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

NTP Technical Report on the Toxicity Studies of Trimethylsilyldiazomethane (CASRN 18107-18-1) Administered by Nose-only Inhalation to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the Toxicity Reports are derived solely from the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds 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 Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

The NTP Toxicity 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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Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Technical Report on the Toxicity Studies of Trimethylsilyldiazomethane (CASRN 18107-18-1) Administered by Nose-only Inhalation to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice* by letter in November 2020 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

(1) Peer review the draft *NTP Technical Report on the Toxicity Studies of Trimethylsilyldiazomethane Administered by Nose-only Inhalation to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice*.

(2) Comment on NTP's interpretations of the data.

NTP carefully considered reviewer comments in finalizing this report.

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Abstract

Trimethylsilyldiazomethane (TMSD) is a methylating reagent widely used in organic chemistry. TMSD is structurally related to the compound diazomethane, which is a known lethal respiratory toxicant in humans and in animal models. TMSD is less reactive (with lower explosive potential) than diazomethane and is considered a safer, less toxic alternative. Few toxicity data are available to support this claim, however, and TMSD is readily available commercially from chemical suppliers. Concern over the inhalation toxicity of TMSD originates from reports of the death of two chemists resulting from lung injury and acute respiratory distress syndrome following exposure to TMSD in the workplace. Other concerns include the known inhalation toxicity of diazomethane and the absence of inhalation toxicity data for TMSD. The National Toxicology Program (NTP) conducted this study to evaluate the acute inhalation toxicity of TMSD in vivo.

Groups of eight male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and eight male B6C3F1/N mice were exposed by nose-only inhalation to targeted vapor concentrations of 0 (air), 0 (hexanes), or 10 ppm TMSD (in hexanes) for 1 day (30 minutes) with or without a 9-day recovery period following exposure (1-day exposure or 1-day exposure – recovery). Groups of eight male rats and eight male mice were also exposed to 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm TMSD (in hexanes) by nose-only inhalation for up to 5 days (30 minutes/day) with or without a 5-day recovery period following exposure (5-day exposure or 5-day exposure – recovery). Animals were either euthanized the day after the single or last exposure or held for 9 or 5 days postexposure, respectively, and euthanized to assess recovery and delayed effects in the absence of TMSD exposure.

All TMSD-exposed rats survived until scheduled termination. In the 5-day exposure – recovery group exposed to 10 ppm, 4/8 (50%) of the rats exhibited labored breathing. Final mean body weights for the 1-day exposure and 1-day exposure – recovery group rats were similar to those of the air control group, but they were significantly decreased in the 5-day exposure (11.7% lower) and 5-day exposure – recovery rat groups (10.4% lower) at 10 ppm TMSD relative to the air control group. Final mean body weight gains in the 5-day exposure and 5-day exposure – recovery rat groups at 10 ppm TMSD were also significantly decreased relative to the air control groups. Absolute and relative lung weights were significantly decreased in the 1-day exposure rat group at 10 ppm TMSD. Absolute and relative lung weights of rats were significantly increased in the three other exposure groups at the same or lower exposure concentrations: 1-day exposure – recovery group at 10 ppm TMSD, 5-day exposure group at 3 and 10 ppm TMSD, and 5-day exposure – recovery group at ≥0.3 ppm TMSD exposure concentrations.

No exposure-related histopathological lesions were present in the 1-day exposure and 1-day exposure – recovery rat groups at 10 ppm TMSD. In the 5-day exposure and 5-day exposure – recovery groups, increased incidences of lung histopathological lesions included chronic-active inflammation (≥3 ppm), histiocyte (alveolar macrophage) infiltration (3 ppm), interstitial fibrosis (10 ppm), pulmonary edema (10 ppm), acute hemorrhage (10 ppm), and alveolar and bronchiolar epithelial hyperplasia (10 ppm). Alveolar and bronchiolar epithelial hyperplasia were also noted at 3 ppm TMSD in the 5-day exposure group. In addition, increased incidences of histiocyte infiltration in the mediastinal lymph nodes were observed in all rats exposed to 10 ppm TMSD in the 5-day exposure and 5-day exposure – recovery groups.

Inhaled TMSD was more toxic in mice than in rats. Five mice in the 1-day exposure – recovery group and all mice in the 5-day exposure and 5-day exposure – recovery groups died or were euthanized prior to scheduled termination after only one to three exposures, and 19/24 (79%) of the 10 ppm-exposed mice exhibited labored breathing. In the 5-day exposure – recovery group exposed to 3 ppm, all mice (8/8) exhibited labored breathing. Final mean body weights for the 1-day exposure group were similar to the air control group. In the 1-day exposure – recovery group, final mean body weights of the three mice remaining at study day 9 were significantly decreased (26.8% less than the air control group); final mean body weight gain was also significantly decreased in this group. Final mean body weights were significantly decreased in the 5-day exposure group mice at 3 ppm (10.2% lower) and 10 ppm (16.5% lower) and also in the 5-day exposure – recovery group mice at 3 ppm (21.7% lower) and 10 ppm (18.4% lower). Final mean body weight gains were also significantly decreased in both the 5-day exposure group (3 ppm) and the 5-day exposure – recovery group (1 and 3 ppm).

Absolute and relative lung weights were significantly increased in the 1-day exposure and 1-day exposure – recovery mouse groups at 10 ppm TMSD, in the 5-day exposure group mice at ≥0.3 ppm, and in the 5-day exposure – recovery group mice at ≥1 ppm. Increased incidences of lung histopathological lesions in mice were similar to those observed in rats, but also included necrosis and acute inflammation and were observed at greater incidences in the 1 and 3 ppm groups. In addition, lesions occurred in the larynx of mice exposed to 3 and 10 ppm TMSD, which included squamous epithelial hyperplasia and ulceration and acute inflammation. Lung and laryngeal lesions in the 1-day exposure – recovery mouse group at 10 ppm TMSD were similar to those in the 5-day exposure – recovery mouse group but occurred at lower incidences.

This in vivo study showed that inhaled TMSD at low vapor concentrations (≤10 ppm) caused acute and progressive lung injury, as reported in human case studies (in particular, pulmonary edema), and that these effects can occur after a single 30-minute exposure to the chemical. These data provide useful information for mitigating exposure risks in the workplace and alerting chemical suppliers to the dangers of TMSD.

Synonyms: (trimethylsilyl) diazomethane; diazo((trimethylsilyl))methane; diazomethyl(trimethyl)silane; (diazomethyl)trimethylsilane; diazomethyltrimethyl silane; silane, (diazomethyl)trimethyl-; TMS-diazomethane; $TMSCHN_2$

Summary of Findings Considered Toxicologically Relevant in Rats and Mice Exposed to Trimethylsilyldiazomethane by Nose-only Inhalation for One and Five Days

	Male Sprague Dawley Rats	Male B6C3F1/N Mice
Exposure Frequency and Recovery Period	Three groups of eight rats each were exposed to 0 (air), 0 (hexanes), or 10 ppm TMSD for 1 day (30 minutes) with or without a 9-day recovery period after exposure (1-day exposure, 1-day exposure – recovery). Six groups of eight rats each were exposed to 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm TMSD for up to 5 days (30 minutes/day) with or without a 5-day recovery period after exposure (5-day exposure, 5-day exposure – recovery).	Three groups of eight mice each were exposed to 0 (air), 0 (hexanes), or 10 ppm TMSD for 1 day (30 minutes) with or without a 9-day recovery period after exposure (1-day exposure, 1-day exposure – recovery). Six groups of eight mice each were exposed to 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm TMSD for up to 5 days (30 minutes/day) with or without a 5-day recovery period after exposure (5-day exposure, 5-day exposure – recovery).
Concentrations in Air	1-day exposure: 0 (air), 0 (hexanes), 10 ppm 1-day exposure – recovery: 0 (air), 0 (hexanes), 10 ppm 5-day exposure: 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm 5-day exposure – recovery: 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm	1-day exposure: 0 (air), 0 (hexanes), 10 ppm 1-day exposure – recovery: 0 (air), 0 (hexanes), 10 ppm 5-day exposure: 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm 5-day exposure – recovery: 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm
Survival Rates	1-day exposure: 8/8, 8/8, 8/8 1-day exposure – recovery: 8/8, 8/8, 8/8 5-day exposure: 8/8, 8/8, 8/8, 8/8, 8/8, 8/8 5-day exposure – recovery: 8/8, 8/8, 8/8, 8/8, 8/8, 8/8	1-day exposure: 8/8, 8/8, 8/8 1-day exposure – recovery: 8/8, 8/8, 3/8 5-day exposure: 8/8, 8/8, 8/8, 8/8, 8/8, 0/8 5-day exposure – recovery: 8/8, 8/8, 8/8, 8/8, 8/8, 0/8
Body Weights	1-day exposure (10 ppm): similar to the air control group at necropsy 1-day exposure – recovery (10 ppm): similar to the air control group at necropsy 5-day exposure (10 ppm): 11.8% less than the air control group at necropsy 5-day exposure – recovery (10 ppm): 10.4% less than the air control group at necropsy	1-day exposure (10 ppm): similar to the air control group at necropsy 1-day exposure – recovery (10 ppm): 26.8% less than the air control group at necropsy 5-day exposure (3 and 10 ppm): 10.2% and 16.5% less, respectively, than the air control group at necropsy 5-day exposure – recovery (3 and 10 ppm): 21.7% and 18.4% less, respectively, than the air control group at necropsy

	Male Sprague Dawley Rats	Male B6C3F1/N Mice
Clinical Observations	1-day exposure (10 ppm): none 1-day exposure – recovery (10 ppm): none 5-day exposure (10 ppm): ruffled coat, discharge from nose/snout 5-day exposure – recovery (10 ppm): ruffled coat, discharge from nose/snout, labored breathing, hunched	1-day exposure (10 ppm): none 1-day exposure – recovery (10 ppm): labored breathing, rapid breathing, ruffled coat, discharge from nose/snout, hunched, lethargic 5-day exposure (3 and 10 ppm): labored breathing, ruffled coat, hunched 5-day exposure – recovery (3 and 10 ppm): labored breathing, ruffled coat, hunched, lethargic
Lung Weights	1-day exposure (10 ppm): ↓ absolute and relative lung weight 1-day exposure – recovery (10 ppm): ↑ absolute and relative lung weight 5-day exposure (3 and 10 ppm): ↑ absolute and relative lung weight 5-day exposure – recovery (0.3, 1, 3, and 10 ppm): ↑ absolute and relative lung weight	1-day exposure (10 ppm): ↑ absolute and relative lung weight 1-day exposure – recovery (10 ppm): ↑ absolute and relative lung weight 5-day exposure (0.3, 1, and 3 ppm)[a]: ↑ absolute and relative lung weight 5-day exposure – recovery (1 and 3 ppm)[a]: ↑ absolute and relative lung weight
Nonneoplastic Effects	1-day exposure: none 1-day exposure – recovery: none 5-day exposure: *Lung*: inflammation, chronic-active (0/8, 0/8, NA, 0/8, 7/8, 8/8); infiltration, cellular, histiocyte (0/8, 0/8, NA, 0/8, 7/8, 0/8); interstitium, fibrosis (0/8, 0/8, NA, 0/8, 0/8, 8/8); edema (0/8, 0/8, NA, 0/8, 0/8, 7/8); hemorrhage, acute (0/8, 0/8, NA, 0/8, 0/8, 8/8); alveolar epithelium, hyperplasia (0/8, 0/8, NA, 0/8, 6/8, 8/8); bronchiole epithelium, hyperplasia (0/8, 0/8, NA, 0/8, 7/8, 8/8) *Mediastinal lymph node*: infiltration, cellular, histiocyte (0/8, 2/8, NA, 0/8, 1/8, 8/8) *Larynx*: epithelial cell, hyperplasia, squamous (0/8, 0/8, NA, NA, NA, 2/8); epiglottis, metaplasia, squamous (0/8, 0/8, NA, NA, NA, 2/8)	1-day exposure: none 1-day exposure – recovery: *Lung:* inflammation, acute (0/8, 0/8, 5/8); edema (0/8, 0/8, 5/8); hemorrhage, acute (0/8, 0/8, 5/8); alveolar epithelium, hyperplasia (0/8, 0/8, 4/8); necrosis (0/8, 0/8, 5/8) *Larynx:* squamous epithelium, hyperplasia (0/8, 0/8, 5/8); inflammation, acute (0/8, 0/8, 4/8) 5-day exposure: *Lung:* inflammation, acute (0/8, 0/8, 0/8, 0/8, 0/8, 8/8); inflammation, chronic-active (0/8, 0/8, 0/8, 0/8, 8/8, 0/8); infiltration, cellular, histiocyte (0/8, 0/8, 0/8, 7/8, 0/8, 0/8); edema (0/8, 0/8, 0/8, 7/8, 8/8, 8/8); hemorrhage, acute (0/8, 0/8, 0/8, 0/8, 8/8, 8/8); alveolar epithelium, hyperplasia (0/8, 0/8, 0/8, 0/8, 8/8, 0/8); bronchiole, epithelium, hyperplasia (0/8, 0/8, 0/8, 0/8, 8/8, 0/8); necrosis (0/8, 0/8, 0/8, 0/8, 8/8, 8/8)

	Male Sprague Dawley Rats	Male B6C3F1/N Mice
	5-day exposure – recovery: *Lung:* inflammation, chronic-active (0/8, 0/8, 0/8, 2/8, 8/8, 8/8); infiltration, cellular, histiocyte (0/8, 0/8, 1/8, 2/8, 8/8, 0/8); interstitium, fibrosis (0/8, 0/8, 0/8, 0/8, 0/8, 8/8); edema (0/8, 0/8, 0/8, 0/8, 0/8, 7/8); hemorrhage, acute (0/8, 0/8, 1/8, 1/8, 3/8, 8/8); alveolar epithelium, hyperplasia (0/8, 0/8, 0/8, 1/8, 2/8, 8/8); bronchiole epithelium, hyperplasia (0/8, 0/8, 0/8, 0/8, 0/8, 6/8)	5-day exposure – recovery: *Lung*: inflammation, acute (0/8, 0/8, 0/8, 0/8, 0/8, 8/8); inflammation, chronic-active (0/8, 0/8, 0/8, 8/8, 8/8, 0/8); interstitium, fibrosis (0/8, 0/8, 0/8, 8/8, 8/8, 0/8); edema (0/8, 0/8, 0/8, 0/8, 6/8, 8/8); hemorrhage, acute (0/8, 0/8, 0/8, 1/8, 7/8, 7/8); alveolar epithelium, hyperplasia (0/8, 0/8, 0/8, 8/8, 8/8, 0/8); bronchiole, epithelium hyperplasia (0/8, 0/8, 0/8, 8/8, 8/8, 0/8); necrosis (0/8, 0/8, 0/8, 0/8, 8/8, 8/8)
	Mediastinal lymph node: infiltration, cellular histiocyte (1/8, 0/8, NA, 0/8, 5/8, 8/8)	*Larynx:* squamous epithelium, ulcer (0/8, 0/8, NA, 0/8, 4/8, 4/8); inflammation, acute (0/8, 0/8, NA, 0/8, 5/8, 0/8)

NA = not assessed.
[a]Organ mean weights are not provided or statistically analyzed for the 10 ppm mouse group because not enough animals survived to scheduled necropsy.

Introduction

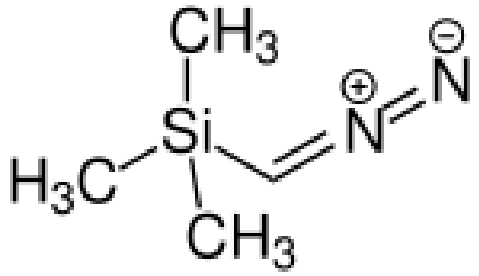

Figure 1. Trimethylsilyldiazomethane (CASRN 18107-18-1; Chemical Formula: $C_4H_{10}N_2Si$; Molecular Weight: 114.22)

Synonyms: (Trimethylsilyl) diazomethane; diazo(trimethylsilyl)methane; diazomethyl(trimethyl)silane; (diazomethyl)trimethylsilane; diazomethyltrimethyl silane; silane, (diazomethyl)trimethyl-; TMS-diazomethane; $TMSCHN_2$.

Trimethylsilyldiazomethane (TMSD) (Figure 1) is a methylating reagent widely used in organic chemistry and analytical methods.[1-7] TMSD is commonly used for the derivatization of carboxylic acid groups (e.g., R-COOH) for the production of methyl esters (e.g., $R\text{-}COOCH_3$). Methyl esters are amenable to analysis by gas chromatography (GC) and thus are ideal derivatives for the characterization of carboxylic acids via GC.

Background

TMSD is related to the toxic compound diazomethane which is highly reactive (flammable and explosive). Diazomethane was previously reported to cause severe pulmonary injury, chemical pneumonitis, and death in humans after inhalation exposure.[2; 8-11] An acute inhalation toxicity study was described for diazomethane in which rabbits were exposed to atmospheres containing 2–12 ppm diazomethane (one to four exposures for 5–20 minutes/exposure). This exposure regimen induced bronchopneumonia and death by day 7.[12] Inhalation exposure of cats to 175 ppm diazomethane for 10 minutes was also reported to be lethal within 3 days.[12] The occupational exposure limit (OEL) for diazomethane is 0.2 ppm (8-hour time-weighted average). This concentration was chosen on the basis of limited exposure data and because diazomethane is thought to have a similar mechanism of action to that of phosgene.[13] These animal toxicity data and the OEL for diazomethane are reported in the Hazardous Substances Data Bank.[12]

Chemical Use and Human Exposure

TMSD is less reactive than diazomethane and is considered a safer, less toxic alternative [2-4; 6]; however, few toxicity data for TMSD are available to support this claim. TMSD is currently readily sold commercially by chemical suppliers in solvents such as hexane or diethyl ether.[2-4; 6] Exposure to TMSD in an occupational setting (e.g., a chemical laboratory) is most likely to occur via dermal contact and/or inhalation, although a dermal toxicity study in rabbits concluded that TMSD (approximately 1,000 mg/kg) was not acutely toxic via dermal exposure.[12; 14] No inhalation studies with TMSD were performed before the current National Toxicology Program (NTP) studies were initiated.

Case Reports

Accidental exposure to TMSD in the workplace resulted in the death of two chemists, one in the United States and one in Canada, in 2008.[15-17] The deaths occurred approximately 15 (U.S. case report) and 26 (Canadian case report) hours after exposure to TMSD. Both deaths were determined to be the result of acute respiratory distress syndrome from lung injury believed to have been caused by exposure to TMSD. Extensive pleural and pericardial effusion, marked congestion of the lung, and histopathological findings that included the presence of delayed, massive, and diffuse pulmonary edema were reported in the autopsy findings from the U.S. incident. Both exposed individuals initially reported "asthma-like" symptoms (including cough, pleuritic chest pain, and severe, progressive dyspnea), suggesting airway/lung effects. In the U.S. case report, TMSD (in diethyl ether solvent) was spilled outside a fume hood and some contacted the individual's skin (potential for both inhalation and dermal exposure). In the Canadian case study, the individual was working with TMSD (in hexane solvent) in a nonfunctioning fume hood and there was no evidence of dermal contact (potential for inhalation exposure only), suggesting that dermal contact was not required for lethal exposure in humans. Inhalation of TMSD was the common route of exposure in both cases. It was not clear whether the chemists were exposed specifically to TMSD vapors or whether the exposure was in fact to diazomethane vapors as a reaction byproduct of TMSD.

Study Rationale

The Occupational Safety and Health Administration (OSHA) nominated TMSD to NTP for toxicity testing largely as the result of the accidental deaths of two chemists in 2008. Other reasons for the nomination included the known inhalation toxicity of the related compound diazomethane and the lack of toxicity data for TMSD.[17] Due to these considerations, the objective of this NTP study was to evaluate the acute inhalation toxicity of TMSD in vivo. Specifically, the aim was to determine whether acute inhalation exposure to TMSD induces asthma-like symptoms and lung injury (including pulmonary edema) in male rats and mice as described in the human case reports and, if so, at what exposure concentrations. The study was conducted only on males given that TMSD is a very reactive chemical and no sex-related differences were expected. Some exposure groups were euthanized 24 hours after the last exposure and some groups were held after cessation of chemical exposure to determine whether lung lesions progressed or resolved over time. These in vivo acute inhalation exposure data can be used by OSHA and other agencies (e.g., National Institute for Occupational Safety and Health) to help mitigate TMSD exposure risks in the production and use of TMSD in chemical laboratories. These data also can be used to update the Material Safety Data Sheet for TMSD (and other chemical reviews) and to alert chemical suppliers to the dangers of TMSD.

Materials and Methods

Procurement and Characterization of Trimethylsilyldiazomethane

Trimethylsilyldiazomethane (TMSD), 2 M in a solution of mixed hexanes, was obtained from Sigma-Aldrich (St. Louis, MO) in a single lot (SHBB9290V). A mixture of hexanes only (no TMSD) was obtained from Sigma-Aldrich (St. Louis, MO) in a single lot (SHBF3717V) and used as a control. Reports on analyses performed to support the TMSD study are on file at the National Institute of Environmental Health Sciences (NIEHS).

