CIMSTAR 3800 was monitored using real-time aerosol monitors (RAMs) (MicroDust *pro*, Casella CEL LTD; Kempson; Bedford, England). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m^3 chamber or the room, a HEPA-filtered room air blank, and two exposure chambers. The output voltage of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Concentration control limits within the program were compared to each measured concentration and, if limits were exceeded, an audible alarm was triggered or, in extreme cases, exposure was terminated.

Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and boron in CIMSTAR 3800 concentrations that were determined by analyzing tandem Tissuquartz 2500QAT-UP or Tissuquartz 2500QAO-UP filters (Pall Corporation, East Hills, NY) collected daily from the exposure chambers.

For the 3-month studies and from April to July 24, 2008, in the 2-year studies, boron in CIMSTAR 3800 was extracted from the filters with approximately 0.25% cesium chloride and shaken on an orbital shaker table, filtered using an Acrodisc syringe filter with a polytetrafluoroethylene membrane, 25 mm, 0.45 µm (Pall Corporation), and analyzed on a Thermo Elemental IRIS Intrepid Inductively Coupled Plasma-Optical Emission Spectrometer (ICP/OES) (Thermo Elemental, Waltham, MA). The results were normalized against certified commercial standards of boron and yttrium as an internal standard obtained from the National Institute of Standards and Technology (NIST).

The ICP/OES instrument was calibrated against serially diluted 10 or 100 µg CIMSTAR 3800/mL containing a NIST-traceable spectrometric internal standard

(2 mg yttrium/mL). Quality control standards and a reagent blank were analyzed after calibration, after approximately every tenth sample, and at the end of the analysis to determine accuracy and calibration drift during analysis.

After July 24, 2008, the calibration of the RAMs was based on gravimetric determination of the amount of test material on the filters to the end of the studies. Samples were acidified with hydrochloric acid, extracted three times with *n*-hexane, dried over sodium sulfate, solvent evaporated, desiccated, and weighed.

H.4. Chamber Atmosphere Characterization

Particle size distribution in each chamber was determined prior to the start of all studies and monthly during the studies. Impactor samples were taken from each exposure chamber using a Mercer-style seven-stage impactor (In-Tox Products, Moriarty, NM) and the stages were initially collected on glass coverslips lightly coated with silicone to prevent particle bounce (stages 1-7) or filters (stage 8) and were analyzed by ICP/OES for boron in CIMSTAR 3800 for the 3-month studies and from April 2008 until July 23, 2008, for the 2-year studies. However, for the remainder of the 2-year studies, gravimetric samples were collected using stainless steel coverslips (stages 1-7) or glass fiber filters coated with Teflon® (stage 8) and analyzed gravimetrically as described previously. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis[65]. The resulting estimates of the mass median aerodynamic diameter and the geometric standard deviation of each set of samples are given in Table H-6, Table H-7, and Table H-8. All samples were within the 1 to 3 µm range required by protocol.

Buildup and decay rates for chamber aerosol concentrations were determined with and without animals present in the chambers. At a chamber air flow rate of 15 cubic feet per minute, the theoretical values for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) was approximately 9.4 minutes for the 3-month and 2-year studies. For rat chambers in the 3-month studies, T_{90} values ranged from 9 to 11 minutes and T_{10} values ranged from 8 to 9 minutes without animals present; for rat/mouse chambers, T_{90} values ranged from 9 to 11 minutes and T_{10} values ranged from 7 to 8 minutes without animals present. For rats and mice in the 3-month studies, T_{90} values ranged from 13 to 17 minutes and T_{10} values ranged from 9 to 10 minutes with animals present.

For rats in the 2-year study, T_{90} values for males ranged from 6.0 to 13.8 minutes and T_{10} values ranged from 6.2 to 9.3 minutes with animals present; T_{90} values for females ranged from 8.3 to 15.9 and T_{10} values ranged from 8.1 to 9.2 minutes with animals present. For mice in the 2-year study, T_{90} values ranged from 8.3 to 15.9 minutes and T_{10} values ranged from 8.1 to 9.2 minutes with animals present. A summary for all exposure chambers with and without animals was made during the 2-year studies. T_{90} values for rats ranged from 6.3 to 15.2 minutes and T_{10} values ranged from 8.8 to 9.2 minutes. For rat/mouse chambers, T_{90} values ranged from 6.3 to 8.4 minutes and T_{10} values ranged from 8.2 to 8.4 minutes. T_{90} values of 18 minutes and 12 minutes were selected for the 3-month and 2-year studies, respectively.

The uniformity of aerosol concentration in the inhalation exposure chambers with animals present was measured once during the 3-month studies and was evaluated before the 2-year studies began without animals present and every 3 months during the 2-year studies. RAM

measurements were taken from 12 different chamber positions. Chamber concentration uniformity was acceptable throughout the 3-month and 2-year studies.

The persistence of CIMSTAR 3800 in the exposure chambers was monitored after aerosol delivery ended by monitoring the concentration overnight in the 400 mg/m^3 rat and mouse chambers during the 3-month studies and the 100 mg/m^3 rat and mouse chambers during the 2-year studies with and without animals present. The average CIMSTAR 3800 concentration decreased to 1% of the target concentration within 18 (rats and mice, 3-month studies), 14 (male rats, 2-year studies), 16 (female rats and mice, 2-year studies), or 15 (2-year studies without animals) minutes.