The identity and purity of lot SHBB9290V of TMSD and of lot SHBF3717V of mixed hexanes were determined at the study laboratory. The identities of TMSD and hexanes were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The concentration of TMSD, determined using ^{1}H NMR, was 2.65 M. Gas chromatography with mass selective detection (GC/MSD) was used to identify the major peaks and any peak areas ≥0.1% of the total peak area.

The mass spectra of lot SHBB9290V of TMSD contained nine peaks ≥0.1% by area. In addition to the primary TMSD peak (36.1%) and a (chloromethyl)trimethylsilane peak (3.30%), two major peaks consistent with n-hexane (33.6%) and methylcyclopentane (25.7%) were identified. The remaining peaks were all <2% of the total peak area and accounted for additional hydrocarbons and impurities related to TMSD (**Error! Reference source not found.**).

The mass spectra of lot SHBF3717V of mixed hexanes consisted of five peaks ≥0.1% by total peak area, all of which were isomers of hexane. The major peaks for n-hexane, methylcyclopentane, and 3-methylpentane were 64.2%, 17.4%, and 16.7% of the total peak area, respectively. Peaks for 2-methylpentane and cyclohexane were <2% of the total peak area (**Error! Reference source not found.**).

After scheduled termination of the animals, bulk chemical and mixed hexanes control materials were reanalyzed by the study laboratory using GC/MSD. Analysis indicated no statistically significant differences in the purities of TMSD and hexanes control materials relative to a frozen reference standard.

Vapor Generation and Exposure System

A schematic of the TMSD vapor generation and delivery system is shown in Figure 2. The system consisted of two parts: the vaporization subsystem and the delivery subsystem. Both subsystems were housed in a glove box. The generation and delivery system for the hexanes control was similar to that for the TMSD system.

TMSD was pumped to the vaporization column using a syringe pump capable of delivering a stable and precisely determined flow of chemical to the generator. The syringe needle was inserted in the vaporizer column through a septum. Air entered the column from the bottom, vaporized the chemical, and carried it to the delivery system.

The exposure vapor was directed to a pneumatic slide valve. This valve served as the exposure on-off valve and was operated by a solenoid actuated by the exposure control unit (ECU). During “on-exposure” periods, the slide valve was actuated, and the vapor was directed through the

valve to the exposure carousel. At each exposure carousel, vapor was delivered uniformly to each nose tube.

During “off-exposure” periods when animals were loaded or removed from each carousel, filtered and conditioned air was supplied to each exposure carousel from the facility compressed air system. Also during this period, the exposure vapor air stream was directed through a scrubber system within the glove box prior to facility exhaust.

The animals were exposed in nose-only inhalation carousels developed at Battelle. The rodent exposure carousel (Figure 2) consisted of stackable tiers with eight nose-only exposure ports per tier. Each stainless-steel exposure unit consisted of three modules providing 24 ports for animal exposure and test atmosphere sampling. Vented enclosures mounted on stainless-steel stands surrounded each exposure carousel and were designed to contain the exposure units. This carousel design provided uniform concentration and fresh aerosol of test chemical to each animal connected to the exposure system. The test atmosphere entered the carousel through the top. After filling an inlet manifold, the test atmosphere flowed radially to each of eight evenly spaced ports into each animal containment tube mounted on one of three different levels of the carousel. This design minimized any effect of the animals on the atmosphere because no exhaled air from one animal could reach the breathing zone of another. The temperature and humidity of the aerosol systems were not monitored or controlled and corresponded to the ambient conditions in the room.

One end of the tube that restrained the individual animal was tapered to approximately fit the shape of the animal’s head, and the diameter of the cylindrical portion of the tube was configured such that it was difficult for the animal to turn in the tube. The back portion of the tube was covered with a plastic cap. The tube containing the animal was fastened to the inhalation carousel by a bracket with the nose portion of the tube protruding into the carousel.

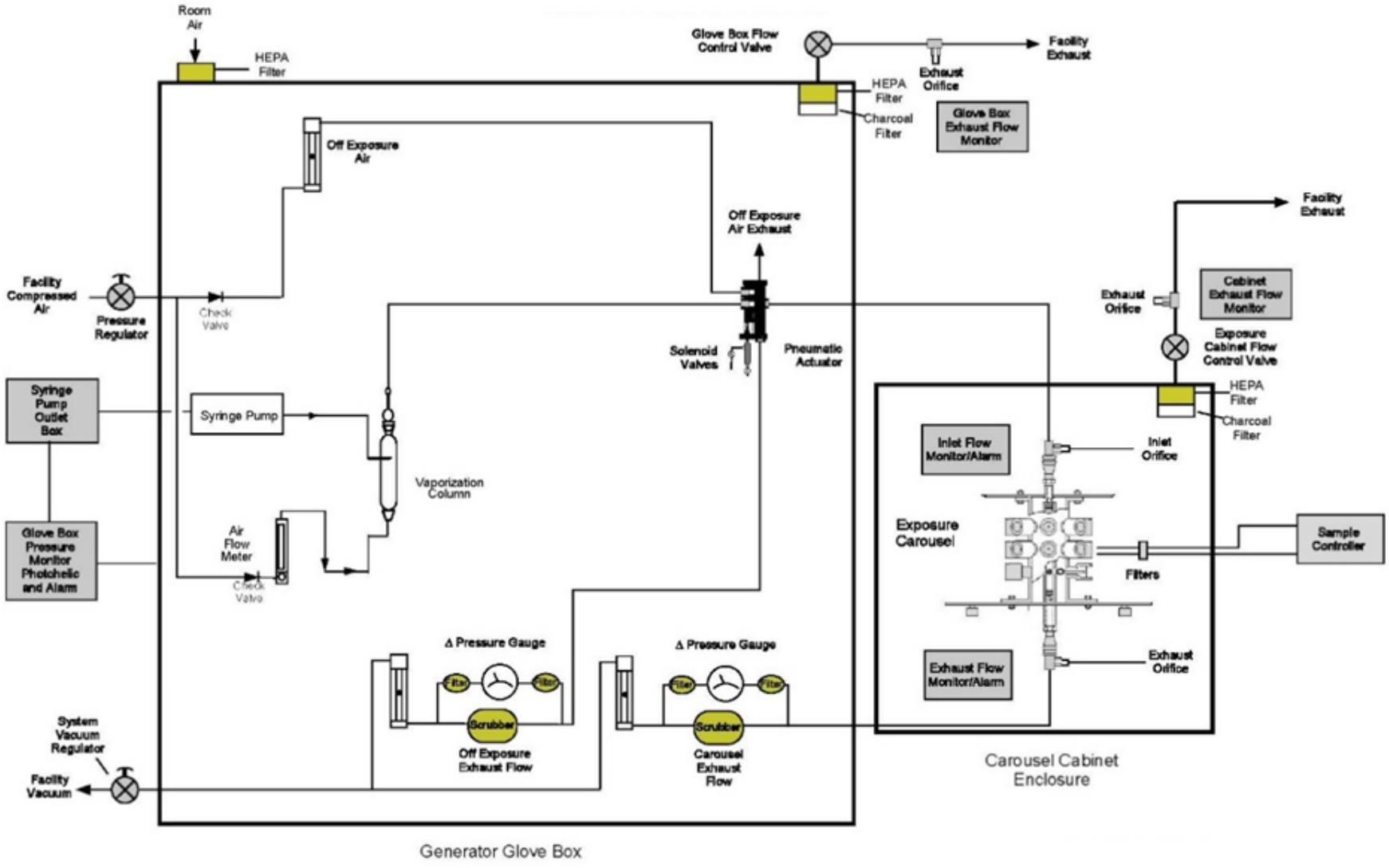

Figure 2. Trimethylsilyldiazomethane Generation, Distribution, and Exposure System

Vapor Concentration Monitoring

XAD-4 sorbent tubes were used for sampling TMSD and hexanes control exposure atmospheres. Sample flow rates and sampling times were documented (approximately 0.45 L/min for 30 minutes). Tube contents (front beds, back beds, and glass wool plugs) were transferred into 15 mL vials and extracted with 4 mL of 1,2,4-trimethylbenzene with vortexing for 15 minutes. Sample aliquots were transferred to GC vials with limited volume inserts for GC/MSD analysis. Samples were compared to solvent standard curves for TMSD and n-hexane as a component of the TMSD test article and n-hexane as a component of the mixed hexanes control article. The analysis was performed at the Battelle Columbus test site using an Agilent 6890 GC equipped with a 5973N MSD using the instrument settings listed in Table A-11. Average measured TMSD and hexanes concentrations for the 1-day and 5-day exposures are shown in Table 1.

Table 1. Summary of Target and Measured Trimethylsilyldiazomethane and Total Hexanes Concentrations for Male Rats and Mice in the One-day and Five-day Nose-only Inhalation Studies[a]

Exposure Group	Target TMSD Concentrations (ppm)	Measured TMSD Concentrations (ppm)		Measured Total Hexanes Concentrations (ppm)	
		Rats	Mice	Rats	Mice
One-day Exposure	Room air	ND	ND	BLOQ	ND
	Filtered air (0)	ND	ND	BLOQ	ND
	Hexanes[b] (0)	NA	NA	25.1 ± 0.7	24.1 ± 0.6
	10	11.3 ± 1.0	11.2 ± 0.2	26.9 ± 1.6	27.1 ± 0.6
Five-day Exposure	Room air	ND	ND	BLOQ	BLOQ
	Filtered air (0)	ND	ND	BLOQ	BLOQ
	Hexanes[b] (0)	NA	NA	24.5 ± 1.4	25.6 ± 1.5
	0.3	0.283 ± 0.047	0.299 ± 0.031	0.845 ± 0.098	1.05 ± 0.07
	1	1.06 ± 0.10	1.04 ± 0.17	3.20 ± 0.21	3.48 ± 0.37
	3	2.87 ± 0.25	2.68 ± 0.16	7.96 ± 0.45	7.90 ± 0.31
	10	10.3 ± 0.8	10.2 ± 0.7	25.4 ± 1.6	25.3 ± 1.2

ND = not detectable; NA = not applicable; BLOQ = below limit of quantitation.
[a]Data shown as mean ± standard deviation.
[b]The target hexanes concentration was 25 ppm.

Chamber Atmosphere Characterization

For homogeneity analysis, TMSD and hexanes sampling ports were sampled at the Battelle West Jefferson testing facility onto XAD-4 sorbent tubes for 30 minutes at a flow rate of approximately 0.45 L/min. At each concentration, six samples were shipped refrigerated to the Battelle Columbus test site: two from the top tier, two from the middle, and two from the bottom, for a total n = 6 for each target concentration. In each tier, two individual samples came from ports 2 and 6. The averaged results by tier for each target concentration to evaluate the homogeneity between the carousel locations and the averaged determined concentrations for each concentration to evaluate homogeneity of the entire carousel are presented in Table A-12, Table A-13, and Table A-14.

A condensation particle counter (Model 3022A; TSI Incorporated; St. Paul, MN) was used to count the particles before the start of generation and during generation. Particle counts <200 particles/cm^3 are typical of an exposure atmosphere when no generation is occurring. Particle counts above this level, especially if the counts increase with exposure concentration and are above the level during the off-exposure period, suggest a contribution to the aerosol concentration due to the generation system. The results of the particle count measurements are given in Table A-15. The data indicate no concentrations >200 particles/cm^3, either before or during the exposures; hence, the generation systems did not add particles to the aerosol concentration.

Analysis of generated vapor concentrations during the evaluation of exposure system homogeneity indicated reproducible TMSD and hexanes concentrations during 30-minute exposures of both rats and mice. The exposure system demonstrated homogeneity, with the carousel sampling within 10% relative standard deviation (RSD) for TMSD and total hexanes component concentrations in TMSD and hexanes. Final evaluation of target concentrations indicated that the TMSD vapor concentrations could be maintained between 15% and 20% of target. Relative consistency of the total hexanes concentrations was observed, with RSDs within 10% to 15% in respective exposure groups. Because the vapor generation was predicated on achieving TMSD targets, values for hexanes concentrations could have been biased toward concentrations associated with specific generator settings; therefore, relative error was not reported for hexanes.

Animal Source

Male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN). Male B6C3F1/N mice were obtained from Taconic Biosciences, Inc. (Germantown, NY).

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 AAALAC International. Studies were approved by the Battelle Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health and National Toxicology Program (NTP) animal care and use policies and applicable federal, state, and local regulations and guidelines.

Exposure Concentration Selection Rationale

A pilot study was performed (data not shown) to select a range of TMSD exposure concentrations to avoid high mortality or, alternatively, no chemical-induced effects. In the pilot study, rats and mice (approximately 8–10 weeks old) were exposed by nose-only inhalation to TMSD at Battelle. During the prestart exposure system characterization, animals were exposed by nose-only inhalation to targeted vapor concentrations of 0 (air), 0.2, 1, 5, or 20 ppm TMSD (n = 2/exposure concentration) for 30 minutes per day for up to three exposures. The highest TMSD exposure concentration tested (20 ppm) was selected on the basis of findings from an acute inhalation toxicity study with diazomethane in rabbits.[12] The lowest TMSD exposure concentration tested (0.2 ppm) was selected for the pilot study because it is the occupational

exposure limit (OEL; 8-hour time-weighted average [TWA]) for diazomethane. Both 20 ppm-exposed mice were found dead following the second exposure, and the lowest achievable exposure concentration was approximately 0.3 ppm. The exposure concentrations for the main study were adjusted accordingly on the basis of the outcomes of this pilot study.

Concentrations of 0 (air), 0 (hexanes), 0.3, 1, 3, and 10 ppm were selected to characterize the acute TMSD inhalation exposure response. The hexanes control generator for the 0 (hexanes) control group was set to a concentration equal to the estimated total hexanes content (25 ppm) in the 10 ppm TMSD generator. This was done to assess the effects of hexanes exposure alone at approximately an equivalent hexanes exposure concentration to that present in the highest TMSD exposure groups. Although the proportions of the individual hexane isomers varied between the TMSD lot and the mixed hexanes lot, the compositions of the test and control articles were sufficiently similar to determine whether hexanes were responsible for toxicity associated with exposure to TMSD.

Health and Safety

As with all NTP studies, extensive effort was expended to ensure the health and safety of study personnel, but this effort was undertaken with utmost consideration in this study of TMSD (see Appendix A) given the reported fatalities. There are no short-term (e.g., 10- to 30-minute) or 8-hour TWA daily occupational exposure limits for TMSD. An OEL of 0.1 ppm, however, was referenced in a Sigma-Aldrich TMSD Safety Data Sheet. For diazomethane, a similar chemical, the 8-hour TWA Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) are both 0.2 ppm. For n-hexane, the OSHA PEL is 500 ppm, the ACGIH TLV is 50 ppm, and the National Institute for Occupational Safety and Health (NIOSH) 10-hour TWA recommended exposure limit (REL) also is 50 ppm.

Occupational action levels typically are 50% of a TLV. Exceeding an action level in a work environment would prompt evaluation of the effectiveness of controls and might trigger additional engineering and administrative controls to limit personnel exposure, supplemented with increased use of appropriate personal protective equipment. Battelle therefore used 0.1 ppm TMSD (a value listed as the OEL in a TMSD Safety Data Sheet and that is 50% of the TLV for diazomethane) as its OEL.

One-day and Five-day Studies

Rats and mice were approximately 7 to 8 weeks old on receipt; they were quarantined for 11 days and were approximately 9 to 11 weeks old on the first day of the studies. Rats and mice were uniquely identified and randomly assigned to one of six exposure groups before the start of the study. Randomization was stratified by body weight that produced similar group mean weights using NTP Provantis software (Instem, Sone, UK).

Before the studies began, 10 rats and 10 mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Additionally, the health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of eight male rats and eight male mice were exposed to 0 (air), 0 (hexanes), or 10 ppm TMSD (in hexanes) by nose-only inhalation for 1 day (30 minutes) with or without a 9-day recovery period after exposure (1-day exposure, 1-day exposure – recovery). In addition, groups of eight male rats and eight male mice were exposed to 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm TMSD (in hexanes) by nose-only inhalation for up to 5 days (30 minutes/day) with or without a 5-day recovery period after exposure (5-day exposure, 5-day exposure – recovery). The 0 (hexanes) control group was exposed to 25 ppm hexanes only (0 ppm TMSD), which is the concentration of hexanes in the highest TMSD exposure concentration (10 ppm). For the groups without a recovery phase (1-day exposure and 5-day exposure), animals were necropsied the day after the single or final exposure, respectively. For the groups with a recovery phase, animals were necropsied 9 days after the single exposure (1-day exposure – recovery) or 5 days after the final exposure (5-day exposure – recovery). Details of the study design are summarized in Figure 3.

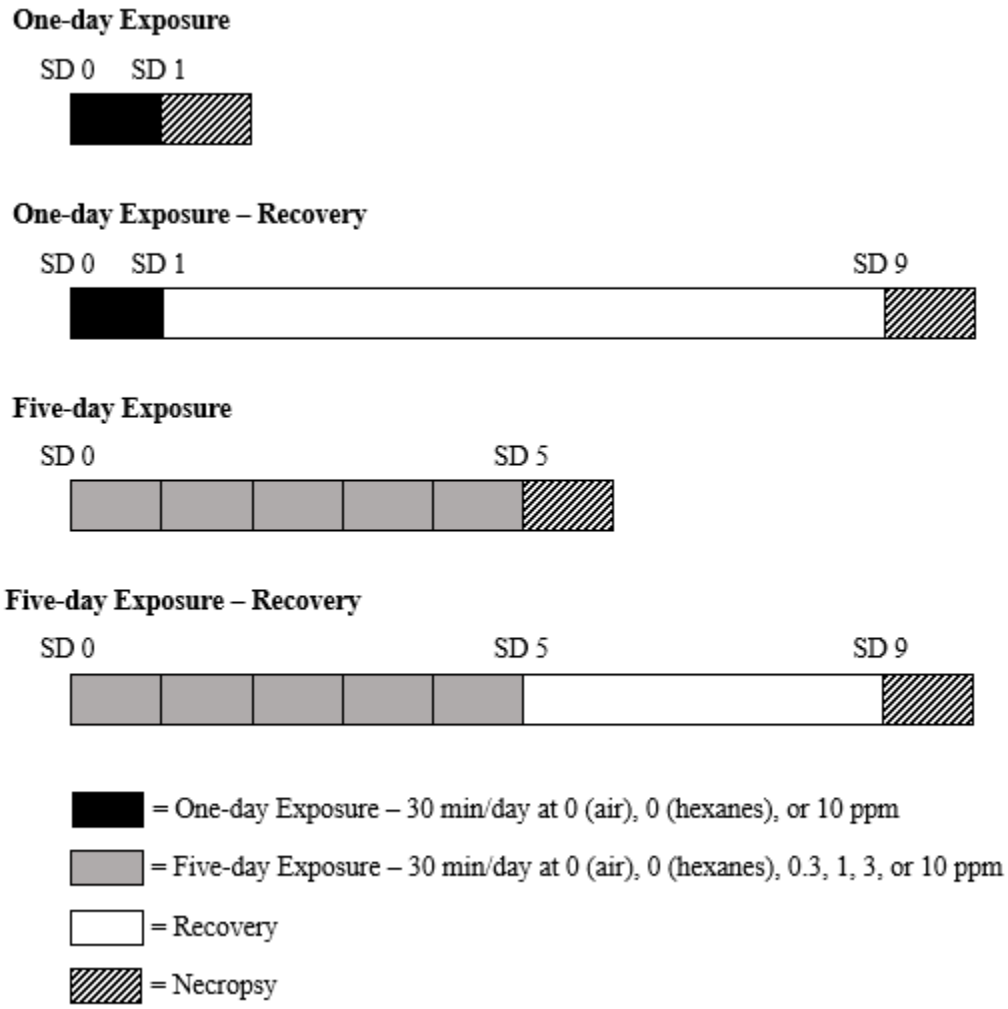

Figure 3. Experimental Design in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

SD = study day.

Feed and water were available ad libitum except during exposures. Rats and mice were pair housed upon receipt and during the first week of quarantine and were individually housed thereafter. Rats and mice were observed twice daily for signs of mortality or moribundity. After the onset of dyspnea following exposure, animals were observed every 1–2 hours postexposure until 11:00 p.m. for signs of moribundity/mortality. Formal clinical observations were recorded twice daily on exposure days (once before and once after exposure), once daily during recovery, and prior to necropsy. Individual body weights were recorded daily for all animals in the study. Further details of animal maintenance are summarized in Table 2. Information on feed composition and contaminants is provided in Appendix B.