Stability studies of the test material in the generation and exposure system were performed before and during the studies by the study laboratory and the analytical chemistry laboratory. Samples of the test atmosphere were taken in the first, middle, and last 2 hours of the generation day from the distribution line, 25 and 400 mg/m^3 chambers (3-month studies), 10 and 100 mg/m^3 chambers (2-year studies), and from the generator reservoir at the end of the generation day. Samples were collected using adsorbent gas sampling tubes (ORBO-52, silica gel, Supelco, Bellefonte, PA) for the determination of fatty acid methyl esters using GC/FID by system D (Table H-1). Samples collected on filters (Tissuquartz 2500QAT-UP) were analyzed using ICP/OES for boron, the filter was followed by a bubbler containing a cesium chloride solution. Samples collected using bubblers filled with water were analyzed for methanol using GC/MS by system C (3-month studies). Samples from the generator reservoir and samples collected in bubblers filled with 10 mM nonafluoropentanoic acid and 2% acetonitrile in water were used to analyze for alkanolamines using HPLC/MS by systems A (lot 60224BBN) or B (lots 71205BN and 90317JN) (Table H-2). In addition, samples from the generator reservoir were also analyzed for fatty acid methyl esters, boron, and alkanolamines. The amount of each constituent in the exposure atmosphere was calculated as a percentage of the expected amount based on concentration as determined by the on-line monitor. For the distribution line, the amount of each constituent was calculated as a percentage of the expected amount based on the gravimetric determination. Reservoir results were calculated relative to the test material.

For the 3-month and the 2-year stability studies without animals present in the exposure chambers, the relative amounts of the major constituents in the exposure atmosphere generally reflected that of the bulk test material. The concentrations of all constituents were higher in the liquid reservoirs at the end of the exposure day indicating the loss of water. Boron, triethanolamine, and fatty acid methyl esters were present primarily as particulates while ethanolamine and 1-amino-2-propanol had significant vapor phase contributions. The percentage of methanol in the liquid reservoirs was lower than that in the bulk test material.

Approximately 4 months into the 2-year studies, exposure chamber analyses with animals present indicated a shift in the relative amounts of several of the constituents relative to boron. Characterization of the test material was performed and compared with the results obtained prior to the study; all results were consistent, indicating that the test material was stable. Sampling and test methods used were the same as those used for previous stability testing with one exception, HPLC/MS by system C used earlier was slightly changed to improve the resolution of alkanolamines (system D). Additional samples were taken from the inlet and exhaust and from the top and bottom of the 10, 30, and 100 mg/m^3 rat/mouse chambers with animals present. The results of the investigation proved that boron was not a good marker for the determination of

CIMSTAR 3800 concentrations. The decision was made to calibrate the RAMs using CIMSTAR 3800 concentrations based on gravimetric determinations for the remainder of the 2-year studies.

Samples were collected, prepared, and analyzed gravimetrically. Instruments were calibrated for each method using gravimetrically prepared dilutions of the bulk test material. Results for each constituent were calculated relative to the concentration determined using the on-line monitor. For the distribution line, the amount of each constituent was calculated as a percentage of the expected amount based on the gravimetric determination. For the generator reservoir samples, results were calculated relative to the bulk test material. Constituents were within approximately 30% of the on-line monitor results in the distribution line and were comparable to the results observed during prestart assays. Triethanolamine concentrations were 95% to 107% of the on-line monitor results in the chamber atmosphere samples; fatty acid methyl ester concentrations were within 35% in the 10 mg/m^3 chambers and 20% in the 100 mg/m^3 chambers. The relative proportions for boron and the volatile alkanolamines were reduced in the 100 mg/m^3 chambers and the reductions were greater in the rat/mouse chambers than in the male rat chambers. The greatest degree of reduction was seen in the 10 mg/m^3 rat/mouse chambers where boron was 34%, ethanolamine was 16%, and 1-amino-2-propanol was 30% of the expected.

Methanol concentrations were increased in the reservoirs as well as the distribution line and exposure chambers. However, other volatile constituents (ethanolamine and 1-amino-2-propanol) were comparable in the reservoirs as well as the distribution line compared to prestart measurements while decreasing in the exposure chamber atmosphere with decreasing exposure concentrations, most significantly in the 10 mg/m^3 exposure chambers.

The concentrations of all constituents were 94% to 108% relative to the bulk test material in the chemical cabinet reservoir at the end of the exposure day. Concentrations of these constituents were 117% to 150% higher in the liquid reservoirs indicating the loss of water, comparable to prestart measurements.

Table H-1. Gas Chromatography Systems Used in the Inhalation Studies of CIMSTAR 3800[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	RTX®-5, 30 m × 0.32 mm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at 10 psi	45°C for 1 minute, then 6°C/minute to 300°C, held for 5 minutes
System B			
Mass spectrometry	RTX®-5, 30 m × 0.25 mm, 1.0 µm film (Restek)	Helium at 8 psi	45°C for 1 minute, then 6°C/minute to 300°C, held for 10 minutes
System C			
Mass spectrometry	DB™-WAX, 30 m × 0.25 mm, 0.5 µm film (J&W Scientific, Folsom, CA)	Helium at 1.2 mL/minute	45°C for 1 minute, then 6°C/minute to 65°C, then 15°C/minute to 130°C, held for 1 minute
System D			
Flame ionization	SP2380, 30 m × 0.32 mm, 0.2 µm film (Supelco, Bellefonte, PA)	Helium at 10 psi	40°C for 2 minutes, then 15°C/minute to 170°C, held for 7 minutes, then 10°C/minute to 270°C, held for 10 minutes

[a]The gas chromatographs and mass spectrometers were manufactured by Agilent Technologies, Inc. (Palo Alto, CA).

Table H-2. High-Performance Liquid Chromatography Systems Used in the Inhalation Studies of CIMSTAR 3800[a]

Detection System	Column	Solvent System
System A		
Ultraviolet light coupled with mass spectrometry	Agilent 1100, Dionex IonPac NS1, 250 mm × 4 mm (Agilent Technologies, Inc., Palo Alto, CA)	A) 10 mM nonafluoropentanoic acid/2% acetonitrile in water and B) 10 mM nonafluoropentanoic acid in acetonitrile; linear gradient from 100% A to 80% A:20% B in 10 minutes, then to 100% A in 2 minutes, held for 3 minutes; flow rate 1.0 mL/minute
System B		
Ultraviolet light coupled with mass spectrometry	Agilent 1200, Dionex IonPac NS1, 250 mm × 4 mm (Agilent Technologies, Inc.)	Isocratic 75% 0.1% formic acid/2% acetonitrile in water and 25% 0.1% formic acid in acetonitrile; flow rate 0.5 mL/minute
System C		
Ultraviolet light coupled with mass spectrometry	Waters Nova-Pak C18 250 mm × 4 mm, 4 µm (Waters Corporation, Milford, MA)	Isocratic 0.1% formic acid/2% acetonitrile in water; flow rate 0.5 mL/minute
System D		
Ultraviolet light coupled with mass spectrometry	Waters Nova-Pak C18 60 Å 300 mm × 3.9 mm, 4 µm (Waters Corporation)	Isocratic 0.1% formic acid/2% acetonitrile in water; flow rate 0.5 mL/minute

[a]The high-performance liquid chromatographs were manufactured by Agilent Technologies, Inc. (Palo Alto, CA). The mass spectrometers were manufactured by Agilent Technologies (systems A and B) or Fisher Scientific, Inc. (Waltham, PA, systems C and D).