Necropsies were performed on all animals. Organ weights were determined for the brain, liver, thymus, left and right kidneys, left and right testes, left and right epididymides, heart, and lung from all animals that survived to scheduled sacrifice or were euthanized early due to moribund condition (no organ weights were determined for animals that died early). Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin. Complete histopathological examinations were performed by the study laboratory pathologist for all animals (including those that died early) on the nasal cavity and turbinates, larynx, pharynx, trachea, left lung and mainstem bronchi, mediastinal lymph nodes, heart and aorta, left kidney, liver, brain, and peripheral nerves (sciatic and trigeminal nerves with ganglion; tibial nerve in rats only). This evaluation was conducted to a no-effect level and included the corresponding tissues in the air control group and hexanes control group. Table 2 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathological slides by a quality assessment (QA) pathologist, the findings in the respiratory system and any discrepancies between the study laboratory pathologist (SP) and QA pathologist were reviewed by an NTP Pathology Working Group (PWG). Any inconsistencies in the diagnoses made by the SP and QA pathologist were resolved through the NTP pathology peer-review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the SP, NTP pathologists, and QA pathologist. Details of these review procedures have been described, in part, by Maronpot and Boorman[18] and Boorman et al.[19]

Table 2. Experimental Design and Materials and Methods in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

Rats	Mice
Study Laboratory	
Battelle (Columbus, OH)	Same as rats
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®)	B6C3F1/N
Animal Source	
Envigo (formerly Harlan Laboratories, Inc.; Indianapolis, IN)	Taconic Biosciences, Inc. (Germantown, NY)

Rats	Mice
Time Held before Studies	
1-day exposure studies: 11 days 5-day exposure studies: 13 days	1-day exposure studies: 12 days 5-day exposure studies: 18 days
Average Age When Studies Began	
1-day exposure studies: 9 weeks 5-day exposure studies: 10 weeks	1-day exposure studies: 9 to 10 weeks 5-day exposure studies: 10 to 11 weeks
Date of First Exposure	
1-day exposure studies: October 12, 2015 5-day exposure studies: October 14, 2015	1-day exposure studies: October 13, 2015 5-day exposure studies: October 19, 2015
Duration of Exposure	
1 day or 5 days	Same as rats
Date of Last Exposure	
1-day exposure studies: October 12, 2015 5-day exposure studies: October 18, 2015	1-day exposure studies: October 13, 2015 5-day exposure studies: October 23, 2015
Necropsy Dates	
1-day exposure: October 13, 2015 1-day exposure – recovery: October 21, 2015 5-day exposure: October 19, 2015 5-day exposure – recovery: October 23, 2015	1-day exposure: October 14, 2015 1-day exposure – recovery: October 22, 2015 5-day exposure: October 24, 2015 5-day exposure – recovery: October 28, 2015
Average Age at Necropsy	
1-day exposure studies: 9 to 11 weeks 5-day exposure studies: 10 to 11 weeks	1-day exposure studies: 10 to 11 weeks 5-day exposure studies: 11 to 12 weeks
Size of Study Groups	
8 males	Same as rats
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as rats
Animals per Cage	
1	Same as rats
Method of Animal Identification	
Tail tattoo and cage card	Same as rats
Diet	
NTP-2000 irradiated wafer feed (Zeigler Brothers Inc., Gardners, PA), available ad libitum except during exposure, changed at least once weekly	Same as rats[a]
Water	
Tap water (West Jefferson, OH municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum except during exposure	Same as rats
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE); changed weekly	Same as rats

Rats	Mice
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as rats
Rack Filters	
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed at least every 2 weeks	Same as rats
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated at least every 2 weeks	Same as rats
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as rats
Exposure Concentrations	
1-day exposure studies: 0 (air), 0 (hexanes), 10 ppm TMSD (in hexanes) by nose-only inhalation 5-day exposure studies: 0 (air), 0 (hexanes), 0.3, 1, 3, or 10 ppm TMSD (in hexanes) by nose-only inhalation	Same as rats
Type and Frequency of Observation	
Observed twice daily and every 1 to 2 hours postexposure following the last scheduled clinical observation of the day until 11:00 p.m. Animals were weighed daily. Clinical observations were recorded twice daily on exposure days and once daily during recovery and on the day of removal from study.	Same as rats
Method of Euthanasia	
Exsanguination while under CO_2/O_2 anesthesia	Same as rats
Necropsy	
Necropsies were performed on all animals. Organs weighed were brain, left and right epididymis, heart, left and right kidney, liver, lung, left and right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were brain, left and right epididymis, heart, left and right kidney, liver, lung, left and right testis, and thymus. No organ weights were determined for animals that died early.
Histopathology	
Histopathological evaluations of brain (7 sections including [1] olfactory bulbs, [2] front-parietal cortex including basal ganglia, [3] mid-parietal cortex and thalamus, [4] mid-brain with substantia nigra and red nucleus, [5] posterior colliculi, [6] mid-cerebellum including cranial nerve VIII, and [7] posterior medulla), gross lesions, heart and aorta, kidney (left), larynx, liver (two sections including left lateral lobe and median lobe), lung and mainstem bronchi (left), nasal cavity and nasal turbinates (3 sections), nerve (sciatic, tibial, and trigeminal with ganglion), mediastinal lymph nodes, pharynx, and trachea were performed as available on all study rats. Organs/tissues were examined to a no-effect level, as were corresponding tissues, in the 0 ppm air and hexanes control animals.	Same as rats

[a]One feeder on one occasion was changed after 11 days.

Statistical Methods

For all statistical analyses, pairwise and trend comparisons were performed between the exposed groups and the air control group. A separate pairwise comparison was performed between the hexanes control group and the air control group.

Calculation and Analysis of Nonneoplastic Lesion Incidences

The incidences of nonneoplastic lesions are presented as numbers of animals bearing such lesions at a specific anatomical site and the numbers of animals with that site examined microscopically. Fisher's exact (pairwise) test,[20] a procedure that uses the overall proportion of affected animals, was used to determine significance between TMSD-exposed and air control animals, and also between the hexanes control animals and air control animals. The Cochran-Armitage trend test was used to test for significant trends.[21] All p values for lesion incidence were calculated using one-sided tests.

Analysis of Continuous Variables

Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey[22] were examined by NTP personnel, and implausible values were eliminated from the analysis.

Pairwise comparisons between exposed and air control groups in the analysis of organ and body weight data, which historically have approximately normal distributions, were performed using the parametric multiple comparison procedures of Dunnett[23] and Williams.[24; 25] The Jonckheere test[26] was used to assess the significance of the exposure-related trends and to determine whether the trend-sensitive test (the Williams test) was more appropriate for pairwise comparisons than would be the test that does not assume a monotonic exposure-related trend (the Dunnett test). For the comparisons between the hexanes control group and the air control group for organ and body weights, a Student t-test was used.[22-26]

Quality Assurance Methods

The 1-day and 5-day studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations.[27] In addition, the 1-day and 5-day study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP Archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity 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 Toxicity Report.

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-TOX-101.[28]

Rats: One-day and Five-day Studies

All trimethylsilyldiazomethane (TMSD)-exposed rats survived until scheduled euthanasia (Table 3). Final mean body weights in the 1-day exposure and 1-day exposure – recovery groups were unaffected by TMSD exposure; however, the final mean body weights for all rats in the 1-day exposure group were lower than the initial mean body weights (Table 4; Figure 4A, Figure 4B). Final mean body weights were significantly decreased in the 5-day exposure and 5-day exposure – recovery groups compared to the air control groups at the highest TMSD exposure concentration (10 ppm) (Table 4; Figure 4C, Figure 4D). Final mean body weight gains in the 5-day exposure and 5-day exposure – recovery groups at 10 ppm TMSD were also significantly decreased relative to the air control groups.

Table 3. Summary of the Disposition of Male Rats in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

Exposure Group	0 ppm (air)	0 ppm (hexanes)[a]	0.3 ppm	1 ppm	3 ppm	10 ppm
One-day Exposure[b]						
Animals Initially in Study	8	8	–[c]	–	–	8
Early Deaths	0	0	–	–	–	0
Scheduled Sacrifice, Terminal	8	8	–	–	–	8
Animals Examined Microscopically	8	8	–	–	–	8
One-day Exposure – Recovery[d]						
Animals Initially in Study	8	8	–	–	–	8
Early Deaths	0	0	–	–	–	0
Scheduled Sacrifice, Terminal	8	8	–	–	–	8
Animals Examined Microscopically	8	8	–	–	–	8
Five-day Exposure[e]						
Animals Initially in Study	8	8	8	8	8	8
Early Deaths	0	0	0	0	0	0
Scheduled Sacrifice, Terminal	8	8	8	8	8	8
Animals Examined Microscopically	8	8	0[f]	8	8	8
Five-day Exposure – Recovery[g]						
Animals Initially in Study	8	8	8	8	8	8

Exposure Group	0 ppm (air)	0 ppm (hexanes)[a]	0.3 ppm	1 ppm	3 ppm	10 ppm
Early Deaths	0	0	0	0	0	0
Scheduled Sacrifice, Terminal	8	8	8	8	8	8
Animals Examined Microscopically	8	8	8	8	8	8

[a]Hexanes control contained an equivalent concentration of hexanes (25 ppm) as the 10 ppm TMSD-exposed group.
[b]Animals were exposed on study day 0 and necropsied on study day 1.
[c]Exposures at this concentration were not conducted for this group.
[d]Animals were exposed on study day 0 and necropsied on study day 9.
[e]Animals were exposed on study days 0–4 and necropsied on study day 5.
[f]Slides were not evaluated because no effects (except for focal chronic-active inflammation) were observed in the 1 ppm TMSD-exposed group.
[g]Animals were exposed on study days 0–4 and necropsied on study day 9.

Table 4. Summary of Mean Body Weights of Male Rats in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane[a,b]

Exposure Group	Initial Body Weight (g)	Final Body Weight (g)	Changes in Body Weight (g)	Final Weight Relative to Controls (%)
One-day Exposure				
0 ppm (air)	268.7 ± 2.6	261.4 ± 2.4	−7.3 ± 1.1	–
0 ppm (hexanes)[c]	263.7 ± 6.5	260.9 ± 3.1	−2.8 ± 4.4	−0.2
10 ppm	267.4 ± 3.3	258.6 ± 2.6	−8.8 ± 1.1	−1.1
One-day Exposure – Recovery				
0 ppm (air)	267.3 ± 2.7	302.5 ± 4.2	35.2 ± 1.9	–
0 ppm (hexanes)	271.3 ± 2.5	307.8 ± 3.3	36.5 ± 1.5	1.7
10 ppm	263.9 ± 3.6	299.8 ± 4.0	35.9 ± 1.4	−0.9
Five-day Exposure				
0 ppm (air)	272.4 ± 3.8	280.5 ± 4.1**	8.1 ± 1.2**	–
0 ppm (hexanes)	276.9 ± 2.9	283.3 ± 4.1	6.4 ± 1.8	1.0
0.3 ppm	279.7 ± 2.9	287.4 ± 3.1	7.8 ± 1.0	2.5
1 ppm	281.6 ± 1.5	288.4 ± 2.1	6.9 ± 1.1	2.8
3 ppm	275.8 ± 2.1	280.4 ± 2.7	4.6 ± 1.6	0.0
10 ppm	280.8 ± 2.6	247.5 ± 4.0**	−33.2 ± 2.9**	−11.7
Five-day Exposure – Recovery				
0 ppm (air)	280.2 ± 3.6	303.2 ± 3.6**	23.0 ± 1.7**	–
0 ppm (hexanes)	275.8 ± 2.7	296.5 ± 3.1	20.7 ± 3.2	−2.2
0.3 ppm	278.9 ± 2.7	306.0 ± 2.9	27.1 ± 1.6	0.9
1 ppm	278.6 ± 2.4	301.7 ± 3.0	23.0 ± 1.0	−0.5
3 ppm	280.0 ± 2.0	303.0 ± 3.6	22.9 ± 2.5	−0.1
10 ppm	277.8 ± 1.8	271.6 ± 1.8**	−6.3 ± 1.5**	−10.4

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Statistical analysis for the hexanes control group compared to the air control group was performed using the t-test.

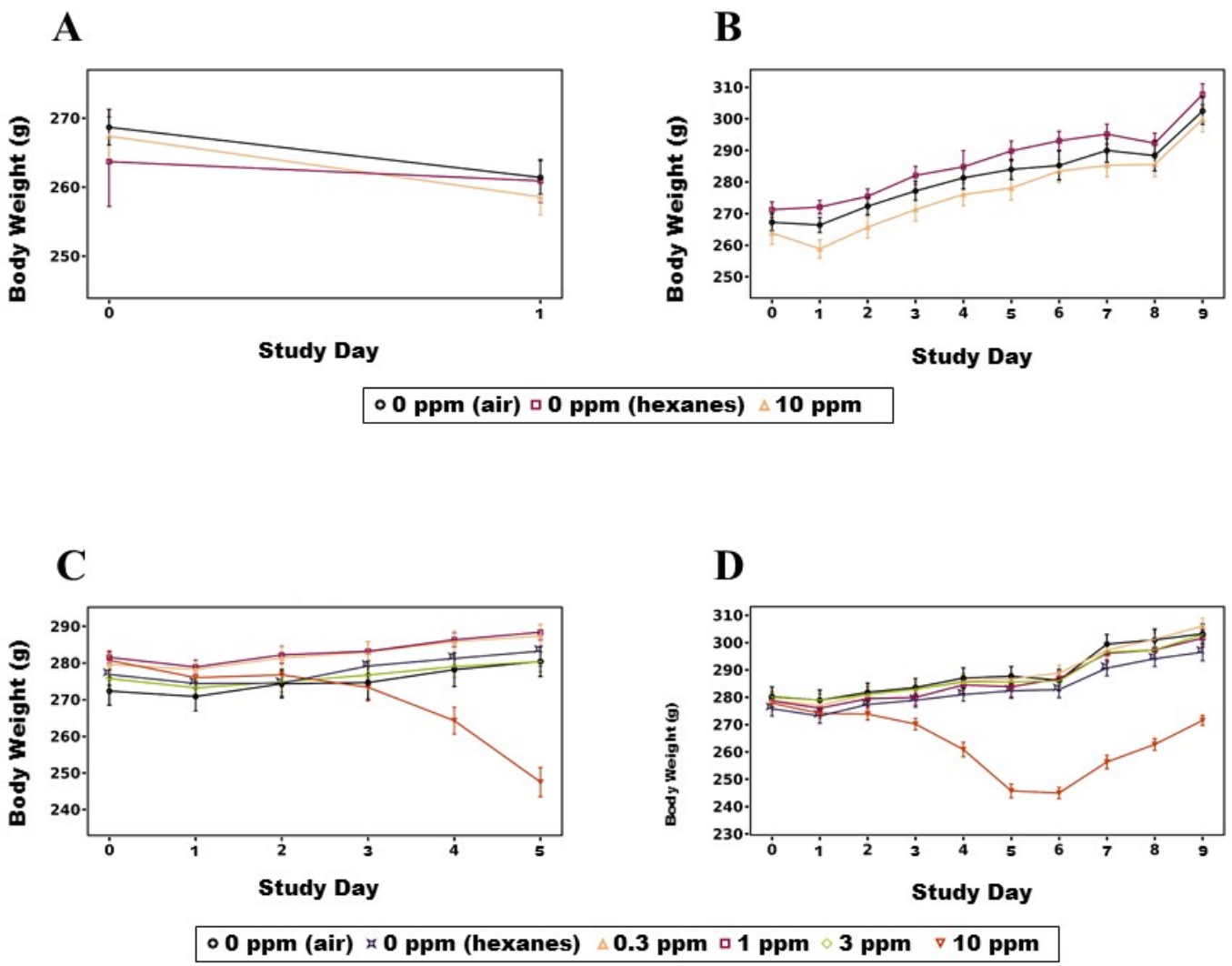

Figure 4. Growth Curves for Male Rats in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

(A) 1-day exposure group, (B) 1-day exposure – recovery group, (C) 5-day exposure group, (D) 5-day exposure – recovery group.

Clinical observations affecting four or more of the eight rats exposed to 10 ppm TMSD in the 5-day exposure and/or 5-day exposure – recovery groups were ruffled coat between study day 2 and 9, discharge from nose/snout, and labored breathing; one rat exhibited hunched posture on study day 7 (Table 5). There were no exposure-related clinical observations in the 1-day exposure and 1-day – exposure recovery groups (Appendix D). Gross pathology observations in rats exposed to 10 ppm TMSD in both the 5-day exposure and 5-day exposure – recovery groups included mottled discoloration of the lung and enlarged, dark mediastinal lymph nodes. (Appendix D).

Table 5. Summary of Clinical Observations for Male Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure[a]						
Ruffled Coat	0/8	0/8	0/8	0/8	0/8	4/8 (SD 2)
Discharge from Nose/Snout; Red	0/8	0/8	0/8	0/8	0/8	4/8 (SD 4)
Five-day Exposure – Recovery						
Ruffled Coat	0/8	0/8	0/8	0/8	0/8	7/8 (SD 4)
Discharge from Nose/Snout; Red	0/8	0/8	0/8	0/8	0/8	6/8 (SD 6)
Labored Breathing	0/8	0/8	0/8	0/8	0/8	4/8 (SD 7)
Hunched Posture	0/8	0/8	0/8	0/8	0/8	1/8 (SD 7)

SD = study day.
[a]Upper row displays cumulative number of animals with observation/total animals started in study. Lower row displays SD of observation onset.

Mean absolute and relative lung weights were significantly decreased at 10 ppm TMSD in the 1-day exposure group and significantly increased at 10 ppm in the 1-day exposure – recovery group compared to the air control group (Table 6). Mean absolute and relative lung weights were significantly increased at 3 and 10 ppm in the 5-day exposure group and at all TMSD exposure concentrations in the 5-day exposure – recovery group compared to the air control group (Table 7). In the 5-day exposure group, mean absolute and relative weights of the right and left kidneys were significantly increased at 1 ppm; mean absolute and relative liver weights were significantly decreased at 10 ppm (Table 7). Mean absolute or relative weight changes for other organs (brain, right epididymis, heart, right and left testes, and thymus in the 5-day exposure group; brain, heart, right and left kidneys, liver, left testis, and thymus in the 5-day exposure – recovery group) were considered secondary to decreased body weight.

Table 6. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the One-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a,b]

	0 ppm (air)	0 ppm (hexanes)	10 ppm
n	8	8	8
One-day Exposure			
Necropsy Body Wt. (g)	261.4 ± 2.4	260.9 ± 3.1	258.6 ± 2.6
Lung			
Absolute (g)	2.25 ± 0.11	2.07 ± 0.13	1.83 ± 0.11*
Relative (mg/g)[c]	8.63 ± 0.43	7.90 ± 0.45	7.06 ± 0.37*
One-day Exposure – Recovery			
Necropsy Body Wt. (g)	302.5 ± 4.2	307.8 ± 3.3	299.8 ± 4.0
Lung			
Absolute (g)	1.84 ± 0.06	1.97 ± 0.11	2.04 ± 0.06*
Relative (mg/g)	6.08 ± 0.18	6.37 ± 0.31	6.82 ± 0.19*

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group.
*Statistically significant at $p \leq 0.05$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Table 7. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a,b]

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
n	8	8	8	8	8	8
Five-day Exposure						
Necropsy Body Wt. (g)	280.5 ± 4.1**	283.3 ± 4.1	287.4 ± 3.1	288.4 ± 2.1	280.4 ± 2.7	247.5 ± 4.0**
Lung						
Absolute (g)	1.51 ± 0.04**	1.56 ± 0.06	1.54 ± 0.04	1.69 ± 0.07	2.04 ± 0.07**	4.52 ± 0.12**
Relative (mg/g)[c]	5.40 ± 0.15**	5.49 ± 0.17	5.37 ± 0.15	5.85 ± 0.23	7.28 ± 0.23**	18.25 ± 0.41**
R. Kidney						
Absolute (g)	0.88 ± 0.03	0.90 ± 0.02	0.94 ± 0.01	1.01 ± 0.03**	0.88 ± 0.03	0.87 ± 0.03
Relative (mg/g)	3.14 ± 0.07*	3.17 ± 0.07	3.27 ± 0.04	3.50 ± 0.11*	3.13 ± 0.07	3.49 ± 0.09*
L. Kidney						
Absolute (g)	0.86 ± 0.03	0.91 ± 0.02	0.94 ± 0.02	0.99 ± 0.03**	0.88 ± 0.03	0.85 ± 0.02
Relative (mg/g)	3.06 ± 0.09*	3.23 ± 0.08	3.26 ± 0.06	3.44 ± 0.09**	3.13 ± 0.08	3.42 ± 0.07**
Liver						
Absolute (g)	10.99 ± 0.35**	10.93 ± 0.30	11.14 ± 0.29	11.84 ± 0.38	10.44 ± 0.42	8.24 ± 0.32**
Relative (mg/g)	39.15 ± 0.87**	38.57 ± 0.70	38.72 ± 0.74	41.04 ± 1.15	37.18 ± 1.23	33.26 ± 1.06**

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure – Recovery						
Necropsy Body Wt. (g)	303.2 ± 3.6**	296.5 ± 3.1	306.0 ± 2.9	301.7 ± 3.0	303.0 ± 3.6	271.6 ± 1.8**
Lung						
Absolute (g)	1.75 ± 0.07**	1.82 ± 0.08	2.33 ± 0.13**	2.14 ± 0.16*	2.67 ± 0.16**	3.58 ± 0.07**
Relative (mg/g)	5.76 ± 0.19**	6.15 ± 0.24	7.59 ± 0.37**	7.08 ± 0.51**	8.78 ± 0.47**	13.19 ± 0.24**

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Pathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the lung and mediastinal lymph nodes in rats. Summaries of the incidences of nonneoplastic lesions and statistical analyses of primary nonneoplastic lesions are presented in Appendix D. All tissues evaluated were assigned a severity score using a scale of minimal (1), mild (2), moderate (3), or marked (4) representing the involvement of approximately <10%, 10%–25%, 26%–50%, or >50% of the affected tissue, respectively.

No exposure-related histopathological lesions were present in the 1-day exposure and 1-day exposure – recovery groups after a single exposure to TMSD in rats. Exposure-related histopathological lesions were observed in the lung and mediastinal lymph nodes in the 5-day exposure and 5-day exposure – recovery groups after 5 days of repeated exposure to TMSD in rats and are described below. No exposure-related histopathological changes were observed in the lung or mediastinal lymph nodes in the hexane control group.

Lung: In the lung, exposure-related histopathological findings were observed at concentrations of 3 and 10 ppm TMSD in the 5-day exposure group compared to the air control group (Table 8, Figure 5). At 3 ppm TMSD, chronic-active inflammation was present in the lungs and was characterized by increased infiltrates of lymphocytes, histiocytes, and scattered neutrophils that infiltrated and expanded the alveolar, bronchiolar, and bronchial interstitia, and extended into the alveolar spaces. These histopathological changes also were associated with hyperplastic alveolar epithelia characterized by increased numbers of cuboidal to pleomorphic type II pneumocytes lining the alveolar walls. Frequently, the hyperplastic alveolar cells displayed increased nuclear:cytoplasmic ratio, prominent nucleoli, cellular pleomorphism, and mitotic figures. Hyperplastic bronchiolar epithelial cells also were observed and were characterized by increased numbers of bronchiolar epithelia lining terminal bronchioles. Histiocytic cellular infiltrates, composed of clusters of macrophages in the alveolar spaces, also were observed at 3 ppm TMSD (Figure 5). At 10 ppm TMSD, more advanced histopathological lesions were observed and consisted of chronic-active inflammation with interstitial fibrosis characterized by deposition of a fibrillar to slightly thickened eosinophilic matrix in the alveolar septa or subpleural areas, in addition to regions of acute hemorrhage and pulmonary edema (Figure 5). The pulmonary edema was present in most rats (7/8) in the 10 ppm TMSD 5-day exposure group and consisted of eosinophilic, homogeneous proteinaceous material that expanded the alveolar spaces and/or the interstitium. Pulmonary edema often was associated with large foamy histiocytic infiltrates in the

alveolar spaces. Average severity scores of lesions observed in rats exposed to 3 ppm TMSD in the 5-day exposure group were classified as minimal; however, mean severity grades for lesions in the 10 ppm TMSD group ranged from minimal to mild (Table 8).

In the lung, exposure-related histopathological findings were observed at concentrations ≥0.3 ppm TMSD in the 5-day exposure – recovery group compared to the air control group (Figure 6). Lesions were similar to those described for the 5-day exposure group. At the lowest exposure concentrations of TMSD (0.3 and 1 ppm), chronic-active inflammation, histiocytic infiltrates, acute hemorrhage, and alveolar epithelial hyperplasia were observed in a few animals in one or both groups (Table 8). At 3 ppm, the predominant lung lesions observed in rats were chronic-active inflammation and histiocytic cellular infiltrates in the alveoli, with a few rats showing alveolar epithelial hyperplasia (2/8) and acute hemorrhage (3/8) (Table 8; Figure 6). At 10 ppm, lung lesions consisted of areas of chronic-active inflammation within the alveolar, bronchiolar, and bronchial interstitia that extended into the alveolar spaces. Also, alveolar or bronchiolar hyperplasia, acute hemorrhage, pulmonary edema, and interstitial fibrosis were present (Table 8; Figure 6). Average severity grades of lesions observed in rats exposed to 0.3, 1, and 3 ppm TMSD in the 5-day exposure – recovery group were minimal; however, mean severity grades for lesions in the 10 ppm TMSD group ranged from minimal to mild (Table 8).

Mediastinal lymph node: In the mediastinal lymph node, the major TMSD-induced histopathological change was infiltrates of histiocytic cells that expanded the paracortex, medullar sinuses, and subcapsular sinuses that were present in the 5-day exposure and 5-day exposure – recovery groups after exposure to 3 or 10 ppm TMSD (Table 8).

Larynx: In the larynx, possible exposure-related histopathological findings were observed in two animals at 10 ppm TMSD in the 5-day exposure group: squamous epithelial hyperplasia and squamous epiglottis metaplasia (Table 8).

Table 8. Incidences of Nonneoplastic Lesions of the Lung, Mediastinal Lymph Node, and Larynx in Male Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a]

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure						
Lung[b]	8	8	0[c]	8	8	8
Inflammation, chronic-active[d]	0**	0	–	0	7** (1.1)[e]	8** (2.8)
Infiltration, cellular, histiocyte	0*	0	–	0	7** (1.0)	0
Interstitium, fibrosis	0**	0	–	0	0	8** (1.0)
Edema	0**	0	–	0	0	7** (2.3)
Hemorrhage, acute	0**	0	–	0	0	8** (1.0)
Alveolar epithelium, hyperplasia	0**	0	–	0	6** (1.0)	8** (2.5)
Bronchiole epithelium, hyperplasia	0**	0	–	0	7** (1.0)	8** (1.0)
Mediastinal Lymph Node	8	8	0	7	8	8
Infiltration, cellular, histiocyte	0**	2 (1.0)	–	0	1 (1.0)	8** (2.0)
Larynx	8	8	0	0	0	8
Epithelial cell, hyperplasia, squamous	0	0	–	–	–	2 (1.0)
Epiglottis, metaplasia, squamous	0	0	–	–	–	2 (1.0)
Five-day Exposure – Recovery						
Lung	8	8	8	8	8	8
Inflammation, chronic-active	0**	0	0	2 (1.0)	8** (1.0)	8** (2.6)
Infiltration, cellular, histiocyte	0**	0	1 (1.0)	2 (1.0)	8** (1.0)	0
Interstitium, fibrosis	0**	0	0	0	0	8** (1.0)
Edema	0**	0	0	0	0	7** (1.9)
Hemorrhage, acute	0**	0	1 (1.0)	1 (1.0)	3 (1.0)	8** (1.0)
Alveolar epithelium, hyperplasia	0**	0	0	1 (1.0)	2 (1.0)	8** (2.1)
Bronchiole epithelium, hyperplasia	0**	0	0	0	0	6** (1.0)
Mediastinal Lymph Node	8	8	0	8	8	8
Infiltration, cellular, histiocyte	1** (1.0)	0	–	0	5 (1.0)	8** (1.9)

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Number of animals with tissue examined microscopically.
[c]Slides not evaluated.
[d]Number of animals with lesion.
[e]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

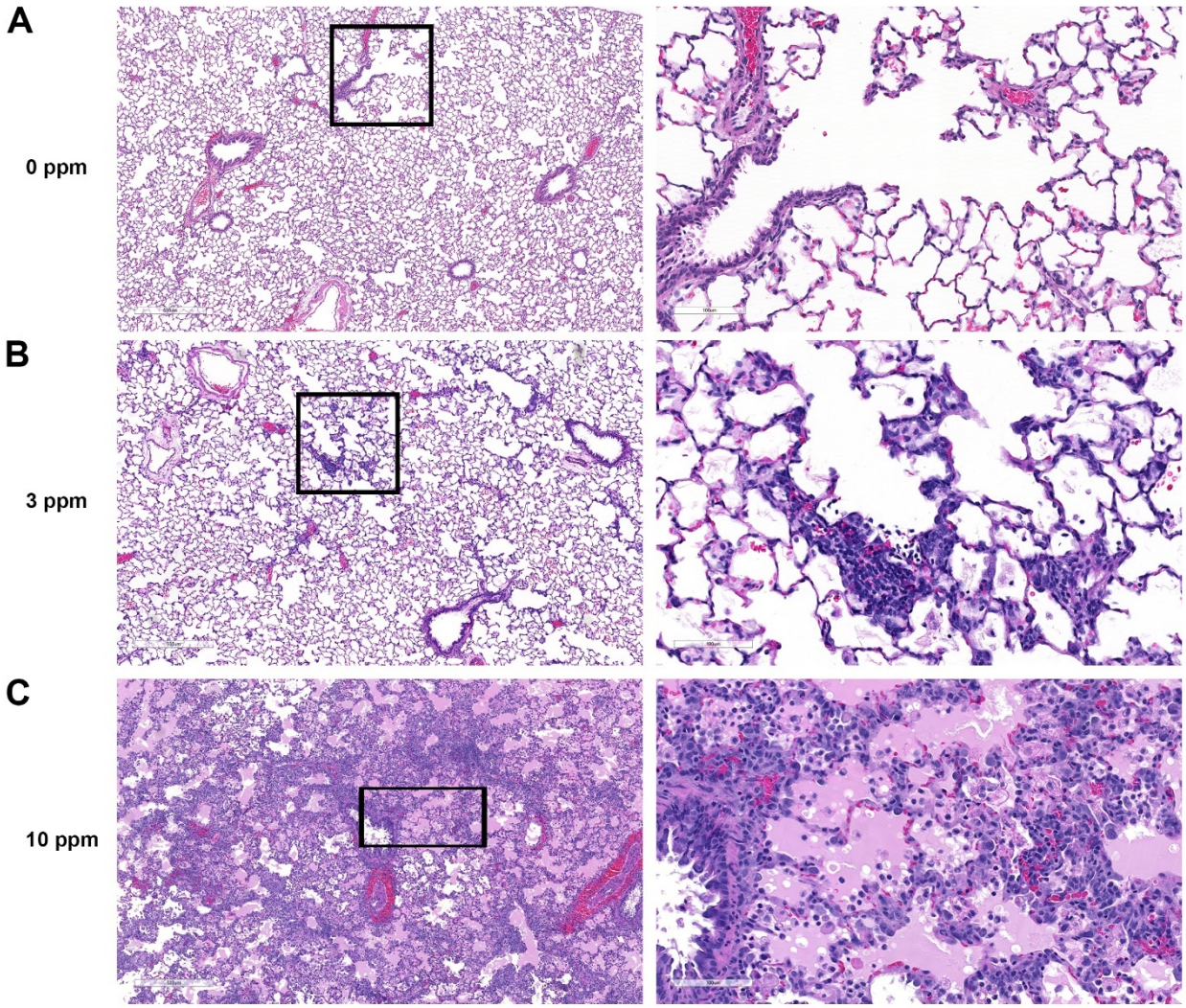

Figure 5. Representative Images of the Lung from Male Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane (Five-day Exposure Group) (H&E)

(A) Lung from rat exposed to 0 ppm (air) showing terminal and respiratory bronchioles, alveolar ducts, and alveoli. (B) Lung from rat exposed to 3 ppm TMSD showing inflammatory cell infiltrates in the alveolar septa (severity grade = 1) with numerous histiocytes in the alveolar spaces (severity grade = 1). (C) Lung from rat exposed to 10 ppm TMSD showing chronic-active inflammation with acute hemorrhage (severity grade = 1) and extensive edema (severity grade = 4). Left panels are 4x and right panels are 20x.

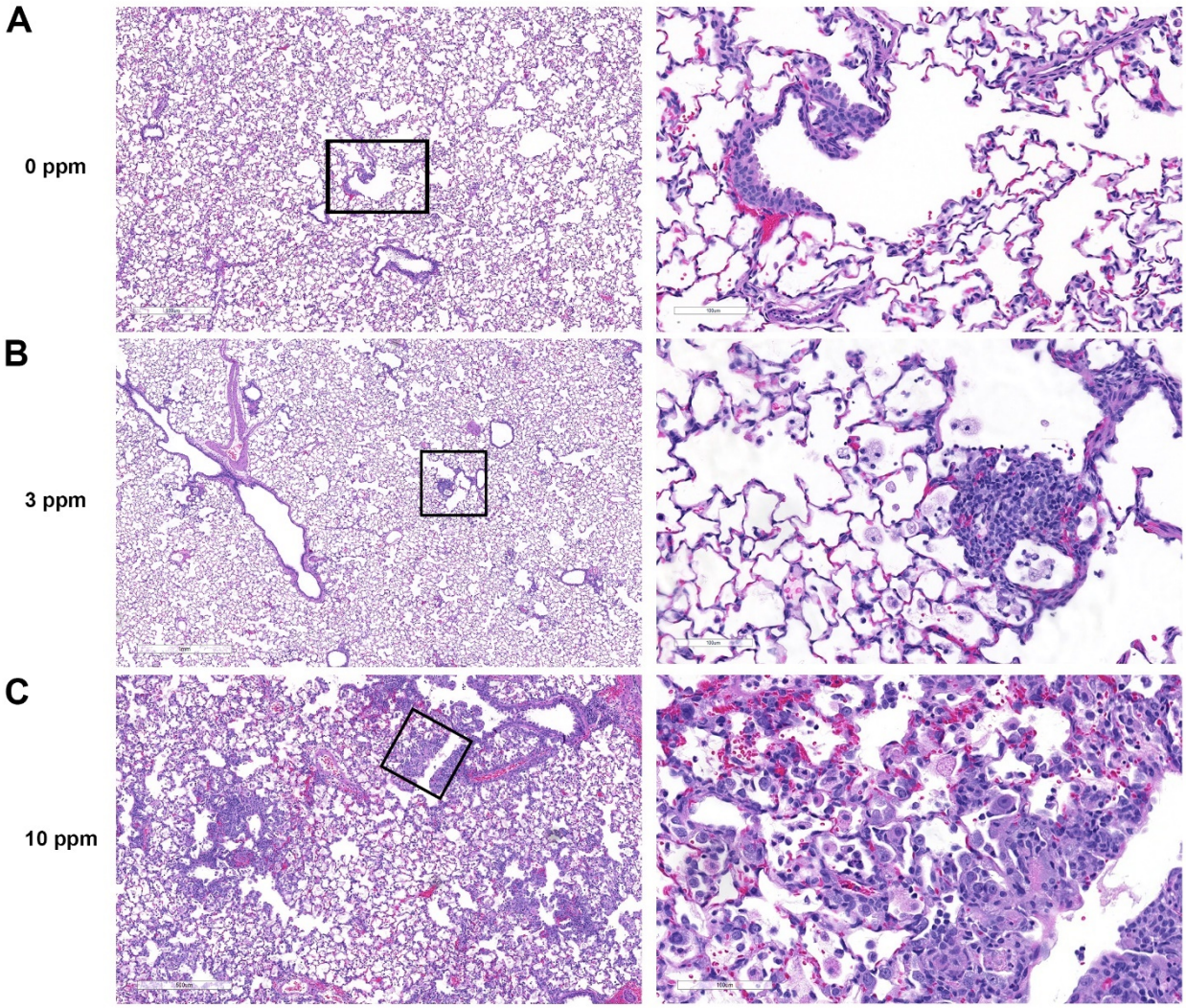

Figure 6. Representative Images of the Lung from Male Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane (Five-day Exposure – Recovery Group) (H&E)

(A) Lung from rat exposed to 0 ppm (air) showing terminal and respiratory bronchioles, alveolar ducts, and alveoli. (B) Lung from rat exposed to 3 ppm TMSD showing chronic-active inflammation of the alveolar septa (severity grade = 1) with infiltrates of histiocytes in the alveolar spaces (severity grade = 1). (C) Lung from rat exposed to 10 ppm TMSD showing chronic-active inflammation (severity grade = 2) with hemorrhage (severity grade = 1) and interstitial fibrosis (severity grade=1). Left panels are 4x and right panels are 20x.

Mice: One-day and Five-day Studies

Inhaled TMSD was more toxic in mice than in rats. All mice in the 1-day exposure group survived to study termination, whereas five mice in the 1-day exposure – recovery group, which were exposed to 10 ppm TMSD, died early between study days 3 and 6 (Table 9). In the 5-day exposure and 5-day exposure – recovery groups, all 10 ppm TMSD-exposed mice died or were euthanized early on study day 2 or 3 after only three exposures (none of the 10 ppm-exposed mice survived to study day 5 or 9, whereas mice in all other exposure groups survived to study termination) (Table 9).

Final mean body weights for the 1-day exposure group were unaffected by TMSD exposure; however, the final mean body weights for all mice in the 1-day exposure group were lower than the initial mean body weights. In the 1-day exposure – recovery group, final mean body weights of the three mice remaining at study day 9 were significantly decreased (26.8% less than the air control group); final mean body weight gain was also significantly decreased in this group (Table 10; Figure 7A and B). Final mean body weights were significantly decreased in the 5-day exposure group mice at 3 ppm (10.2% lower) and 10 ppm (16.5% lower) and also in the 5-day exposure – recovery group mice at 3 ppm (21.7% lower) and 10 ppm (18.4% lower) compared to the air control group (Table 10; Figure 7C and D). Final mean body weight gains were also significantly decreased in both the 5-day exposure group (3 ppm) and the 5-day exposure – recovery group (1 and 3 ppm).

Table 9. Summary of the Disposition of Male Mice in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

Exposure Group	0 ppm (air)	0 ppm (hexanes)[a]	0.3 ppm	1 ppm	3 ppm	10 ppm
One-day Exposure[b]						
Animals Initially in Study	8	8	–[c]	–	–	8
Early Deaths	0	0	–	–	–	0
Scheduled Sacrifice, Terminal	8	8	–	–	–	8
Animals Examined Microscopically	8	8	–	–	–	8
One-day Exposure – Recovery[d]						
Animals Initially in Study	8	8	–	–	–	8
Early Deaths[e]						
Found dead	0	0	–	–	–	5
Scheduled Sacrifice, Terminal	8	8	–	–	–	3
Animals Examined Microscopically	8	8	–	–	–	8
Five-day Exposure[f]						
Animals Initially in Study	8	8	8	8	8	8
Early Deaths[g]						
Euthanized, moribund	0	0	0	0	0	2
Found dead	0	0	0	0	0	6
Scheduled Sacrifice, Terminal	8	8	8	8	8	0
Animals Examined Microscopically	8	8	8	8	8	8

Exposure Group	0 ppm (air)	0 ppm (hexanes)[a]	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure – Recovery[h]						
Animals Initially in Study	8	8	8	8	8	8
Early Deaths[i]						
Euthanized, moribund	0	0	0	0	0	1
Found dead	0	0	0	0	0	7
Scheduled Sacrifice, Terminal	8	8	8	8	8	0
Animals Examined Microscopically	8	8	8	8	8	8

TMSD = trimethylsilyldiazomethane.
[a]Hexanes control contained an equivalent concentration of hexanes (25 ppm) as the 10 ppm TMSD-exposed group.
[b]Animals were exposed on study day 0 and necropsied on study day 1.
[c]Exposures at this concentration were not conducted for this group.
[d]Animals were exposed on study day 0 and necropsied on study day 9.
[e]Two animals found dead on both study day 3 and study day 4 and one animal found dead on study day 6.
[f]Animals were exposed on study days 0–4 and scheduled to be necropsied on study day 5.
[g]Two animals euthanized moribund on study day 3; two animals found dead on study day 2; four animals found dead on study day 3 in the 10 ppm TMSD-exposed exposure group.
[h]Animals were exposed on study days 0–4 and scheduled to be necropsied on study day 9.
[i]One animal euthanized moribund on study day 3; two animals found dead on study day 2; five animals found dead on study day 3 in the 10 ppm TMSD-exposed exposure group.

Table 10. Summary of Mean Body Weights of Male Mice in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane[a,b]

Exposure Group	Initial Body Weight (g)	Final Body Weight (g)	Changes in Body Weight (g)	Final Weight Relative to Controls (%)
One-day Exposure				
0 ppm (air)	28.1 ± 0.6	27.9 ± 0.5	−0.2 ± 0.1	–
0 ppm (hexanes)[c]	28.0 ± 0.5	27.7 ± 0.8	−0.3 ± 0.4	−0.7
10 ppm	27.5 ± 0.9	27.2 ± 0.5	−0.3 ± 0.5	−2.7
One-day Exposure – Recovery				
0 ppm (air)	28.5 ± 0.4	29.3 ± 0.3*	0.7 ± 0.3*	–
0 ppm (hexanes)	28.2 ± 0.7	29.1 ± 0.8	0.9 ± 0.3	−0.6
10 ppm[d]	28.1 ± 0.3	21.4 ± 1.5**	−6.5 ± 1.3**	−26.8
Five-day Exposure				
0 ppm (air)	28.8 ± 0.4	28.0 ± 0.6**	−0.8 ± 0.2*	–
0 ppm (hexanes)	28.5 ± 0.6	27.9 ± 0.4	−0.6 ± 0.5	−0.2
0.3 ppm	28.0 ± 0.2	27.6 ± 0.1	−0.4 ± 0.2	−1.3
1 ppm	28.6 ± 0.2	28.3 ± 0.2	−0.3 ± 0.1	1.1
3 ppm	28.6 ± 0.4	25.1 ± 0.5**	−3.5 ± 0.3**	−10.2
10 ppm[e]	28.8 ± 0.6	23.8 ± 0.4**	NR	−16.5
Five-day Exposure – Recovery				
0 ppm (air)	28.4 ± 0.5	28.6 ± 0.5**	0.3 ± 0.3**	–
0 ppm (hexanes)	29.1 ± 0.6	29.9 ± 0.5	0.8 ± 0.2	4.5
0.3 ppm	28.8 ± 0.3	29.9 ± 0.4	1.1 ± 0.3	4.4
1 ppm	28.8 ± 0.4	28.0 ± 0.3	−0.9 ± 0.3*	−2.3
3 ppm	29.8 ± 0.5	22.4 ± 0.4**	−7.4 ± 0.3**	−21.7
10 ppm[f]	28.1 ± 0.6	23.0 ± 0.4**	NR	−18.4

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
NR = not recorded.
[a]Weights and weight changes are given as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Statistical analysis for the hexanes control group compared to the air control group was performed using the t-test.
[d]Final body weight at study day (SD) 9 (n = 3).
[e]Final body weight at SD 3 (n = 5). Change in body weight not reported for SD 3.
[f]Final body weight at SD 3 (n = 6). Change in body weight not reported for SD 3.