Table H-3. Specific Components of the Three Lots of CIMSTAR 3800 Used in the Inhalation Studies of CIMSTAR 3800[a]

Component	Lot 60224BBN[b]	Lot 71205BN[c]	Lot 90317JN[c]
Water	60.0	60.0	60.0
Methanol	0.30	0.30	0.35
Ethanolamine	4.7	5.7	5.6
Triethanolamine	3.4	3.3	3.2
1-Amino-2-propanol	1.8	1.4	1.6
Methyl palmitate	0.24	0.21	0.20
Methyl stearate	0.71	0.77	0.78
Methyl oleate	2.60	2.26	2.66
Methyl linoleate	1.68	1.54	1.77
Hexane extractable material	26.0	25.0	26.0
Total	101.43	100.48	102.16

[a]All values are percentages.
[b]Used in the 3-month studies.
[c]Used in the 2-year studies.

Table H-4. Summary of Chamber Concentrations in the Three-month Inhalation Studies of CIMSTAR 3800

	Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Rat Chambers			
	25	728	24.9 ± 1.5
	50	713	49.8 ± 2.6
	100	727	102 ± 4
	200	715	198 ± 8
	400	728	400 ± 16
Mouse Chambers			
	25	752	24.9 ± 1.5
	50	736	49.7 ± 2.6
	100	751	102 ± 4
	200	738	197 ± 8
	400	752	400 ± 16

[a]Mean ± standard deviation.

Table H-5. Summary of Chamber Concentrations in the Two-year Inhalation Studies of CIMSTAR 3800

	Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Male Rat Chambers			
	10	5,328	10.1 ± 0.5
	30	5,327	29.5 ± 1.3
	100	5,380	99.8 ± 3.9
Female Rat Chambers			
	10	5,401	9.9 ± 0.5
	30	5,400	29.6 ± 1.2
	100	5,346	99.1 ± 4.2
Mouse Chambers			
	10	5,400	9.9 ± 0.5
	30	5,399	29.7 ± 1.2
	100	5,347	99.2 ± 4.2

[a]Mean ± standard deviation.

Table H-6. Summary of Aerosol Size Measurements for the Rat and Mouse Exposure Chambers in the Three-month Inhalation Studies of CIMSTAR 3800

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
June 2006	25	1.6	2.2
	50	1.7	2.1
	100	1.7	2.0
	200	1.8	2.0
	400	1.6	2.0
July 2006	25	1.6	2.2
	50	1.6	2.0
	100	1.6	2.0
	200	1.7	1.9
	400	1.7	1.8
August 2006	25	1.6	1.9
	50	1.6	2.0
	100	1.6	1.9
	200	1.7	1.8
	400	1.7	1.9

Table H-7. Summary of Aerosol Size Measurements for the Rat Exposure Chambers in the Two-year Inhalation Study of CIMSTAR 3800

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
Male			
April 2008	10	1.8	2.4
	30	1.7	2.9
	100	1.6	2.5
May 2008	10	1.7	2.5
	30	1.7	2.3
	100	1.6	2.2
June 2008	10	1.6	2.7
	30	1.7	2.2
	100	1.6	2.2
July 2008	10	1.6	2.7
	30	1.6	2.2
	100	1.6	2.1
August 2008	10	1.3	2.9
	30	1.6	1.9
	100	1.6	2.1
September 2008	10	1.3	2.3
	30	1.3	2.6
	100	1.5	2.2
October 2008	10	1.7	2.8
	30	1.6	2.6
	100	1.3	2.4
November 2008	10	1.3	2.5
	30	1.6	2.3
	100	1.5	2.4
December 2008	10	1.6	2.4
	30	1.4	2.4
	100	1.5	2.4
January 2009	10	1.3	2.4
	30	1.4	2.4
	100	1.6	2.2
February 2009	10	1.3	2.6
	30	1.3	2.6
	100	1.2	2.6

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2009	10	1.4	2.7
	30	1.4	2.3
	100	1.6	2.3
April 2009	10	1.5	2.4
	30	1.6	2.2
	100	1.9	2.4
May 2009	10	1.5	2.1
	30	1.6	2.0
	100	1.6	2.1
June 2009	10	1.7	1.7
	30	1.6	1.8
	100	1.7	1.7
July 2009	10	1.4	2.2
	30	1.5	2.2
	100	1.5	2.4
August 2009	10	1.4	2.5
	30	1.6	2.2
	100	1.6	2.4
September 2009	10	1.3	2.7
	30	1.3	2.7
	100	1.8	2.0
October 2009	10	1.6	2.4
	30	1.5	2.5
	100	1.5	2.2
November 2009	10	1.6	2.2
	30	1.9	2.6
	100	1.5	2.6
December 2009	10	1.6	2.3
	30	1.7	2.2
	100	1.7	2.2
January 2010	10	1.5	2.4
	30	1.8	2.5
	100	1.7	2.3
February 2010	10	1.5	2.4
	30	1.6	2.3