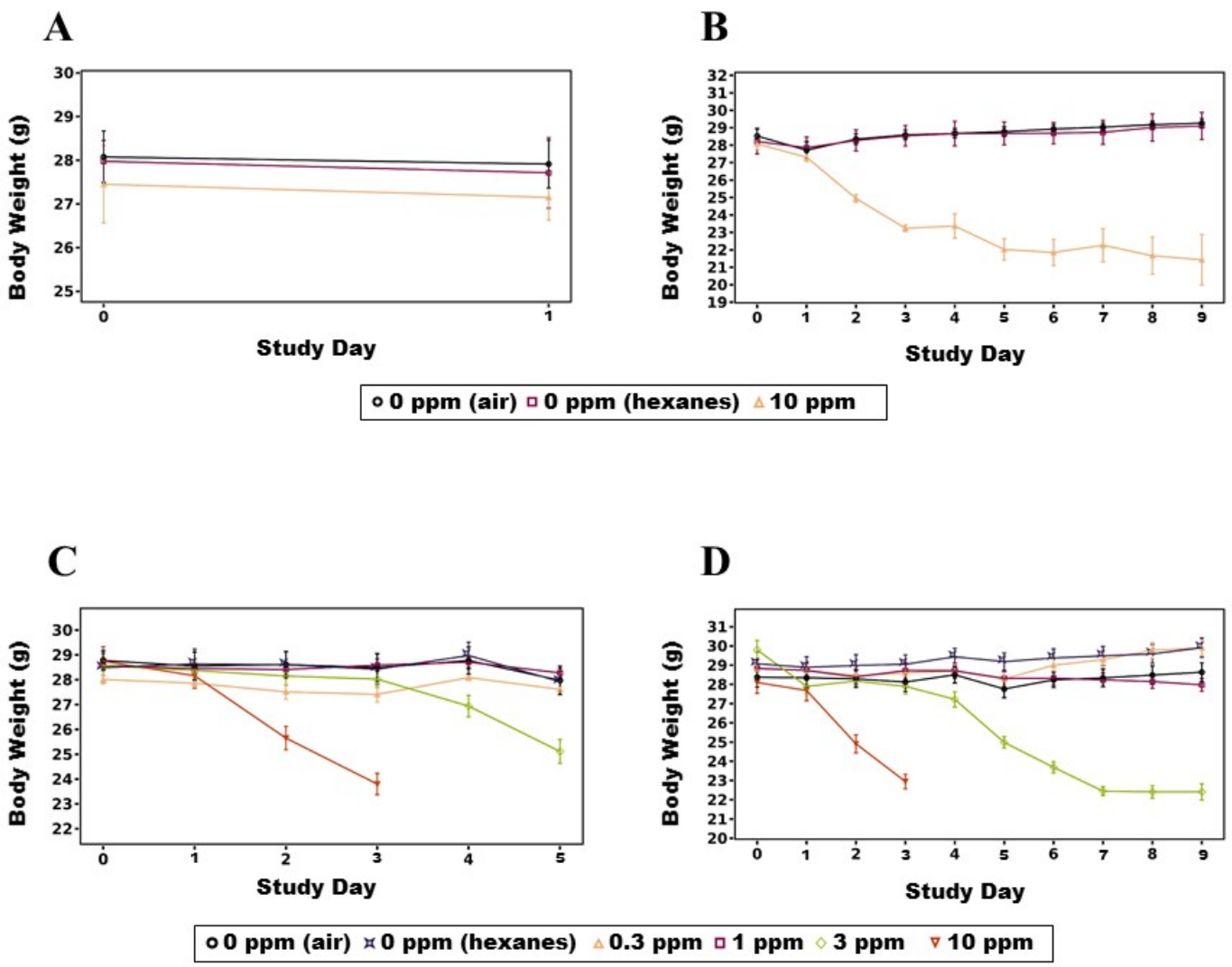

Figure 7. Growth Curves for Male Mice in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

(A) 1-day exposure group, (B) 1-day exposure – recovery group, (C) 5-day exposure group, (D) 5-day exposure – recovery group. The number of animals in each exposure group for each study day is included in Table 9.

No abnormal clinical findings were observed in the 1-day exposure group. In the 1-day exposure – recovery group at 10 ppm, all mice exhibited labored breathing, ruffled coat, and lethargy beginning on study day 2; seven mice exhibited hunched posture beginning on study day 2; one mouse exhibited rapid breathing beginning on study day 4; and one mouse exhibited nasal discharge beginning on study day 3 (Table 11).

Clinical observations affecting five or more of the 3 ppm and/or 10 ppm-exposed mice in the 5-day exposure and 5-day exposure – recovery groups were labored breathing, ruffled coat, and hunched posture that began between study days 2 and 6; two mice were lethargic beginning on study day 7; one mouse was lethargic beginning on study day 3; and one mouse exhibited hunched posture beginning on study day 5 (Table 11). It is notable that when the 1-day exposure – recovery group and both 5-day exposure groups were combined, 8 of 16 (50%) of the 3 ppm-exposed mice and 19 of 24 (79%) of the 10 ppm-exposed mice exhibited labored breathing (Table 11).

Table 11. Summary of Clinical Observations for Male Mice in the One-day and Five-day Nose-only Inhalation Studies of Trimethylsilyldiazomethane

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
One-day Exposure[a]						
No Abnormalities Found	8/8	8/8	–	–	–	8/8
One-day Exposure – Recovery						
Labored Breathing	0/8	0/8	–	–	–	8/8 (SD 2)
Rapid Breathing	0/8	0/8	–	–	–	1/8 (SD 4)
Ruffled Coat	0/8	0/8	–	–	–	8/8 (SD 2)
Discharge from Nose/Snout, Clear	0/8	0/8	–	–	–	1/8 (SD 3)
Hunched Posture	0/8	0/8	–	–	–	7/8 (SD 2)
Lethargic	0/8	0/8	–	–	–	8/8 (SD 2)
Five-day Exposure						
Labored Breathing	0/8	0/8	0/8	0/8	0/8	5/8 (SD 2)
Ruffled Coat	0/8	0/8	0/8	0/8	5/8 (SD 2)	8/8 (SD 2)
Hunched Posture	0/8	0/8	0/8	0/8	1/8 (SD 5)	5/8 (SD 2)

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure – Recovery						
Labored Breathing	0/8	0/8	0/8	0/8	8/8 (SD 6)	6/8 (SD 2)
Ruffled Coat	0/8	0/8	0/8	0/8	8/8 (SD 5)	8/8 (SD 2)
Hunched Posture	0/8	0/8	0/8	0/8	8/8 (SD 6)	8/8 (SD 2)
Lethargic	0/8	0/8	0/8	0/8	2/8 (SD 7)	1/8 (SD 3)

SD = study day.
[a]Upper row displays cumulative number of animals with observation/total animals started in study. Lower row displays SD of observation onset.

In the 1-day exposure group, mean absolute and relative lung weights were significantly increased, whereas mean absolute and relative thymus weights were significantly decreased at 10 ppm (Table 12). In the 1-day exposure – recovery group, only three mice survived to scheduled necropsy on study day 9 due to early deaths. In the 1-day exposure – recovery group, mean absolute and relative weights of the lung were significantly increased at 10 ppm. Mean absolute and relative weights of the liver and thymus were significantly decreased at 10 ppm. Mean absolute weight of the heart was significantly decreased, whereas mean relative weight was significantly increased at 10 ppm (Table 12).

Mean absolute and relative lung weights were significantly increased at 0.3, 1, and 3 ppm in the 5-day exposure groups (n = 2 at 10 ppm due to early deaths or euthanasia) and at 1 and 3 ppm in the 5-day exposure – recovery group (n = 1 at 10 ppm due to early deaths or euthanasia) (Table 13). In both the 5-day exposure and the 5-day exposure – recovery groups, mean absolute and relative liver weights were significantly decreased at 3 ppm (Table 13). In the 5-day exposure – recovery group, significant decreases were observed in the mean absolute and relative weight of the right kidney and thymus at 3 ppm (Table 13). Mean absolute or relative weight changes for other organs (liver for 1-day exposure group; brain, right epididymis, right and left kidneys, and right and left testes for 1-day exposure – recovery group; brain, heart, right and left testes, right and left kidney, and thymus for 5-day exposure group; brain, right epididymis, heart, and right and left testes for 5-day exposure – recovery group) were considered secondary to decreased body weight.

Table 12. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the One-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a,b]

	0 ppm (air)	0 ppm (hexanes)	10 ppm
One-day Exposure			
n	8	8	8
Necropsy Body Wt. (g)	27.9 ± 0.5	27.7 ± 0.8	27.2 ± 0.5
Lung			
Absolute (g)	0.24 ± 0.02	0.23 ± 0.02	0.32 ± 0.02*
Relative (mg/g)[c]	8.43 ± 0.59	8.39 ± 0.49	11.68 ± 0.75**
Thymus			
Absolute (g)	0.040 ± 0.001	0.036 ± 0.002	0.032 ± 0.002**
Relative (mg/g)	1.43 ± 0.08	1.29 ± 0.05	1.18 ± 0.07*
One-day Exposure – Recovery			
n	8	8	3
Necropsy Body Wt. (g)	29.3 ± 0.3	29.1 ± 0.8	21.4 ± 1.5**
Lung			
Absolute (g)	0.25 ± 0.02	0.25 ± 0.02	0.42 ± 0.02**
Relative (mg/g)	8.62 ± 0.51	8.48 ± 0.62	19.70 ± 2.09**
Heart			
Absolute (g)	0.15 ± 0.00	0.15 ± 0.00	0.12 ± 0.01**
Relative (mg/g)	5.00 ± 0.08	5.04 ± 0.14	5.61 ± 0.15**
Liver			
Absolute (g)	1.50 ± 0.04	1.47 ± 0.06	0.95 ± 0.11**
Relative (mg/g)	51.31 ± 0.88	50.38 ± 1.11	44.22 ± 1.99**
Thymus			
Absolute (g)	0.049 ± 0.002	0.048 ± 0.004	0.013 ± 0.003**
Relative (mg/g)	1.67 ± 0.06	1.65 ± 0.11	0.60 ± 0.07**

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Table 13. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a,b]

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm
Five-day Exposure						
n	8	8	8	8	8	2
Necropsy Body Wt. (g)	28.0 ± 0.6**	27.9 ± 0.4	27.6 ± 0.1	28.3 ± 0.2	25.1 ± 0.5**	–[c]
Lung						
Absolute (g)	0.25 ± 0.02**	0.25 ± 0.02	0.31 ± 0.01**	0.38 ± 0.01**	0.45 ± 0.01**	–
Relative (mg/g)[d]	8.92 ± 0.53**	8.78 ± 0.55	11.04 ± 0.49**	13.31 ± 0.30**	17.96 ± 0.45**	–
Liver						
Absolute (g)	1.38 ± 0.03**	1.47 ± 0.05	1.31 ± 0.02	1.36 ± 0.02	1.06 ± 0.03**	–
Relative (mg/g)	49.31 ± 0.69**	52.54 ± 1.44	47.57 ± 0.42	48.14 ± 0.58	42.10 ± 0.52**	–
Five-day Exposure – Recovery						
n	8	8	8	8	8	1
Necropsy Body Wt. (g)	28.6 ± 0.5**	29.9 ± 0.5	29.9 ± 0.04	28.0 ± 0.3	22.4 ± 0.4**	–
Lung						
Absolute (g)	0.26 ± 0.02**	0.23 ± 0.01	0.27 ± 0.01	0.41 ± 0.02**	0.52 ± 0.01**	–
Relative (mg/g)	9.17 ± 0.60**	7.82 ± 0.29	8.91 ± 0.33	14.50 ± 0.51**	23.30 ± 0.82**	–
R. Kidney						
Absolute (g)	0.23 ± 0.00**	0.24 ± 0.00	0.24 ± 0.00	0.22 ± 0.00*	0.17 ± 0.01**	–
Relative (mg/g)	8.04 ± 0.09**	7.90 ± 0.11	7.91 ± 0.13	7.78 ± 0.04	7.41 ± 0.14**	–
L. Kidney						
Absolute (g)	0.21 ± 0.01**	0.21 ± 0.00	0.21 ± 0.00	0.19 ± 0.00*	0.16 ± 0.00**	–
Relative (mg/g)	7.29 ± 0.11*	7.15 ± 0.13	7.07 ± 0.07	6.92 ± 0.07	6.92 ± 0.17	–
Liver						
Absolute (g)	1.40 ± 0.02**	1.49 ± 0.02	1.50 ± 0.03	1.35 ± 0.02	0.93 ± 0.03**	–
Relative (mg/g)	49.05 ± 0.65**	49.82 ± 0.58	50.06 ± 0.69	48.36 ± 0.43	41.54 ± 0.70**	–
Thymus						
Absolute (g)	0.042 ± 0.002**	0.040 ± 0.001	0.041 ± 0.002	0.038 ± 0.003	0.013 ± 0.001**	–
Relative (mg/g)	1.48 ± 0.06**	1.33 ± 0.04	1.38 ± 0.08	1.36 ± 0.11	0.59 ± 0.04**	–

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Weights are not provided because not enough animals survived to scheduled necropsy.
[d]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

Gross pathology findings in the 10 ppm TMSD-exposed mice included mottled discoloration of the lung in the 1-day exposure – recovery group; this finding also was present in the 3 ppm and 10 ppm TMSD-exposed mice in the 5-day exposure and 5-day exposure – recovery groups (Appendix D).

Pathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the lung and larynx in mice. Summaries of the incidences of nonneoplastic lesions and statistical analyses of primary nonneoplastic lesions are presented in Appendix D. All tissues evaluated were assigned a severity grade using a scale of minimal (1), mild (2), moderate (3), or marked (4) representing involvement of approximately <10%, 10%–25%, 26%–50%, or >50% of the affected tissue, respectively.

No exposure-related histopathological lesions were present in mice in the 1-day exposure group after a single exposure to TMSD. As described below, exposure-related histopathological lesions were observed in the lung and larynx in the 1-day exposure – recovery group at 10 ppm (Table 14) and in the 5-day exposure and 5-day exposure – recovery groups after 5 days of repeated exposure to 1 or 3 ppm TMSD and 2 or 3 days of exposure to 10 ppm TMSD (Table 15). No exposure-related histopathological changes were observed in the lung or larynx in the hexane control group.

Lung: No histopathological changes were observed in the lungs of mice in the 1-day exposure group at 10 ppm TMSD compared to the air control group. Histopathological changes observed in the 1-day exposure – recovery group mice at 10 ppm TMSD included acute and chronic-active inflammation, histiocytic infiltrates within alveoli, interstitial fibrosis, pulmonary edema, necrosis, acute hemorrhage, and hyperplasia of the alveolar or bronchiolar epithelium (Table 14). Average severity grades of these lesions were minimal to mild (Table 14); five mice in this exposure group were found dead between study day 3 and 6 (Table 9). Exposure-related histopathological findings were observed at concentrations of 1, 3, and 10 ppm TMSD in the 5-day exposure group (Table 15; Figure 8). At 1 ppm TMSD, lesions consisted of histiocytic cellular infiltrates in the alveoli, characterized by variable numbers of histiocytes in the alveolar spaces, and pulmonary edema that consisted of faint eosinophilic fibrillar fluid within the alveolar spaces (Figure 8). At 3 ppm TMSD, histopathological findings were more severe and consisted of chronic-active inflammation characterized by infiltrating histiocytes, lymphocytes, fewer neutrophils that were admixed with fibrin, in addition to necrosis and necrotic cellular debris, acute hemorrhage, and pulmonary edema that ranged from an eosinophilic fibrillar to homogenous fluid. Areas of alveolar epithelial hyperplasia also occurred, which were characterized by alveolar walls lined by cuboidal to pleomorphic type II pneumocytes with large, round nuclei, prominent nucleoli, and variably frequent mitoses. Bronchioles also were hyperplastic and were occasionally lined by piling epithelial cells (Figure 8). At 10 ppm TMSD, acute hemorrhage characterized by red blood cells within the alveolar spaces and interstitium and acute inflammation, pulmonary edema, and necrosis were observed in mice exposed for 2 or 3 days and euthanized or found dead on study day 2 or 3. Necrosis in the lung was quite evident and characterized by loss of alveolar septal architecture and replacement with necrotic cellular debris and fibrin (Figure 8).

In the 5-day exposure group, average severity grades of lesions observed in mice exposed to 1 and 3 ppm TMSD were minimal to mild. At 10 ppm TMSD, severity grades for lesions ranged from minimal to moderate (Table 15); all mice in this exposure group were euthanized or found dead on study day 2 or 3 (Table 9).

In the 5-day exposure – recovery group, exposure-related histopathological lesions of the lung were identified in mice exposed to 1 and 3 ppm TMSD at scheduled necropsy on study day 9

(Table 15; Figure 9). Histopathological findings included chronic-active inflammation characterized by infiltrating histiocytes, lymphocytes, fewer neutrophils that were admixed with fibrin, and pulmonary edema (3 ppm only). Alveolar and bronchiolar epithelial cell hyperplasia, acute hemorrhage, necrosis (3 ppm only), and interstitial fibrosis characterized by increased collagen fibers expanding the alveolar septa or surrounding bronchioles were present (Figure 9). Often, the fibrosis was associated with inflammation. At 10 ppm TMSD, acute hemorrhage, acute inflammation, pulmonary edema, and necrosis were observed in mice exposed for 2 or 3 days and euthanized or found dead at study day 2 or 3. In the 5-day exposure – recovery group, average severity grades of lesions observed at necropsy in mice exposed to 1, 3, or 10 ppm TMSD were minimal to moderate (Table 15); all mice in the 10 ppm group were euthanized or found dead on study day 2 or 3 (Table 9).

Larynx: In the larynx, exposure-related histopathological lesions were observed at concentrations of 10 ppm TMSD in the 1-day exposure – recovery group (Table 14) and in the 5-day exposure group (Table 15) and at concentrations of 3 and 10 ppm TMSD in the 5-day exposure – recovery group (Table 15; Figure 9) compared to the air control groups. These histopathological lesions consisted of hyperplasia and ulceration of the squamous epithelium in both the 3 and 10 ppm TMSD groups, with acute inflammation characterized by neutrophils infiltrating the mucosa and submucosa of the larynx present in mice in the 3 ppm group (Figure 10).

Table 14. Incidences of Nonneoplastic Lesions of the Lung and Larynx in Male Mice in the One-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a]

One-day Exposure – Recovery Group	0 ppm (air)	0 ppm (hexanes)	10 ppm
n[b]	8	8	8[c]
Lung			
Inflammation, acute[d]	0	0	5* (1.2)[e]
Inflammation, chronic-active	0	0	3 (1.7)
Infiltration, cellular, histiocyte	2 (1.0)	0	3 (1.3)
Interstitium, fibrosis	0	0	3 (2.0)
Edema	0	0	5* (2.4)
Hemorrhage, acute	0	0	5* (1.0)
Alveolar epithelium, hyperplasia	0	0	4* (2.3)
Bronchiole epithelium, hyperplasia	0	0	3 (1.0)
Necrosis	0	0	5* (2.0)
Larynx			
Squamous epithelium, hyperplasia	0	0	5* (1.2)
Squamous epithelium, ulcer	0	0	1 (1.0)
Inflammation, acute	0	0	4* (1.0)

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group.
*Statistically significant at $p \leq 0.05$.
[a]Statistical analysis performed by Fisher's exact (pairwise) test.
[b]Number of animals examined microscopically.
[c]Only three animals survived until scheduled termination; two animals each were found dead on study day (SD) 3 and SD 4; one animal was found dead on SD 6.
[d]Number of animals with lesion.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Table 15. Incidences of Nonneoplastic Lesions of the Lung and Larynx in Male Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane[a]

	0 ppm (air)	0 ppm (hexanes)	0.3 ppm	1 ppm	3 ppm	10 ppm[b]
Five-day Exposure						
Lung[c]	8	8	8	8	8	8
Inflammation, acute[d]	0**	0	0	0	0	8** (1.8)[e]
Inflammation, chronic-active	0**	0	0	0	8** (1.9)	0
Infiltration, cellular, histiocyte	0**	0	0	7** (1.0)	0	0
Edema	0**	0	0	7** (1.0)	8** (1.4)	8** (2.0)
Hemorrhage, acute	0*	0	0	0	8** (1.0)	8** (1.1)
Alveolar epithelium, hyperplasia	0	0	0	0	8** (1.1)	0
Bronchiole epithelium, hyperplasia	0	0	0	0	8** (1.0)	0
Necrosis	0**	0	0	0	8** (2.3)	8** (2.8)
Larynx	8	8	0	0	8	7
Squamous epithelium, hyperplasia	0	0	–[f]	–	0	2 (1.0)
Squamous epithelium, ulcer	0*	0	–	–	0	3 (1.0)
Inflammation, acute	0	0	–	–	0	1 (1.0)
Five-day Exposure – Recovery						
Lung	8	8	8	8	8	8
Inflammation, acute	0**	0	0	0	0	8** (1.9)
Inflammation, chronic-active	0	0	0	8** (1.9)	8** (2.9)	0
Infiltration, cellular, histiocyte	0	1 (1.0)	0	0	0	0
Interstitium, fibrosis	0	0	0	8** (1.0)	8** (1.6)	0
Edema	0**	0	0	0	6** (1.7)	8** (2.3)
Hemorrhage, acute	0**	0	0	1 (1.0)	7** (1.0)	7** (1.0)
Alveolar epithelium, hyperplasia	0	0	0	8** (1.8)	8** (3.4)	0
Bronchiole epithelium, hyperplasia	0	0	0	8** (1.0)	8** (3.0)	0
Necrosis	0**	0	0	0	8** (1.5)	8** (2.4)
Larynx	8	8	0	8	8	8
Squamous epithelium, hyperplasia	0	0	–	0	3 (1.0)	1 (1.0)
Squamous epithelium, ulcer	0*	0	–	0	4* (1.0)	4* (1.0)
Inflammation, acute	0	0	–	0	5* (1.0)	0

Statistical significance for an exposure group indicates a significant pairwise test compared to the air control group. Statistical significance for the air control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]No animals survived until scheduled termination. In the 5-day exposure group, two animals were found dead on study day (SD) 2 and four on SD 3, and two animals were euthanized moribund on SD 3. In the 5-day exposure – recovery group, two animals were found dead on SD 2 and five on SD 3, and one animal was euthanized moribund on SD 3.
[c]Number of animals examined microscopically.
[d]Number of animals with lesion.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[f]Slides not evaluated because no effect was observed at 3 ppm.

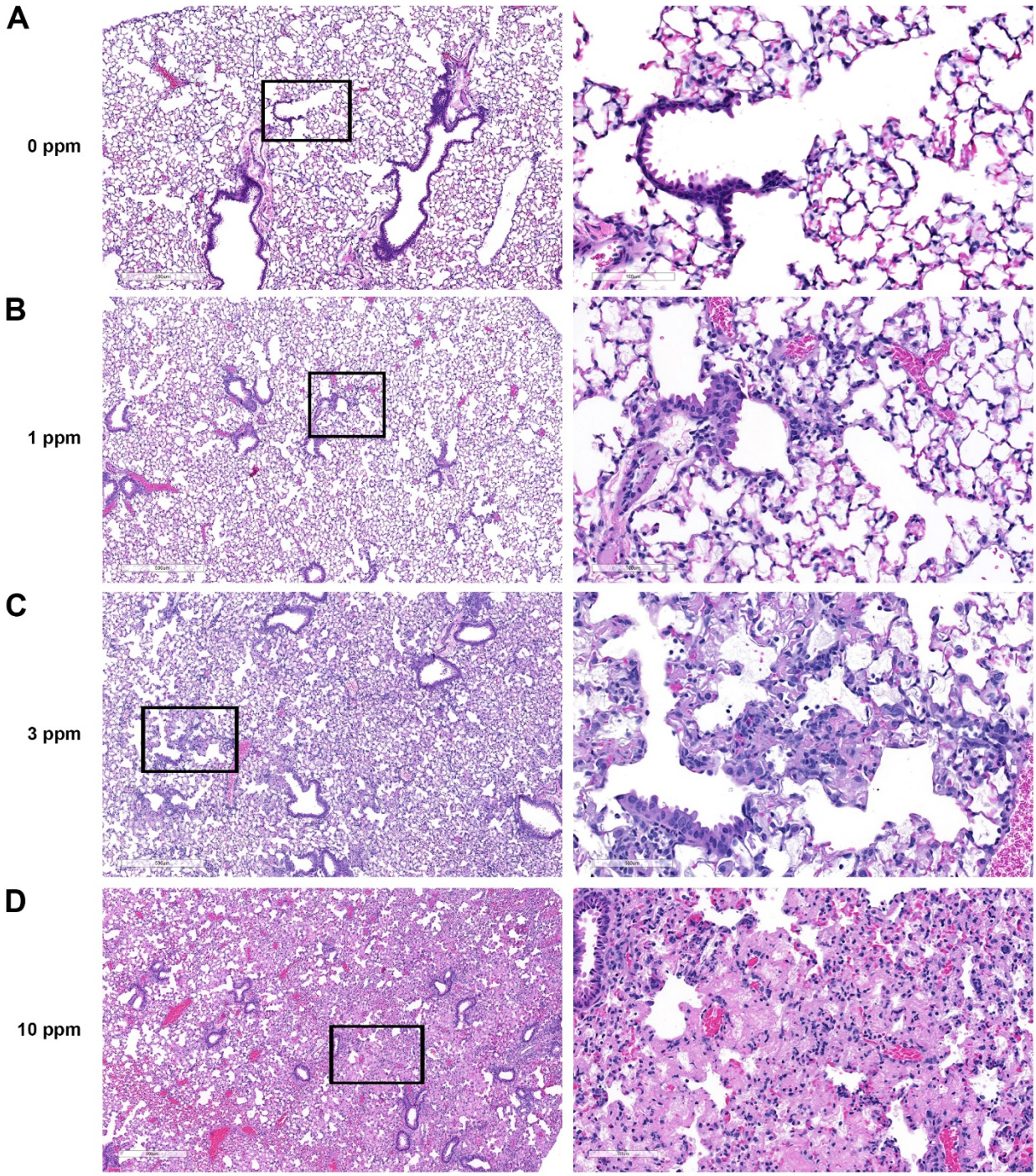

Figure 8. Representative Images of the Lung from Male Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane (Five-day Exposure Group) (H&E)

(A) Lung from mouse exposed to 0 ppm (air) showing terminal bronchiole and alveoli. (B) Lung from mouse exposed to 1 ppm TMSD showing histiocytic cell infiltrates (severity grade = 1) in the alveolar sacs mixed with fine eosinophilic fibrillar fluid. (C) Lung from mouse exposed to 3 ppm TMSD showing inflammatory cell infiltrates (severity grade = 2) in the alveolar spaces mixed with eosinophilic fibrillar fluid; also, focal necrosis of the alveolar septa (severity grade = 2). (D) Lung from mouse exposed to 10 ppm TMSD (necropsy on study day 2) showing area of necrosis (severity grade = 4) mixed with acute inflammatory cell infiltrates (severity grade = 1), fibrinous debris, edema (severity grade = 3), and focal hemorrhage (severity grade = 2). Left panels are 4x and right panels are 20x.

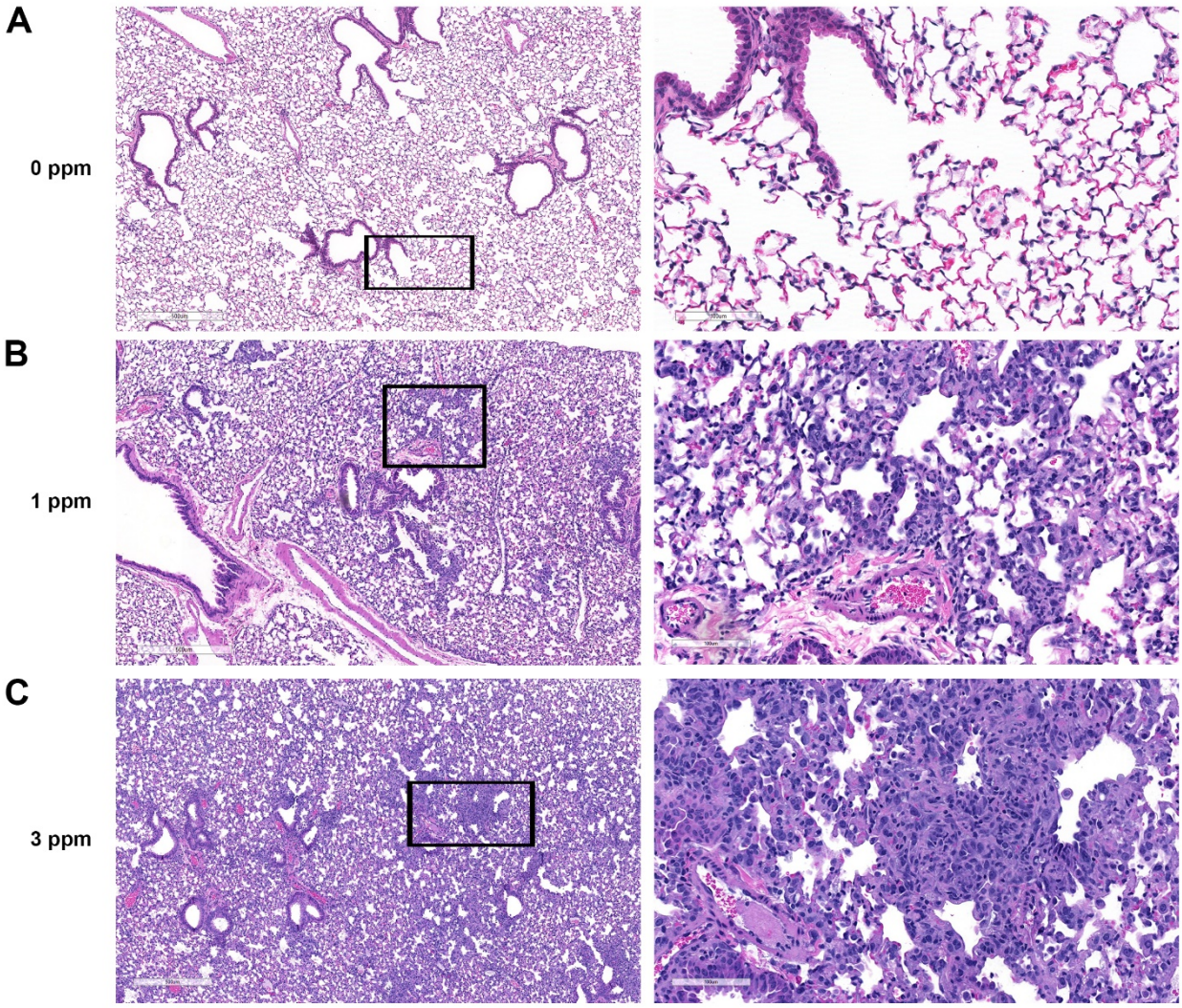

Figure 9. Representative Images of the Lung from Male Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane (Five-day Exposure – Recovery Group) (H&E)

(A) Lung from mouse exposed to 0 ppm (air) showing terminal bronchiole, alveolar duct, and alveoli. (B) Lung from mouse exposed to 1 ppm TMSD showing chronic-active inflammation (severity grade = 2) of the alveolar septa with early interstitial fibrosis (severity grade = 1). (C) Lung from mouse exposed to 3 ppm TMSD showing chronic-active inflammation (severity grade = 3) with alveolar and bronchiolar epithelial hyperplasia (severity grade = 4 and severity grade = 3, respectively), necrosis (severity grade = 1), and interstitial fibrosis (severity grade = 2). Left panels are 4x and right panels are 20x.

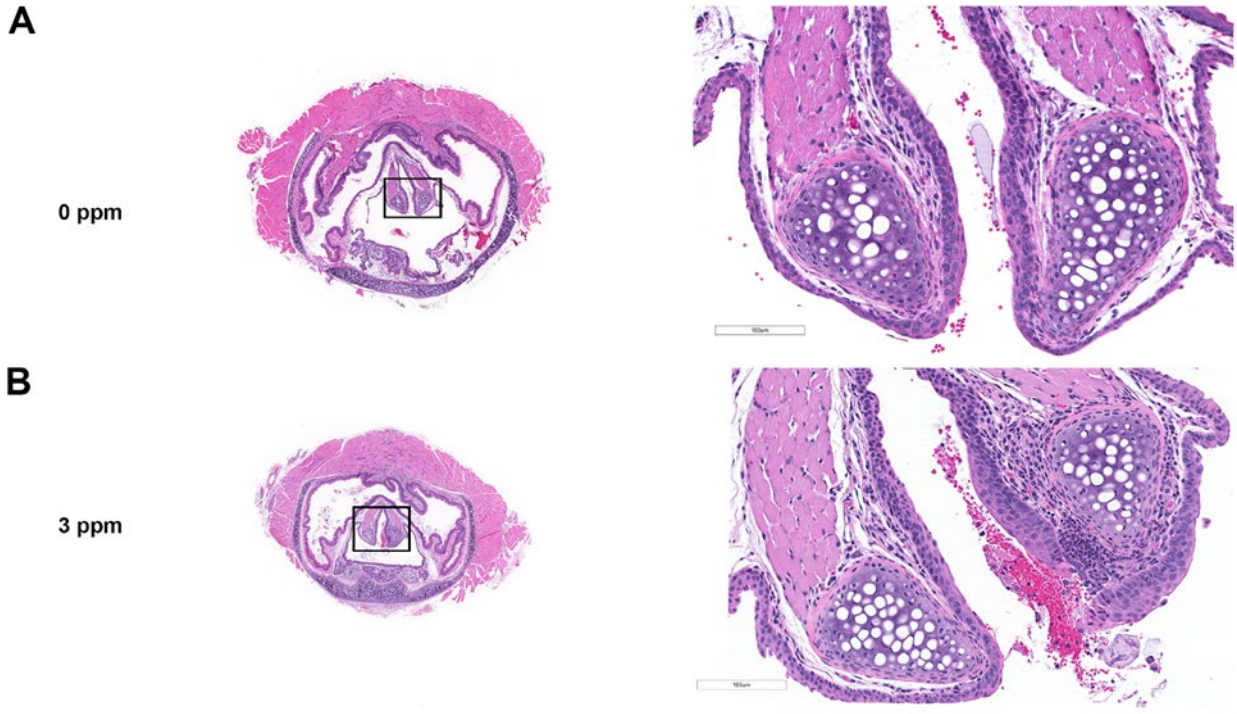

Figure 10. Representative Images of the Larynx from Male Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane (Five-day Exposure – Recovery Group) (H&E)

(A) Larynx from mouse exposed to 0 ppm (air). (B) Larynx from mouse exposed to 3 ppm TMSD showing hyperplasia and ulceration (severity grade = 1) of the squamous epithelium with acute inflammatory cell infiltrates (severity grade = 1) in the submucosa and mucosa and adjacent hemorrhagic debris. Left panels are 4x and right panels are 20x.

Discussion

Acute inhalation toxicity testing of trimethylsilyldiazomethane (TMSD), a synthetic methylating reagent widely used in organic chemistry, was performed in male Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6C3F1/N mice by the National Toxicology Program (NTP) because of reports of the death of two chemists exposed to TMSD in the workplace. Other concerns included the known inhalation toxicity of the related compound diazomethane and the absence of inhalation toxicity data for TMSD. Lung injury, peribronchial inflammation, pulmonary edema, and death have been reported in humans exposed to diazomethane.[8; 10; 11]

The two human case reports appeared to involve single, acute exposures to unknown concentrations of TMSD vapors. In the NTP studies, these exposure scenarios were mimicked in vivo by single exposures to TMSD in rats and mice; however, no significant adverse effects were observed in rats after a single exposure so multiple exposures were also tested in both species. A potential scenario involving more chronic or persistent (multiple) exposures in an occupational setting cannot be ruled out but has not been reported.

In the NTP studies, inhaled TMSD caused acute and progressive lung injury in both rats and mice at low vapor concentrations (≤10 ppm). Also, pulmonary toxicity in rats and mice was due to exposure specifically to TMSD vapors and not exposure to diazomethane vapors as a reaction byproduct of TMSD. Under the experimental conditions and with the exposure concentrations tested in this study, TMSD was more toxic in mice than in rats in that it caused labored breathing in 8/16 and 19/32 mice exposed to 3 and 10 ppm, respectively (compared to 4/32 rats exposed to 10 ppm) and was lethal after one to three exposures at 10 ppm in mice. The potency of TMSD was most evident in the findings of extensive lung injury, labored breathing, and death induced in mice after just a single 30-minute exposure to 10 ppm TMSD.

Peripheral lung effects at the bronchiolar/alveolar level were observed in rats and mice after exposure to TMSD. Histopathological effects in the lungs of both species included chronic-active inflammation, acute hemorrhage often with accompanying vascular congestion, pulmonary edema, bronchiolar and alveolar epithelial hyperplasia, interstitial fibrosis, and necrosis (only in mice). Overall, these adverse effects were observed in the lung at lower exposure concentrations in mice than in rats. The no-observed-effect level (NOEL) for pulmonary edema, which is an adverse lung effect previously reported after lethal exposure in humans, was <10 ppm in rats and <1 ppm in mice after only five repeated 30-minute exposures on 5 consecutive days. The NOEL for lung necrosis in mice was <3 ppm. TMSD was also observed to be profibrotic, inducing interstitial fibrosis in the lungs of rats and mice. The NOEL for interstitial fibrosis was <10 ppm in rats and <1 ppm in mice. The NOEL for this study overall was <0.3 ppm, based on increased lung weights in rats and mice. A NOEL of <0.3 ppm is approximately equal to the 8-hour time-weighted average occupational exposure limit of 0.2 ppm for diazomethane. Exposure-related adverse effects also were observed in the mediastinal lymph nodes (rats) and larynx (mice).

The vehicle for TMSD was a solution of mixed hexanes in air; however, hexanes at an exposure concentration (25 ppm) equivalent to the level of hexanes in the highest tested exposure concentration of TMSD (10 ppm) caused no adverse effects in rats or mice, which is consistent with a previous NTP study that showed n-hexane is toxic after 13 weeks of exposure, but only at much higher concentrations.[29] The cause of death in mice was likely attributable to extensive

lung necrosis and pulmonary edema resulting from TMSD-induced lung injury. Why rats are less sensitive than mice to the toxic effects of inhaled TMSD is unclear, but might be related to higher breathing rates in mice compared to rats[30] and/or tissue dosimetry in the distal lung at the alveolar level.

In summary, the histopathological findings in rats and mice suggest that the toxic effects to the lung following short-term inhalation exposure to TMSD are progressive over time and consistent with the adverse effects (in particular, pulmonary edema) observed in the two human case reports for which pulmonary intoxication was lethal. The progressive nature of the lung injury induced by TMSD was most apparent by the presence of interstitial fibrosis in the 1 and 3 ppm TMSD-exposed mice at study termination in the 5-day exposure – recovery group, but not in the 5-day exposure group, which suggests that ongoing adverse post-inhalation exposure changes did not show evidence of resolving during the recovery period. In addition, adverse lung effects were observed in the 10 ppm-exposed mice of the 1-day exposure – recovery group but not in the 1-day exposure group.

These in vivo acute inhalation exposure data, including NOELs for increased lung weights and specific deleterious lung effects (such as necrosis, pulmonary edema, and interstitial fibrosis), can be used by the Occupational Safety and Health Administration and other agencies (e.g., the National Institute for Occupational Safety and Health) to help mitigate TMSD exposure risks in the production and use of TMSD in chemical laboratories. These data also can be used to update the Material Safety Data Sheet for TMSD (and other chemical reviews) and to alert chemical suppliers to the dangers of TMSD, which is readily available commercially and is considered a safer, less toxic alternative to diazomethane.

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Appendix A. Chemical Characterization and Generation of Exposure Concentrations

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A.1. Procurement and Characterization of Trimethylsilyldiazomethane

Trimethylsilyldiazomethane (TMSD), 2 M in a solution of mixed hexanes (CASRN 18107-18-1, lot SHBB9290V), and the hexanes control article, a mixture of hexanes only (no TMSD) (referred to as hexanes control; CASRN not provided, lot SHBF3717V), were originally obtained from Sigma-Aldrich (Saint Louis, MO) via Government Scientific Source, Inc. (Reston, VA). Both the test article and hexanes control article were received from the National Institute of Environmental Health Sciences (NIEHS) chemistry support services contractor, Battelle, on May 6, 2015, at the Battelle Columbus test site. At the time of receipt, aliquots of the test article and hexanes control article were taken and stored frozen at −20°C to serve as frozen reference samples. Reports on analyses performed in support of the TMSD studies are on file at NIEHS.