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
	100	1.5	2.4
March 2010	10	1.4	2.7
	30	1.7	2.3
	100	1.7	2.5
April 2010	10	1.6	2.3
	30	1.5	2.3
	100	1.7	2.3
Range	10	1.3–1.8	1.7–2.9
	30	1.3–1.9	1.8–2.9
	100	1.2–1.9	1.7–2.6
Female			
April 2008	10	1.8	2.5
	30	1.4	2.6
	100	1.6	2.3
May 2008	10	1.6	2.5
	30	1.5	2.6
	100	1.5	2.4
June 2008	10	1.6	2.7
	30	1.6	2.3
	100	1.6	2.3
July 2008	10	1.6	2.4
	30	1.5	2.3
	100	1.6	2.1
August 2008	10	1.7	2.6
	30	1.6	2.0
	100	1.6	2.1
September 2008	10	1.4	2.2
	30	1.3	2.3
	100	1.5	2.2
October 2008	10	1.4	2.4
	30	1.4	2.2
	100	1.3	2.2
November 2008	10	1.4	2.4
	30	1.5	2.2
	100	1.6	2.2

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
December 2008	10	1.2	2.7
	30	1.9	2.7
	100	1.3	2.6
January 2009	10	1.2	2.2
	30	1.4	2.1
	100	1.5	2.1
February 2009	10	1.4	2.5
	30	1.3	2.4
	100	1.1	2.3
March 2009	10	1.3	2.4
	30	1.4	2.3
	100	1.6	2.2
April 2009	10	1.5	2.3
	30	1.5	2.2
	100	1.4	2.4
May 2009	10	1.4	2.1
	30	1.5	2.0
	100	1.5	2.2
June 2009	10	1.4	2.1
	30	1.5	2.0
	100	1.6	1.6
July 2009	10	1.5	2.2
	30	1.6	1.9
	100	1.5	2.2
August 2009	10	1.5	2.1
	30	1.5	2.1
	100	1.4	2.3
September 2009	10	1.5	2.4
	30	1.4	2.4
	100	1.6	2.2
October 2009	10	1.3	2.7
	30	1.5	2.4
	100	1.4	2.7
November 2009	10	1.4	2.4
	30	1.4	2.8

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
	100	2.0	2.5
December 2009	10	1.4	2.3
	30	1.6	2.1
	100	1.7	2.1
January 2010	10	1.4	2.4
	30	1.5	2.3
	100	1.6	2.3
February 2010	10	1.5	2.4
	30	1.4	2.9
	100	1.6	2.6
March 2010	10	1.3	2.7
	30	1.5	2.5
	100	1.6	2.4
April 2010	10	1.6	2.2
	30	1.5	2.2
	100	1.6	2.3
Range	10	1.2–1.8	2.1–2.7
	30	1.3–1.9	1.9–2.9
	100	1.1–2.0	1.6–2.7

Table H-8. Summary of Aerosol Size Measurements for the Mouse Exposure Chambers in the Two-year Inhalation Study of CIMSTAR 3800

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
Female			
April 2008	10	1.8	2.5
	30	1.4	2.6
	100	1.6	2.3
May 2008	10	1.6	2.5
	30	1.5	2.6
	100	1.5	2.4
June 2008	10	1.6	2.7
	30	1.6	2.3
	100	1.6	2.3
July 2008	10	1.6	2.4
	30	1.5	2.3
	100	1.6	2.1
August 2008	10	1.7	2.6
	30	1.6	2.0
	100	1.6	2.1
September 2008	10	1.4	2.2
	30	1.3	2.3
	100	1.5	2.2
October 2008	10	1.4	2.4
	30	1.4	2.2
	100	1.3	2.2
November 2008	10	1.4	2.4
	30	1.5	2.2
	100	1.6	2.2
December 2008	10	1.2	2.7
	30	1.9	2.7
	100	1.3	2.6
January 2009	10	1.2	2.2
	30	1.4	2.1
	100	1.5	2.1
February 2009	10	1.4	2.5
	30	1.3	2.4
	100	1.1	2.3

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2009	10	1.3	2.4
	30	1.4	2.3
	100	1.6	2.2
April 2009	10	1.5	2.3
	30	1.5	2.2
	100	1.4	2.4
May 2009	10	1.4	2.1
	30	1.5	2.0
	100	1.5	2.2
June 2009	10	1.4	2.1
	30	1.5	2.0
	100	1.6	1.6
July 2009	10	1.5	2.2
	30	1.6	1.9
	100	1.5	2.2
August 2009	10	1.5	2.1
	30	1.5	2.1
	100	1.4	2.3
September 2009	10	1.5	2.4
	30	1.4	2.4
	100	1.6	2.2
October 2009	10	1.3	2.7
	30	1.5	2.4
	100	1.4	2.7
November 2009	10	1.4	2.4
	30	1.4	2.8
	100	2.0	2.5
December 2009	10	1.4	2.3
	30	1.6	2.1
	100	1.7	2.1
January 2010	10	1.4	2.4
	30	1.5	2.3
	100	1.6	2.3
February 2010	10	1.5	2.4
	30	1.4	2.9
	100	1.6	2.6

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2010	10	1.3	2.7
	30	1.5	2.5
	100	1.6	2.4
April 2010	10	1.6	2.2
	30	1.5	2.2
	100	1.6	2.3
May 2010	10	1.6	2.3
	30	1.7	2.2
	100	1.8	2.2
Range	10	1.2–1.8	2.1–2.7
	30	1.3–1.9	1.9–2.9
	100	1.1–2.0	1.6–2.7

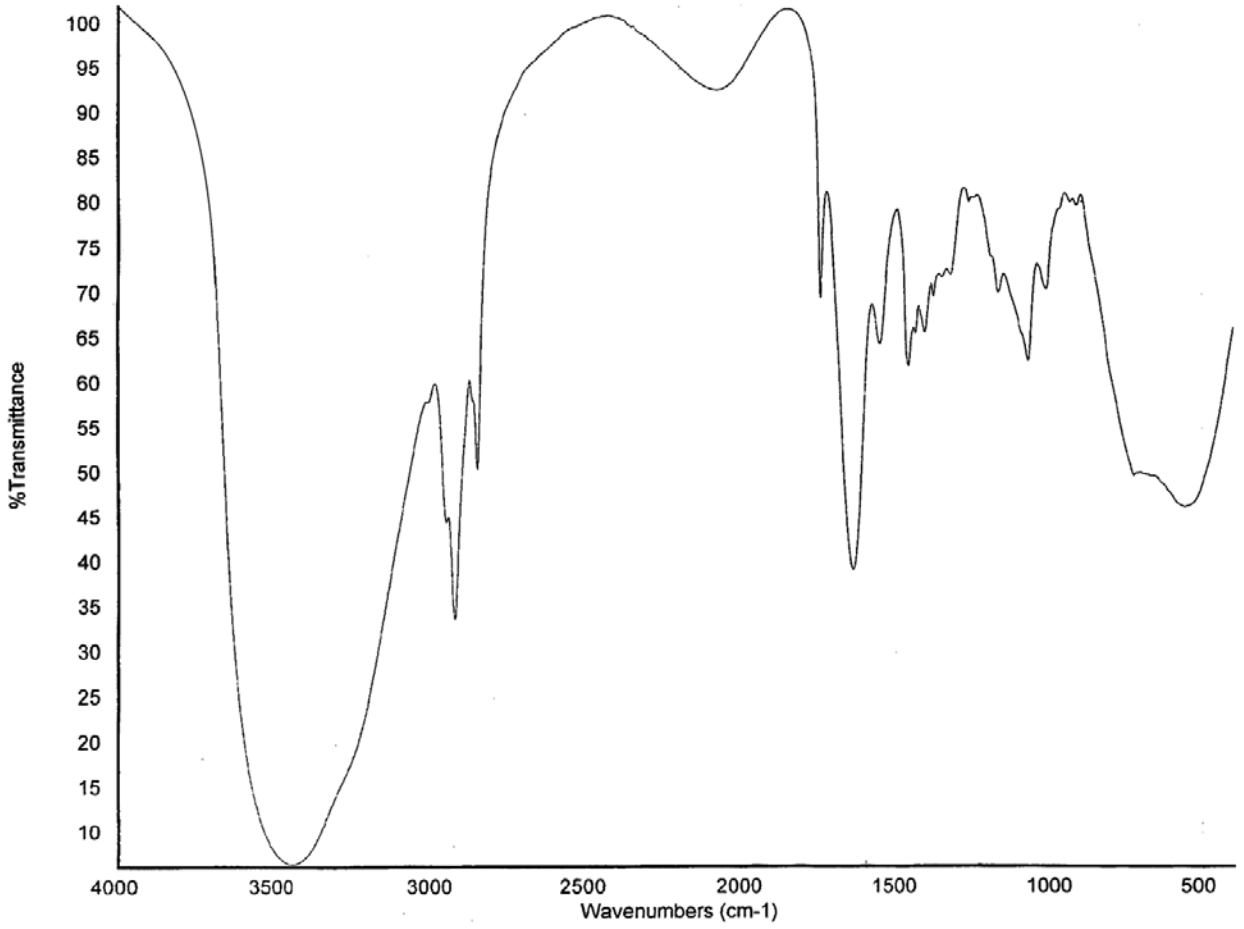

Figure H-1. Fourier Transform Infrared Absorption Spectrum of CIMSTAR 3800

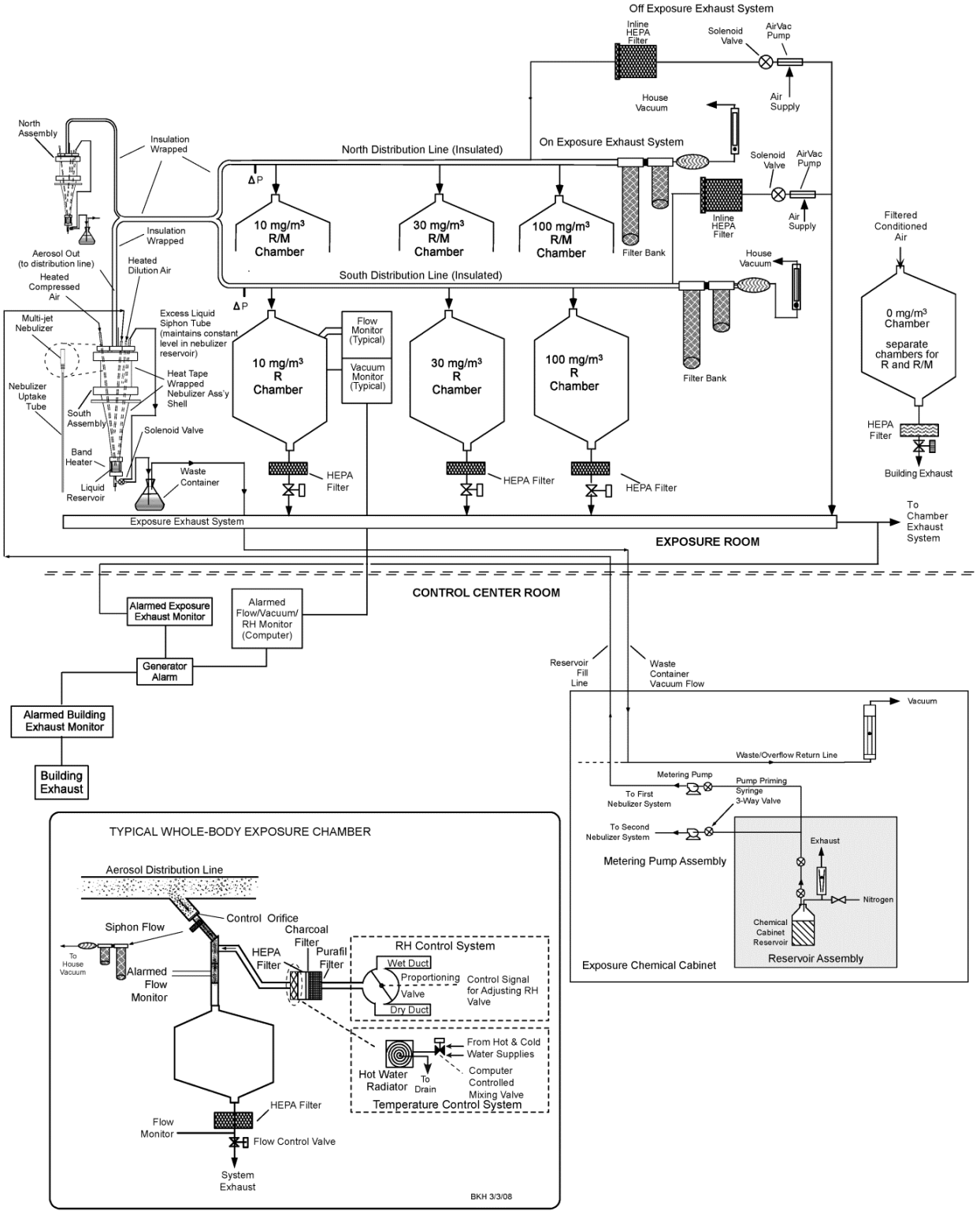

Figure H-2. Schematic of the Aerosol Generation and Delivery System in the Three-month and Two-year Inhalation Studies of CIMSTAR 3800

Appendix I. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table I-1. 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 I-2. 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		

	Amount	Source
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 I-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.55	13.7–15.9	25
Crude fat (% by weight)	8.2 ± 0.25	7.7–8.6	25
Crude fiber (% by weight)	9.3 ± 0.90	7.1–11.1	25
Ash (% by weight)	5.1 ± 0.16	4.9–5.4	25
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.67–0.97	23
Cystine	0.220 ± 0.024	0.15–0.25	23
Glycine	0.700 ± 0.040	0.62–0.80	23
Histidine	0.351 ± 0.080	0.27–0.68	23
Isoleucine	0.546 ± 0.043	0.43–0.66	23
Leucine	1.095 ± 0.066	0.96–1.24	23
Lysine	0.700 ± 0.116	0.31–0.86	23
Methionine	0.409 ± 0.045	0.26–0.49	23
Phenylalanine	0.628 ± 0.039	0.54–0.72	23
Threonine	0.506 ± 0.042	0.43–0.61	23
Tryptophan	0.150 ± 0.028	0.11–0.20	23
Tyrosine	0.405 ± 0.063	0.28–0.54	23
Valine	0.664 ± 0.042	0.55–0.73	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.96 ± 0.254	3.49–4.55	23
Linolenic	0.30 ± 0.031	0.21–0.35	23
Vitamins			
Vitamin A (IU/kg)	3,684 ± 94.6	2,110–5,720	25
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.3 ± 21.6	27.0–124.0	23
Thiamine (ppm)[b]	7.2 ± 1.26	5.1–11.0	25
Riboflavin (ppm)	7.7 ± 2.87	4.20–17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4–98.2	23
Pantothenic acid (ppm)	27 ± 12.35	17.4–81.0	23

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Pyridoxine (ppm)[b]	9.54 ± 1.94	6.44–13.7	23
Folic acid (ppm)	1.61 ± 0.47	1.15–3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	23
Vitamin B_{12} (ppb)	53.4 ± 38.7	18.3–174.0	23
Choline (ppm)[b]	2,773 ± 590	1,160–3,790	23
Minerals			
Calcium (%)	0.901 ± 0.038	0.81–0.97	25