Identification and purity analyses were performed by the testing laboratory. The identities were determined by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Figure A-2, Figure A-3, Figure A-4, Figure A-5). The concentration of TMSD in the mixed hexanes solution was determined by ^{1}H NMR spectroscopy using an internal standard and was 2.65 M, which was slightly higher than 2.1 M specified in the manufacturer's certificate of analysis. Gas chromatography with mass selective detection (GC/MSD) was used to identify the major peaks and any degradants or impurities with areas ≥0.1% of the total peak area (Figure A-6, Figure A-7). Additional purity analyses were performed throughout the study by GC/MSD using area percent purity profiles and major peak area comparisons to frozen reference samples of each material.

For the TMSD and mixed hexanes test and control articles, triplicate replicates of the bulk articles were prepared in 1,2,4-trimethylbenzene. In addition, standards of potential hexane components or impurities, n-hexane, methylcyclopentane, (chloromethyl)trimethylsilane, and 3-methylpentane observed in the chemical identity and purity screen were prepared in 1,2,4-trimethylbenzene. All samples were analyzed using an Agilent (Santa Clara, CA) 6890 GC equipped with a 5973N MSD. The mass spectra of lot SHBB9290V of TMSD contained 9 peaks ≥0.1% by area. In addition to the primary TMSD peak (36.1%) and a (chloromethyl)trimethylsilane peak (3.30%), two major peaks were consistent with the mixed hexanes solution: n-hexane (33.6%) and methylcyclopentane (25.7%). The remaining peaks were all <2% of the total peak area and accounted for additional hydrocarbons and impurities related to TMSD (**Error! Reference source not found.**). The mass spectra of lot SHBF3717V of mixed hexanes consisted of five peaks ≥0.1% by total peak area, all of which were isomers of hexane. The major peaks for n-hexane, methylcyclopentane, and 3-methylpentane were 64.2%, 17.4%, and 16.7% of the total peak area, respectively. Peaks for 2-methylpentane and cyclohexane were <2% of the total peak area (**Error! Reference source not found.**).

Stability analysis of the TMSD and hexanes standard solutions in 1,2,4-trimethylbenzene met the criterion for stability up to 7 days when stored refrigerated, with the relative error (RE) within 15% of the initial concentrations. Stability of TMSD and hexanes on XAD-4 sorbent (unextracted) and in solution with 1,2,4-trimethylbenzene (extracted) was demonstrated at refrigerated temperatures up to 7 days. The stability of TMSD during storage on unextracted sorbent at refrigerated temperatures was better than storage in extracted form. The average RE of the low- and high-exposure unextracted samples were within 6% of the respective initial concentrations for TMSD, whereas the extracted samples were within 18%. No significant

difference was found between the two methods of storage for total hexanes in either TMSD or hexanes control groups. The extracted and unextracted samples demonstrated REs within 6% for TMSD low- and high-exposure samples. For the hexanes control group, RE was within 12% for both low- and high-exposure samples.

After scheduled termination of the animals, bulk chemical and mixed hexanes control articles were reanalyzed by the study laboratory using GC/MSD along with respective frozen reference standards taken at the time of receipt at the testing facility. The TMSD bulk test article was a yellow liquid upon receipt and remained so over the course of the study. The hexanes bulk control article was a colorless liquid upon receipt and remained so over the course of the study. For the bulk chemical reanalysis of TMSD, analysis indicated that hexamethyldisiloxane was present at a higher percentage (1.02%) in the sample compared to the initial analysis (0.79%) or frozen reference (0.44%). Aside from this result, the data indicated no statistically significant differences in the purities of TMSD and hexanes control articles and their respective frozen reference standards (data not shown).

A.2. Vapor Generation and Exposure System

A schematic of the TMSD vapor generation and delivery system is shown in Figure A-1. The system consisted of two parts: the vaporization subsystem and the delivery subsystem. Both subsystems were housed in a glove box. The generation and delivery system for the hexanes control was similar to that for the TMSD system. The test chemical was pumped to the vaporization column using a syringe pump capable of delivering a stable and precisely determined flow of chemical to the generator. The syringe needle was inserted in the vaporizer column through a septum. Air entered the column from the bottom, vaporized the chemical, and carried it to the delivery system. The exposure vapor was directed to a pneumatic slide valve. This valve served as the exposure on-off valve and was operated by a solenoid actuated by the exposure control unit (ECU).

During “on-exposure” periods, the slide valve was actuated and the vapor directed through the valve to the nose-only exposure carousel. At each exposure carousel, vapor was delivered uniformly to each nose tube. During “off-exposure” periods when animals were loaded or removed from each carousel, filtered and conditioned air was supplied to each exposure carousel from the facility compressed air system. Also during this period, the exposure vapor air stream was directed through a scrubber system within the glove box prior to facility exhaust.

The animals were exposed in nose-only inhalation carousels developed at Battelle. The rodent exposure carousel (Figure A-1) consisted of stackable tiers with eight nose-only exposure ports per tier. Each stainless-steel exposure unit consisted of three modules providing 24 ports for animal exposure and test atmosphere sampling. Each port received approximately 500 mL/min of exposure atmosphere, for a total exposure unit inlet flow of approximately 12 L/min. Vented enclosures mounted on stainless-steel stands surrounded each exposure carousel and were designed for containment of the exposure units. This carousel design provided uniform concentration and fresh vapor of test chemical to each animal connected to the exposure system. Internal tubing was constructed of electropolished stainless steel. The materials of construction reduced turbulence within the aerosol flow path and reduced the potential for particle loss to internal surfaces.

The test atmosphere entered the carousel through the top. After filling an inlet manifold, the test atmosphere then flowed radially to each of eight evenly spaced ports into each animal containment tube mounted on one of three different levels of the carousel. This design minimized any effect of the animals on the atmosphere because no exhaled air from one animal could reach the breathing zone of another. The temperature and humidity of the aerosol systems were not monitored or controlled and corresponded to the ambient conditions in the room.

One end of the tube that restrained the individual animal was tapered to approximately fit the shape of the animal's head, and the diameter of the cylindrical portion of the tube was configured such that it was difficult for the animal to turn in the tube. The back portion of the tube was covered with a plastic cap. The tube containing the animal was fastened to the inhalation carousel by a bracket with the nose portion of the tube protruding into the carousel.

Each exposure carousel was surrounded by a ventilated enclosure maintained at a pressure slightly negative relative the room's pressure. The purpose of the enclosure was to minimize the release of test atmosphere from the carousel to the room. Each enclosure was ventilated at a rate of approximately 450 L/min. Three separate exposure carousels and cabinets were used for the studies: one for the TMSD exposures, one for the hexanes control group, and a third for the air control group.

Exposure operation proceeded in stages: equilibration, normal operation, and shutdown. The equilibration period began by initiating the syringe pump, transferring test chemical to the vaporization column. During this period, the test chemical vapor was exhausted through a scrubber in the glove box and filtered off-exposure air was delivered to the exposure carousel inlet. After the system equilibrated, exposure was initiated using the on-exposure switch on the ECU. Concurrent with this action, a solenoid valve was actuated, directing exposure vapor from the bypass line to the inlet of the carousel and placing the generation system in the normal operating stage. Shutdown at the end of the exposure period began with an ECU-initiated actuation of the solenoid valve that diverted the flow of test chemical vapor to exhaust and returned off-exposure air to the inlet of the exposure carousel. Exposures for the TMSD-exposed groups were conducted sequentially from high concentration to low.

A.3. Vapor Concentration Monitoring

Samples from each exposure (TMSD) and control (hexanes and air) atmosphere were collected at the Battelle West Jefferson testing facility from nose-only ports onto XAD-4 sorbent tubes (8 mm × 150 mm, SKC, Inc., Eighty Four, PA) for 30 minutes at flow rates ranging from 0.4 to 0.5 L/min. Room air samples also were collected for the duration of the exposure sampling. Samples were collected on October 12, 14, 15, 16, 17, and 18, 2015 (rat studies) and on October 13, 19, 20, 21, 22, and 23, 2015 (mouse studies).

Analysis of the filtered air and room air samples produced TMSD and total hexanes results that were either nondetectable (ND) or below the limit of quantitation (BLOQ) for the respective analyte. The analytical limits of quantitation (LOQs) for TMSD and n-hexane were approximately 2.04 μg/mL and 2.00 μg/mL, respectively. These amounts correspond to vapor concentrations of approximately 0.13 ppm TMSD and 0.29 ppm total hexanes, using their respective solution-to-vapor concentration conversion equations with a dilution volume of 4 mL and assuming a sampling flow rate of 0.45 L/min for 30 minutes.

A.3.1. Rats

Summaries of TMSD and total hexanes concentration results are shown in Table A-3 and Table A-4 (1-day exposure) and in Table A-5 and Table A-6 (5-day exposure) for rat exposures. The relative standard deviations (RSDs) for all TMSD exposure concentrations were within 13%. TMSD concentrations were within 20% of the respective target concentrations, except for the 0.3 ppm exposure on October 15, 2015, which was 22.5% below target. Over the 5 days of exposure, the grand average RSDs for the TMSD exposure concentrations were within 10% for the 1, 3, and 10 ppm exposure concentrations and within 17% for the 0.3 ppm exposure concentration. The grand average determined TMSD concentrations were within 7% of the respective target exposure concentrations for all concentrations.

The RSDs for all hexanes exposure concentrations were within 11% for each exposure concentration. Over the 5 days of exposure, the grand average RSD for the total hexanes exposure concentrations were within 7% for the 1, 3, and 10 ppm TMSD exposure concentrations and within 12% for the 0.3 ppm exposure concentration.

A.3.2. Mice

Summaries of TMSD and total hexanes concentration results are shown in Table A-7 and Table A-8 (1-day exposure) and in Table A-9 and Table A-10 (5-day exposure) from mouse exposures. The RSDs for all TMSD exposure concentrations were within 13%, except for the 1 ppm exposure on October 23, 2015, which was 16%. TMSD concentrations were within 18% of the respective target concentrations, with the exception of the 1 ppm exposure on October 23, 2015, which was 21.4% below target. Over the 5 days of exposure, the grand average RSDs for the TMSD exposure concentrations were within 8% for the 3 and 10 ppm exposure concentrations and within 12% for the 0.3 ppm exposure concentration. The grand average RSD for the 1 ppm exposure concentration was within 16%. The grand average determined TMSD concentrations were within 4% of the respective target exposure concentrations for the 0.3, 1, and 10 ppm exposures. The grand average determined TMSD concentration for the 3 ppm TMSD exposure was within 11% of the target concentration.

The RSDs for all hexanes exposure concentrations were within 12%, except for the 1 ppm exposure on October 23, 2015, which was 14.3%. Over the 5 days of exposure, the grand average RSDs for the total hexanes exposure concentrations were within 8% for the 0.3, 3, and 10 ppm TMSD exposure concentrations and within 11% for the 1 ppm exposure concentration. Comparison of the hexanes control to the total hexanes concentration for the highest TMSD exposure concentration indicated comparable results according to the delivery settings used and provided a high-exposure benchmark for the effect of hexanes only on animal exposure.

Analysis of the filtered air and room air samples produced TMSD and total hexanes results that were either ND or BLOQ for the respective analyte. The analytical LOQs for TMSD and n-hexane were approximately 2.04 µg/mL and 2.00 µg/mL, respectively. These amounts correspond to vapor concentrations of approximately 0.13 ppm TMSD and 0.29 ppm total hexanes, using their respective solution-to-vapor concentration conversion equations with a dilution volume of 4 mL and assuming a sampling flow rate of 0.45 L/min for 30 minutes.

A.4. Chamber Atmosphere Characterization

Sample analyses were performed at the Battelle Columbus test site. All analyses used a GC/MSD method validated before the start of the study (Table A-11).

For homogeneity, TMSD and hexanes sampling ports were sampled at the Battelle West Jefferson testing facility onto XAD-4 sorbent tubes for 30 minutes at a flow rate of approximately 0.45 L/min. At each concentration, six samples were shipped refrigerated to the Battelle Columbus test site: two from the top tier, two from the middle, and two from the bottom, for a total n = 6 for each target concentration. In each tier, two individual samples came from ports 2 and 6. Table A-12 shows the averaged results by tier (n = 2) for each target concentration level (n = 6) to evaluate the homogeneity between the carousel locations. Table A-13 and Table A-14 show the averaged determined concentrations for each concentration level (n = 6) to evaluate homogeneity of the entire carousel. For the purposes of homogeneity evaluation, the low concentration (0.2 ppm) exposed group was generated at an elevated concentration to ensure no results were BLOQ. The hexanes control generator was set to a concentration equal to the estimated total hexanes content in the TMSD high-exposure group generator to assess the highest hexanes concentration present in the TMSD-exposed groups.

Evaluation of the homogeneity for both TMSD and total hexanes in the TMSD and hexanes exposure samples indicated that the carousel sampling from all three tiers was within 10% RSD for all concentrations. The TMSD concentrations were within 15% of target except for the 0.2 ppm and 5 ppm groups. The higher RE value was a result of comparison with the 0.2 ppm target. The actual concentration was purposely set to a higher value to ensure detection during analysis. RE for total hexanes was not reported. The hexanes control results were provided as a relative comparison with the highest total hexanes concentrations present in TMSD vapor concentrations as the system generator settings were identical. These results were comparable.

Consistent delivery of TMSD was observed in the exposure atmosphere at the nose-only ports. Total hexanes were measured as a ratio of a given TMSD concentration because TMSD was in a solution of mixed hexanes. Because the vapor generation was predicated on achieving TMSD targets, values for hexanes concentrations could have been biased toward concentrations associated with specific generator settings. Therefore, a comparison with a target concentration was not reported for total hexanes. Consistent delivery of hexanes was observed. Comparison of the hexanes control with the total hexanes concentration for the highest TMSD exposure concentration indicated comparable results according to the delivery settings used and provided a high-exposure benchmark for the effect of hexane-only exposure on animals.

A.4.1. Test for Aerosol in Exposure Atmosphere

A condensation particle counter (Model 3022A; TSI Incorporated; St. Paul, MN) was used to count the particles before the start of generation and during generation. Particle counts <200 particles/cm^3 are typical of an exposure atmosphere when no generation is occurring. Particle counts above this level, especially if the counts increase with exposure concentration and are above the level during the off-exposure period, would suggest a contribution to the aerosol concentration due to the generation system. The results of the particle count measurements are given in Table A-15. The data indicates no concentrations >200 particles/cm^3 either before or

during the exposure, hence no contribution to the aerosol concentration due to the generation systems (a single before-exposure measurement was used for the TMSD-exposed groups).

A.4.2. Health and Safety

As with all NTP studies, extensive effort was expended to ensure the health and safety of study personnel. This section summarizes selected health and safety aspects applicable to all NTP inhalation studies, as well as those specific to TMSD and hexanes. Detailed information is on file at NIEHS.

There are no short-term (e.g., 10- to 30-minute) or 8-hour time-weighted average (TWA) daily occupational exposure limits for TMSD. An occupational exposure limit (OEL) of 0.1 ppm, however, was referenced in a Sigma-Aldrich TMSD Safety Data Sheet. For diazomethane, a similar chemical, the 8-hour TWA Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) are both 0.2 ppm. For n-hexane, the OSHA PEL is 500 ppm, the ACGIH TLV is 50 ppm, and the National Institute for Occupational Safety and Health (NIOSH) 10-hour TWA recommended exposure limit (REL) also is 50 ppm.

Occupational action levels typically are 50% of a TLV. Exceeding an action level in a work environment would have prompted evaluation of the effectiveness of controls and might have triggered additional engineering and administrative controls to limit personnel exposure, supplemented with increased use of appropriate personal protective equipment (PPE). Battelle therefore used 0.1 ppm TMSD (a value listed as the OEL in a TMSD Safety Data Sheet and that is 50% of the TLV for diazomethane) as its OEL.

Protection from explosion was considered. The high concentration on study is <10% of the lower explosive limit (1.1% LEL; 10% is 1,110 ppm).

No incompatible chemicals (e.g., oxidizing materials) were used or introduced into the generation and delivery systems for TMSD and hexanes.

Primary engineering control was achieved by the use of local exhaust ventilation systems. Personnel were instructed to confine the opening of container, transferring, and use of TMSD and hexanes, to include handling of samples, in a functional laboratory hood or ventilated enclosure. Ventilation systems were monitored for airflow efficiency and monitoring records were maintained.

Staff were trained on the Battelle Chemical Safety Summaries for both TMSD and hexanes. In addition, all personnel involved in the TMSD and hexanes studies were provided health and safety training to comply with the Occupational Safety and Health Administration (OSHA) Laboratory Standard requirements. Personnel who worked with TMSD or hexanes or exposed study animals were assigned to the Battelle occupational health surveillance program.

To complement engineering and administrative controls, additional hazard control was accomplished with the use of personal protective clothing and equipment. In addition, during initial startup of exposure generation activities, a photoionization detector (PID) was run. TMSD and hexanes generation handling activities were performed in a ventilated glove box. The purpose of the PID was to continuously monitor for changes at the exposure carousel enclosure (based on hexanes) to detect abnormal conditions. Passive sampling, which has a greater

resolution and specificity, was also conducted and demonstrated that containment was effective. This allowed selection of appropriate PPE, including full-face respiratory protection. The PID was also run during the in-life phase to monitor for unexpected concentrations or release throughout the duration of the studies. Chemistry activities were performed in a working laboratory hood with appropriate PPE.

The design of the generation and exposure system and the minimum exhaust flow for the block of exposure rooms used for the studies suggested that detectable concentrations would not be expected in the building exhaust. In support of inhalation system design and safety evaluation, exposure and exhaust atmospheres of hexanes and TMSD were evaluated. Sampling for both TMSD and hexanes exhaust analyses occurred at the nose-only port, collecting front (primary sample delivery) and backup tubes (check for sample breakthrough) in quadruplicate. Additional samples were acquired (no backup tubes) within the carousel cabinet, at the exhaust scrubber, inside the glove box (housing the syringe pump), and within the room (field blank). These samples were acquired in duplicate. Samples were shipped refrigerated from the Battelle West Jefferson testing facility to the Battelle Columbus test site to be analyzed.

The evaluation of hexanes control exhaust was performed using an arbitrarily high concentration as a target for the nose-only port to ensure detection of potential leaks in the system. Results for this exhaust analysis are summarized in Table A-16. For the carousel cabinet with two replicates, standard deviation is not calculated, so precision of duplicates was reported in place of RSD.

In the TMSD exhaust analysis, no TMSD was detected, but hexanes were present in small amounts (≤0.13% of the hexanes concentration measured at the nose-only port) in the carousel cabinet, at the exhaust flow scrubber, and inside the glove box. This concentration is roughly the same order of magnitude of hexanes present during the hexanes exhaust analysis, with differences likely due to secure sampling fixtures. Comparison of the mixed hexanes control with the hexanes component in the TMSD test article indicated that comparable concentrations were obtained. The absence of TMSD and hexanes in the backup tubes for the nose-only port samples indicated that no breakthrough occurred.

Specific procedures were implemented for spill control, clean-up, and emergency planning and response.