Phosphorus (%)	0.558 ± 0.061	0.49–0.82	25
Potassium (%)	0.667 ± 0.030	0.626–0.733	23
Chloride (%)	0.385 ± 0.038	0.300–0.474	23
Sodium (%)	0.189 ± 0.016	0.160–0.222	23
Magnesium (%)	0.216 ± 0.060	0.185–0.490	23
Sulfur (%)	0.170 ± 0.030	0.116–0.209	14
Iron (ppm)	186 ± 38.64	135–311	23
Manganese (ppm)	51.02 ± 10.19	21.0–73.1	23
Zinc (ppm)	53.61 ± 8.34	43.3–78.5	23
Copper (ppm)	7.1 ± 2.540	3.21–16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158–0.972	23
Chromium (ppm)	0.696 ± 0.270	0.330–1.380	23
Cobalt (ppm)	0.248 ± 0.163	0.094–0.864	23

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

Table I-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.038	0.16–0.31	25
Cadmium (ppm)	0.06 ± 0.009	0.05–0.10	25
Lead (ppm)	0.12 ± 0.161	0.06–0.90	25
Mercury (ppm)	<0.02	–	25
Selenium (ppm)	0.2 ± 0.46	0.14–0.34	25
Aflatoxins (ppb)	<5.00	–	25
Nitrate nitrogen (ppm)[c]	22.02 ± 8.46	10.0–42.3	25
Nitrite nitrogen (ppm)[c]	<0.61	–	25
BHA (ppm)[d]	<1.0	–	25
BHT (ppm)[d]	<1.0	–	25
Aerobic plate count (CFU/g)	10 ± 0	10	25
Coliform (MPN/g)	3.0 ± 0	3.0	25
Escherichia coli (MPN/g)	<10	–	25
Salmonella (MPN/g)	Negative	–	25

	Mean ± Standard Deviation[b]	Range	Number of Samples
Total nitrosoamines (ppb)[e]	10 ± 4.49	2.0–17.2	25
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 2.77	0.9–11.1	25
N-Nitrosopyrrolidine (ppb)[e]	7.8 ± 3.50	1.0–13.9	25
Pesticides (ppm)			
α-BHC	<0.01	–	25
β-BHC	<0.02	–	25
γ-BHC	<0.01	–	25
δ-BHC	<0.01	–	25
Heptachlor	<0.01	–	25
Aldrin	<0.01	–	25
Heptachlor epoxide	<0.01	–	25
DDE	<0.01	–	25
DDD	<0.01	–	25
DDT	<0.01	–	25
HCB	<0.01	–	25
Mirex	<0.01	–	25
Methoxychlor	<0.05	–	25
Dieldrin	<0.01	–	25
Endrin	<0.01	–	25
Telodrin	<0.01	–	25
Chlordane	<0.05	–	25
Toxaphene	<0.10	–	25
Estimated PCBs	<0.20	–	25
Ronnel	<0.01	–	25
Ethion	<0.02	–	25
Trithion	<0.05	–	25
Diazinon	<0.10	–	25
Methyl chlorpyrifos	0.131 ± 0.122	0.020–0.553	25
Methyl parathion	<0.02	–	25
Ethyl parathion	<0.02	–	25
Malathion	0.10 ± 0.089	0.020–0.395	25
Endosulfan I	<0.01	–	25
Endosulfan II	<0.01	–	25
Endosulfan sulfate	<0.03	–	25

[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 J. Sentinel Animal Program

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Tables

J.1. Methods

Rodents used in 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 test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from each animal and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and evaluated for the presence of pathogens. For the 3-month study, samples were tested in-house or sent to BioReliance Corporation (Rockville, MD). For the 2-year study, samples were tested in-house or sent to the Research Animal Diagnostic Laboratory, University of Missouri (Columbia, MO). The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed (Table J-1).

Table J-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

	Method/Test	Time of Collection
Rats		
Three-month Study		
ELISA		
	Mycoplasma pulmonis	2 weeks
	PVM (pneumonia virus of mice)	2 weeks, study termination
	RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	2 weeks, study termination
	RPV (rat parvovirus)	2 weeks
	Sendai	2 weeks, study termination
Immunofluorescence Assay		
	Parvovirus	Study termination
Two-year Study		
ELISA		
	M. pulmonis	2 weeks
	PVM	2 weeks
	RCV/SDA	2 weeks
	RPV	2 weeks
	Sendai	2 weeks

	Method/Test	Time of Collection
Multiplex Fluorescent Immunoassay		
	KRV (Kilham's rat virus)	6, 12, and 18 months, study termination
	M. pulmonis	6, 12, and 18 months, study termination
	Parvo NS-1	6, 12, and 18 months, study termination
	PVM	6, 12, and 18 months, study termination
	RMV (rat minute virus)	6, 12, and 18 months, study termination
	RPV	6, 12, and 18 months, study termination
	RTV (rat theilovirus)	6, 12, and 18 months, study termination
	RCV/SDA	6, 12, and 18 months, study termination
	Sendai	6, 12, and 18 months, study termination
	TMEV (Theiler's murine encephalomyelitis virus)	6, 12, and 18 months, study termination
	Toolan's H-1	6, 12, and 18 months, study termination
Immunofluorescence Assay		
	M. pulmonis	12 months
	RPV	Study termination
	RTV	Study termination
Mice		
Three-month Study		
ELISA		
	Ectromelia virus	Study termination
	EDIM (epizootic diarrhea of infant mice)	Study termination
	LCM (lymphocytic choriomeningitis virus)	Study termination
	MAd-1 (mouse adenovirus)	Study termination
	MHV (mouse hepatitis virus)	2 weeks, study termination
	MMV VP2 (mouse minute virus viral protein 2)	Study termination
	MPV (mouse parvovirus)	2 weeks
	MPV VP2 (mouse parvovirus viral protein 2)	Study termination
	M. pulmonis	2 weeks
	PVM	2 weeks, study termination
	REO3 (reovirus 3)	Study termination
	Sendai	2 weeks, study termination
	TMEV	2 weeks, study termination
Immunofluorescence Assay		
	EDIM	Study termination
	MAd-1	Study termination
	MPV	Study termination

	Method/Test	Time of Collection
	PVM	Study termination
Two-year Study		
ELISA		
	GDVII (mouse poliovirus, strain GDVII)	2 weeks
	MHV	2 weeks
	MPV	2 weeks
	PVM	2 weeks
	Sendai	2 weeks
	M. pulmonis	2 weeks
Multiplex Fluorescent Immunoassay		
	Ectromelia virus	6, 12, and 18 months, study termination
	EDIM	6, 12, and 18 months, study termination
	LCM	6, 12, and 18 months, study termination
	M. pulmonis	6, 12, and 18 months, study termination
	MHV	6, 12, and 18 months, study termination
	MNV (mouse norovirus)	6, 12, and 18 months, study termination
	Parvo NS-1	6, 12, and 18 months, study termination
	MPV	6, 12, and 18 months, study termination
	MVM (minute virus of mice)	6, 12, and 18 months, study termination
	PVM	6, 12, and 18 months, study termination
	REO3	6, 12, and 18 months, study termination
	Sendai	6, 12, and 18 months, study termination
	TMEV/GDVII (Theiler's murine encephalomyelitis virus/mouse poliovirus, strain GDVII)	6, 12, and 18 months, study termination
Immunofluorescence Assay		
	Sendai	12 months
	MPV	18 months
	MVM	6 and 18 months
	MNV	Study termination
Polymerase Chain Reaction		
	Helicobacter species	18 months

J.2. Results

All test results were negative.

Appendix K. Summary of Peer Review Panel Comments

On May 22, 2014, the draft Technical Report on the toxicology and carcinogenesis studies of CIMSTAR 3800 received public review by the National Toxicology Program's Technical Reports Peer Review Panel. 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 CIMSTAR 3800, a semi-synthetic metalworking fluid by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *equivocal evidence of carcinogenic activity* in male and female Wistar Han rats, *no evidence of carcinogenic activity* in male B6C3F1/N mice, and *some evidence of carcinogenic activity* in female B6C3F1/N mice.

Dr. Mirsalis asked Dr. Morgan about the justification for using inhalation versus dermal exposure, and Dr. Morgan replied that inhalation is likely the most common occupational route of exposure. Dr. P.C. Howard, FDA, asked if the target droplet size was based on industry standards for conducting inhalation toxicology studies, not necessarily the droplet size to which workers could be exposed. Dr. Morgan stated droplet size was based on NTP standards for inhalation studies in rodents.

Dr. Carpenter noted receipt and distribution to the Panel of written comments from Mr. J.L. Leiter on behalf of the Independent Lubricant Manufacturers Association (ILMA).

Dr. F.E. Mirer, CUNY School of Public Health, spoke by telephone on his own behalf and described his background related to the subject of metalworking fluids. He related a history of the project, which dated back to a petition by the United Automobile Workers to NTP for bioassay of respiratory effects and carcinogenicity of representative metalworking fluids and stated that respiratory effects were of as much public health concern as carcinogenicity. He recommended that the report note that nearly 100% of the rats in the 3-month and 2-year studies had some upper respiratory lesions even at the lowest exposures. He said the adjusted incidences should be calculated for non-tumor pathology so the results could be included in a benchmark dose analysis, and only NTP could do so. He argued that the lung neoplasms in female mice should be seen as *clear evidence* and asked for clearer discussion of composition in the report, listing some of the details for clarification.

Dr. W. Dalbey, DalbeyTox, LLC, speaking on behalf of ILMA, questioned the characterization of exposures in the report, particularly whether a vapor phase was monitored during exposures. He questioned the method used for gravimetric measurement of the aerosol phase, and noted the composition of the aerosol was not apparent. He said the criteria for evidence of carcinogenicity were sometimes unclear in the report, referring to the conclusions related to prostate gland and brain neoplasms in male rats and the lung neoplasms in mice. Similarly, he raised questions about the basis for the *equivocal evidence* conclusion for skin neoplasms in female rats. He approved of labelling CIMSTAR 3800 "weakly mutagenic."

Dr. J.K. Howell, GHS Resources, Inc., also spoke on behalf of ILMA. He provided general information about metalworking fluids and the characterization of CIMSTAR 3800. He stressed

that metalworking fluids are complex mixtures, with thousands of formulations commercially available. He suggested several editorial changes to the report, including additional references to unintended substances in metalworking fluids and addition of language describing occupational levels of aerosol exposure to metalworking fluids as opposed to experimental concentrations used in the current studies. He questioned the characterization of CIMSTAR 3800 in the report, and recommended adding a table describing the composition of CIMSTAR 3800. He asked for correction on the statement that no biocide was present, citing the updated Material Safety Data Sheet (MSDS).

Dr. Regan, the first primary reviewer, said this was a difficult study from a pathology standpoint and was very well done. She said comments about the incidences of uterine neoplasms should be limited to the combined final numbers. She asked if there were any animals that had both granulosa cell tumors of the ovary and adenocarcinomas or Müllerian tumors of the uterus and if the skin neoplasms in rats were associated with the chronic active inflammation that was observed. She suggested adding a table for the components analyzed in CIMSTAR 3800 along with information indicating if there was any evidence of bacterial or fungal growth or bacterial endotoxin in the test material. She asked whether the historical control data presented also had residual uterine tissue examinations performed.

Dr. Morgan said he could add a table as suggested by Dr. Regan and noted the information on bacterial and fungal counts was in Appendix H; however, the information could be brought into the Materials and Methods section. He said endotoxin was not measured. Regarding the discussion about relative exposures, he noted it was addressed in the Discussion section, but it could be expanded.

Dr. M.F. Cesta, NIEHS, said none of the animals had both granulosa cell tumors of the ovary and adenocarcinomas or Müllerian tumors of the uterus. He stated that none of the skin neoplams in rats were associated with chronic active inflammation. Regarding the historical control data, he said it only included the original sections, not residual sections. Dr. Regan noted the overall historical incidence of adenocarcinomas in rats was seven in 150, and asked if that incidence was based solely on original sections, which Dr. Cesta confirmed.

Dr. Fanucchi, the second primary reviewer, agreed with Dr. Regan’s comments. She said in Appendix H, it was shown that boron was originally used as a marker for the aerosol monitors, and later a gravimetric method was used; she asked how the methods were correlated. She noted that the report reviewed epidemiologic studies that suggest laryngeal cancer may be associated with occupational exposure to machining fluids, but the observed increased incidences of metaplasia in the larynges of treated animals in the current studies were not considered in the evidence of carcinogenicity. Dr. Fanucchi also asked about the presence of a biocide in CIMSTAR 3800, noting that two compounds tentatively identified in the chemical analysis of the test material may act as biocides. She noted the 2-year studies began in 2008, and a 2008 MSDS did not list a biocide.

Dr. Morgan said a biocide had tentatively been identified, and the report could be changed to reflect its presence. He said the switch to the gravimetric method had been made 4 months into the 2-year studies and was done to eliminate drift in the relative amounts of compounds.
Dr. Fanucchi said what was written was unclear as to whether boron was a bad marker, and she asked how it would be known that the first 4 months matched with the remainder of the studies.

Dr. Morgan said chamber concentrations were measured with and without animals present and he said that boron worked well in the 3-month studies with fewer animals in the chambers. After 4 months of the 2-year studies, with more animals present in the chambers, drift was noted, and a switch was made to the gravimetric method. He said the issue could be clarified in the report.

Dr. Cesta said that squamous metaplasia is not considered to be a preneoplastic lesion, and it does not lead to laryngeal neoplasia. Dr. Fanucchi asked for this point to be clarified in the report. Dr. Conner said that laryngeal changes are commonly seen in respiratory studies.

Dr. Mahrt, the third primary reviewer, asked whether NTP considered looking at the literature or other sources for Wistar Han rats to add to the information on historical controls. Dr. Morgan said that had not been done, as such information would be very study-dependent and may not be relevant to the NTP study.

Dr. Mahrt asked for clarity in the report on the historical controls involved with the original uterine sectioning and if there were some historical controls with the residual longitudinal uterine sectioning. Dr. Cesta responded it could be clarified in the report which animals were included in the historical controls.

Dr. Regan moved that the conclusion regarding incidences of benign or malignant granular cell tumors (combined) of the brain in male Wistar Han rats be changed from *equivocal evidence* to *no evidence.* Dr. Mirsalis seconded the motion, noting that the incidence of three tumors at the lowest exposure was not strong. Dr. Regan added there was also the occurrence of such tumors in the chamber control female rats, and Dr. Mahrt agreed, noting there were limited historical control data. The panel voted six to zero to accept the conclusions as amended.

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