Table A-1. Area Percent Purity Profile − Trimethylsilyldiazomethane

Peak ID	Average Percent of Total Area
n-Hexane	33.6
Methylcyclopentane	25.7
Trimethylsilanol	0.13
Cyclohexane	0.21
Hexamethyldisiloxane	0.79
Heptane	0.15
Trimethylsilyldiazomethane	36.1
(Chloromethyl)trimethylsilane	3.30
Trimethylsilylmethanol	0.07

Table A-2. Area Percent Purity Profile – Mixed Hexanes

Peak ID	Average Percent of Total Area
2-Methylpentane	0.84
3-Methylpentane	16.7
n-Hexane	64.2
Methylcyclopentane	17.4
Cyclohexane	0.87

Table A-3. Summary of Trimethylsilyldiazomethane Chamber Concentrations for Rats in the One-day Nose-only Inhalation Study

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined TMSD Concentration[b] (ppm)	RSD	Mean RE
October 12, 2015	October 12, 2015	Room air	ND	NA	NA
		0 (air)	ND	NA	NA
		10	11.3 ± 1.0	8.8	12.9

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; RE = relative error; ND = not detected; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-4. Summary of Total Hexanes Chamber Concentrations for Rats in the One-day Nose-only Inhalation Study of Trimethylsilyldiazomethane

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined Total Hexanes Concentration[b] (ppm)	RSD
October 12, 2015	October 12, 2015	Room air	BLOQ	NA
		0 (air)	BLOQ	NA
		0 (hexanes)	25.1 ± 0.7	2.8
		10	26.9 ± 1.6	6.0

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; BLOQ = below limit of quantitation; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-5. Summary of Trimethylsilyldiazomethane Chamber Concentrations for Rats in the Five-day Nose-only Inhalation Study

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined TMSD Concentration[b] (ppm)	Grand RSD	Grand Mean RE
October 14–18, 2015	October 14–19, 2015	Room air	ND	NA	NA
		0 (air)	ND	NA	NA
		0.3	0.283 ± 0.047	16.5	−5.5
		1	1.06 ± 0.10	9.1	6.5
		3	2.87 ± 0.25	8.8	−4.4
		10	10.3 ± 0.8	8.0	3.0

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; RE = relative error; ND = not detected; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-6. Summary of Total Hexanes Chamber Concentrations for Rats in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined Total Hexanes Concentration[b] (ppm)	Grand RSD
October 14–18, 2015	October 14–19, 2015	Room air	BLOQ	NA
		0 ppm (air)	BLOQ	NA
		0 ppm (hexanes)	24.5 ± 1.4	5.9
		0.3	0.845 ± 0.098	11.6
		1	3.20 ± 0.21	6.4
		3	7.96 ± 0.45	5.7
		10	25.4 ± 1.6	6.3

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; BLOQ = below limit of quantitation; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-7. Summary of Trimethylsilyldiazomethane Chamber Concentrations for Mice in the One-day Nose-only Inhalation Study

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined TMSD Concentration[b] (ppm)	RSD	Mean RE
October 13, 2015	October 13, 2015	Room air	ND	NA	NA
		Filtered air (0)	ND	NA	NA
		10	11.2 ± 0.2	2.1	11.9

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; RE = relative error; ND = not detected; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-8. Summary of Total Hexanes Chamber Concentrations for Mice in the One-day Nose-only Inhalation Study of Trimethylsilyldiazomethane

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined Total Hexanes Concentration[b] (ppm)	RSD
October 13, 2015	October 13, 2015	Room air	ND	NA
		Filtered air (0)	ND	NA
		Hexanes (0)	24.1 ± 0.6	2.7
		10	27.1 ± 0.6	2.2

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; ND = not detected; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-9. Summary of Trimethylsilyldiazomethane Chamber Concentrations for Mice in the Five-day Nose-only Inhalation Study

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined TMSD Concentration[b] (ppm)	Grand RSD	Grand Mean RE
October 19–23, 2015	October 22–27, 2015	Room air	ND	NA	NA
		Filtered air (0)	ND	NA	NA
		0.3	0.299 ± 0.031	10.4	−0.5
		1	1.04 ± 0.17	16.0	3.9
		3	2.68 ± 0.16	5.9	−10.6
		10	10.2 ± 0.7	7.4	1.5

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; RE = relative error; ND = not detected; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-10. Summary of Total Hexanes Chamber Concentrations for Mice in the Five-day Nose-only Inhalation Study of Trimethylsilyldiazomethane

Exposure Date	Date Analyzed[a]	Target TMSD Concentration (ppm)	Determined Total Hexanes Concentration[b] (ppm)	Grand RSD
October 19–23, 2015	October 22–27, 2015	Room air	BLOQ	NA
		Filtered air (0)	BLOQ	NA
		Hexanes (0)	25.6 ± 1.5	6.0
		0.3	1.05 ± 0.07	6.6
		1	3.48 ± 0.37	10.7
		3	7.90 ± 0.31	3.9
		10	25.3 ± 1.2	4.8

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; BLOQ = below limit of quantitation; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation.

Table A-11. Gas Chromatography/Mass Selective Detection System Settings

Column	RTX-1301 (Restek, Bellefonte, PA)
Column Dimensions	30 m × 0.25 mm (ID); 1-µm film thickness
Autosampler	CombiPAL (CTC Analytics, Leap Technologies, Carrboro, NC)
Carrier Gas and Flow Rate	Helium at 1.5 mL/minute
Oven Temperature	35°C hold for 12 minutes, increase at 40°C/minute to 240°C, final hold 2 minutes
Injector Temperature	140°C
Injection Volume/Mode	2 µL, Split 10:1
Detector/Mode	MSD, EI+, Scan 35–300 m/z
Auxiliary Temperature	260°C

Column	RTX-1301 (Restek, Bellefonte, PA)
Source Temperature	230°C
Quad Temperature	150°C
Run Time	19 minutes
Data Acquisition System	ChemStation Version D.02.00.275
Data Analysis System	MassLynx Version 4.0

ID = internal diameter; MSD = mass selective detection; EI+ = electron impact ionization + .

Table A-12. Trimethylsilyldiazomethane and Total Hexanes Homogeneity Results for Carousel Sampling Locations

Target TMSD Exposure Concentration (ppm)	Average Determined Concentration (ppm)					
	Top Tier		Middle Tier		Bottom Tier	
	TMSD	Total Hexanes	TMSD	Total Hexanes	TMSD	Total Hexanes
0 ppm (hexanes)	NA	53.9	NA	53.7	NA	56.4
0.2	0.572	1.52	0.580	1.49	0.568	1.36
1	1.18	3.04	1.14	3.06	1.11	3.03
5	5.72	14.3	5.80	14.6	5.80	14.7
20	22.8	52.8	19.8	49.5	21.6	51.0

TMSD = trimethylsilyldiazomethane; NA = not applicable.

Table A-13. Trimethylsilyldiazomethane Homogeneity Results for Carousel

Target TMSD Exposure Concentration (ppm)	Average Determined TMSD Concentration (ppm)[a]	RSD	Average RE
0.2	0.573 ± 0.028	4.9	186.7
1	1.14 ± 0.06	5.6	14.2
5	5.78 ± 0.29	5.0	15.5
20	21.4 ± 2.0	9.4	7.2

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation; RE = relative error.
[a]Data are presented as mean ± standard deviation.

Table A-14. Total Hexanes Homogeneity Results for Carousel

Target TMSD Exposure Concentration (ppm)	Average Determined Total Hexanes Concentration (ppm)[a]	RSD
0 ppm (hexanes)	54.7 ± 3.0	5.4
0.2	1.45 ± 0.12	8.3
1	3.05 ± 0.06	2.0
5	14.5 ± 0.7	4.7
20	51.1 ± 2.5	4.9

TMSD = trimethylsilyldiazomethane; RSD = relative standard deviation.
[a]Data are presented as mean ± standard deviation.

Table A-15. Particle Counts in Exposure Atmosphere before and during Generation

Exposure Group	Particle Count before Exposure (particle/cm^3)[a]	Particle Count during Exposure (particle/cm^3)[a]
Hexanes Control	10	34
0.3 ppm TMSD	9	15
1 ppm TMSD	9	18
3 ppm TMSD	9	18
10 ppm TMSD	9	9
Room 1–560 (collected after exposure)	730	NA

TMSD = trimethylsilyldiazomethane; NA = not applicable.
[a]Values shown are rounded.

Table A-16. Summary of Mixed Hexanes Exhaust Analysis

Sampling Description	Average Determined Total Hexanes Concentration[a] (ppm)	RSD
Nose-only Port – Front[b]	125 ± 8	6.2
Nose-only Port – Backup	BLOQ	NA
Carousel Cabinet	1.05	0.864[c]
Exhaust Flow Scrubber	ND	NA
Inside Glove Box	BLOQ	NA
Field Blank (Exposure Room)	ND	NA

RSD = relative standard deviation; BLOQ = below the limit of quantitation; NA = not applicable; ND = not detected.
[a]Data shown are mean ± standard deviation.
[b]n = 3; one replicate excluded as an outlier via Q-test.
[c]n = 2; precision of duplicates reported.

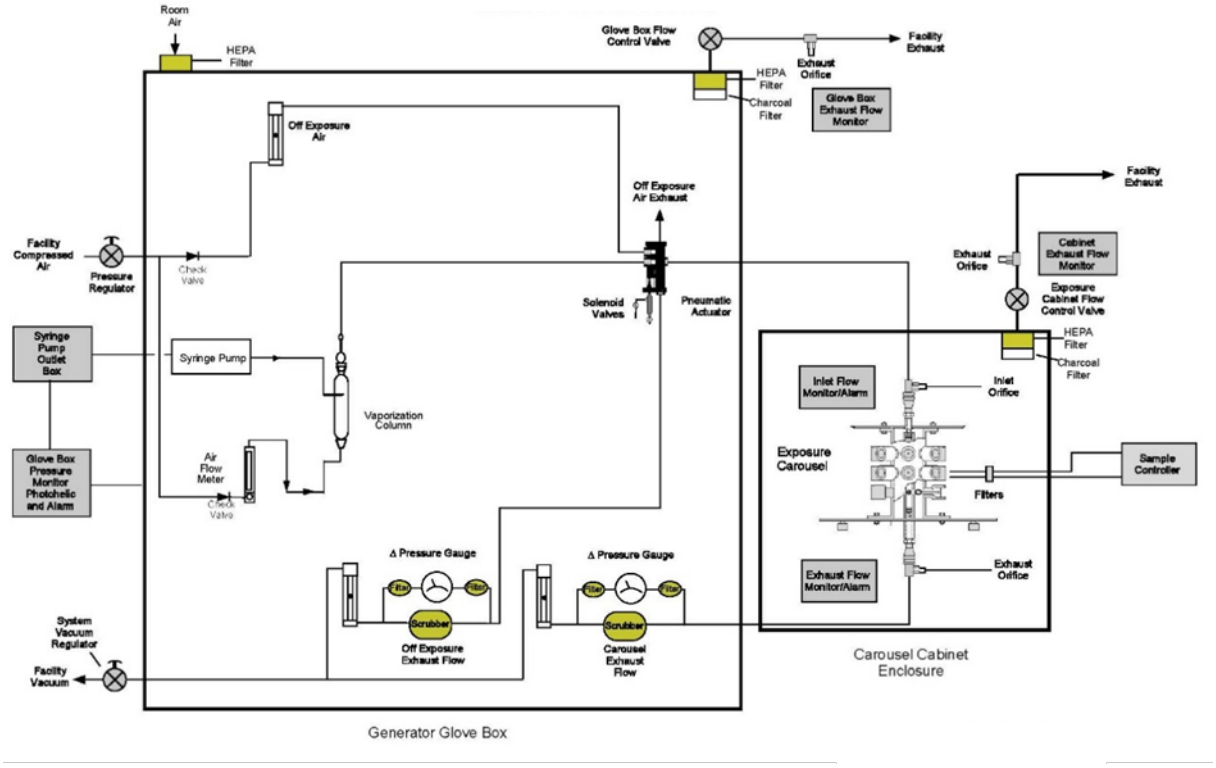

Figure A-1. Trimethylsilyldiazomethane Generation, Distribution, and Exposure System

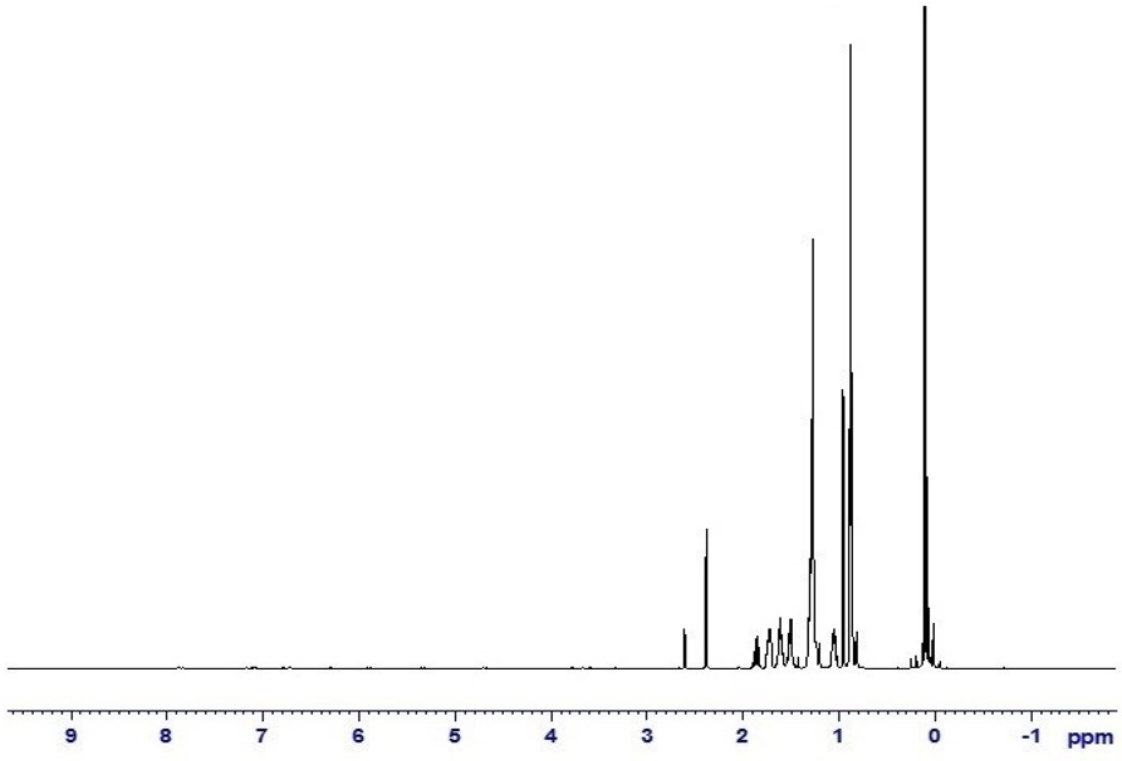

Figure A-2. ^{1}H Nuclear Magnetic Resonance Spectrum of Trimethylsilyldiazomethane

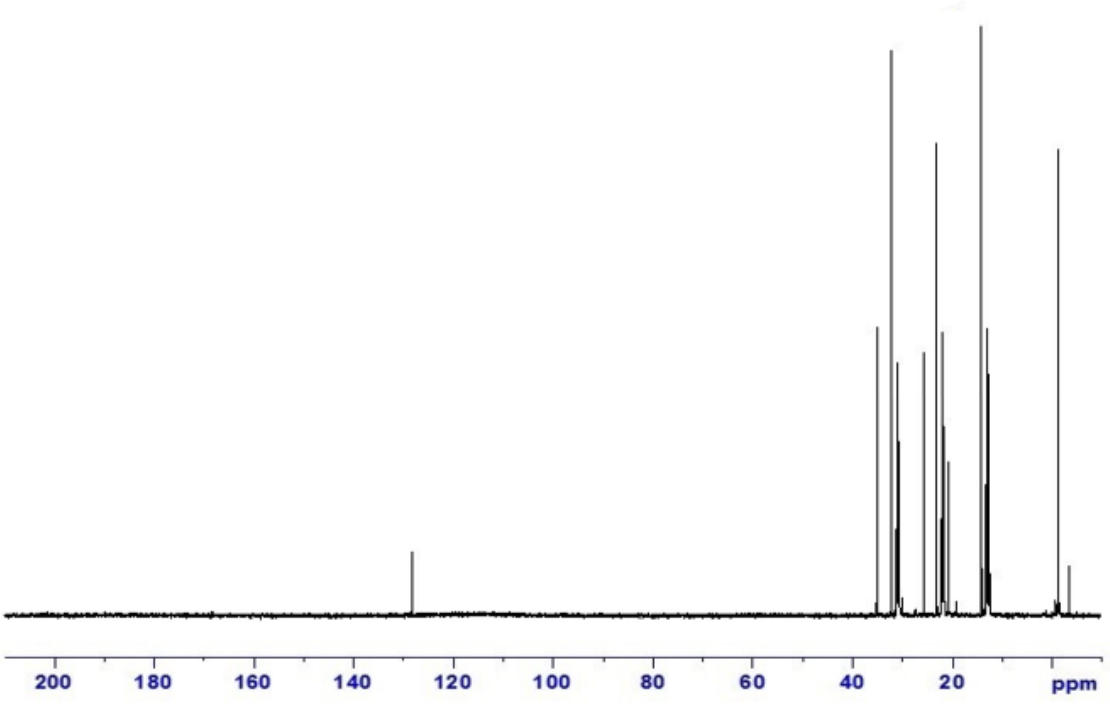

Figure A-3. ^{13}C Nuclear Magnetic Resonance Spectrum of Trimethylsilyldiazomethane

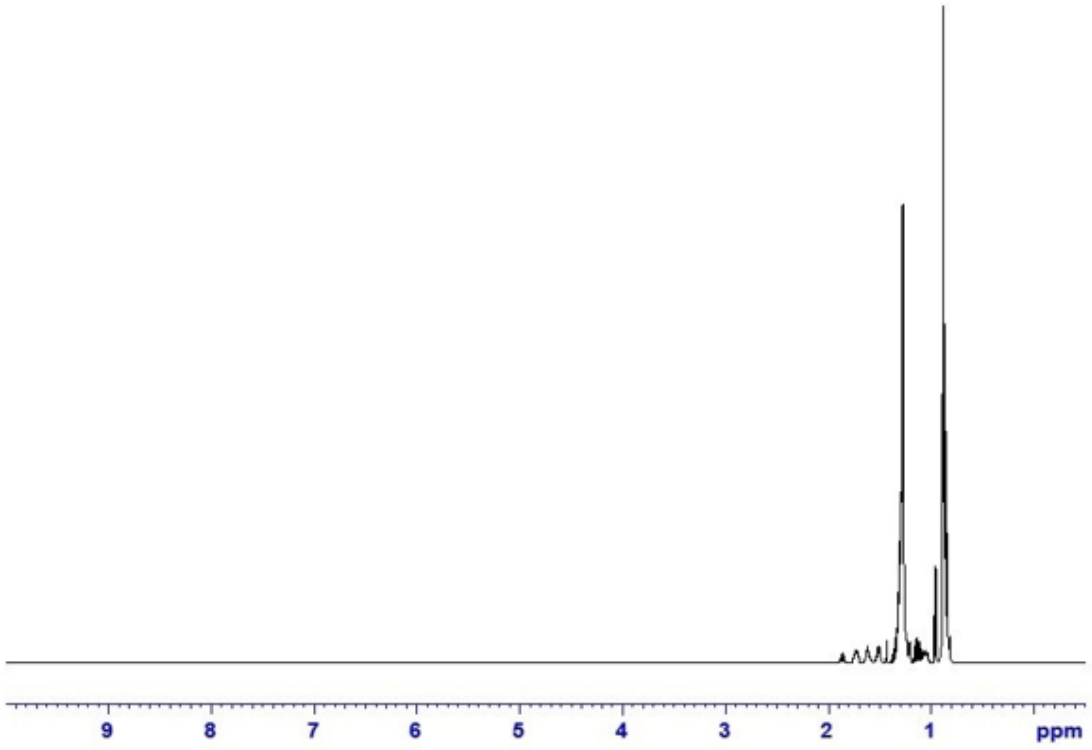

Figure A-4. ^{1}H Nuclear Magnetic Resonance Spectrum of Mixed Hexanes

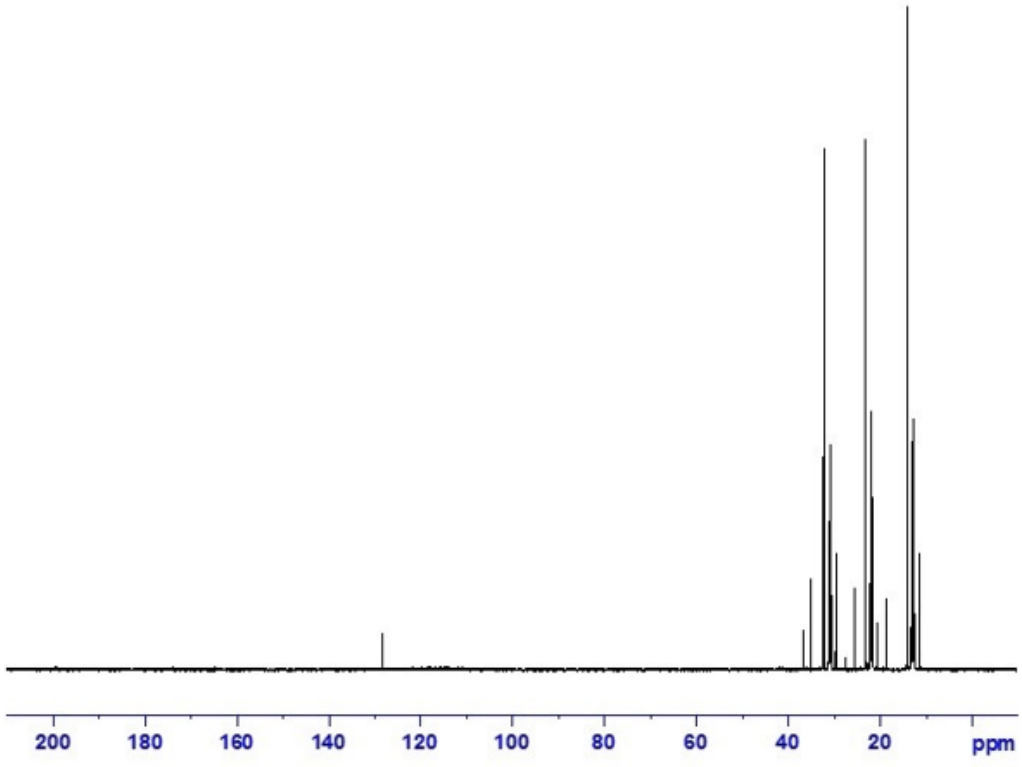

Figure A-5. ^{13}C Nuclear Magnetic Resonance Spectrum of Mixed Hexanes

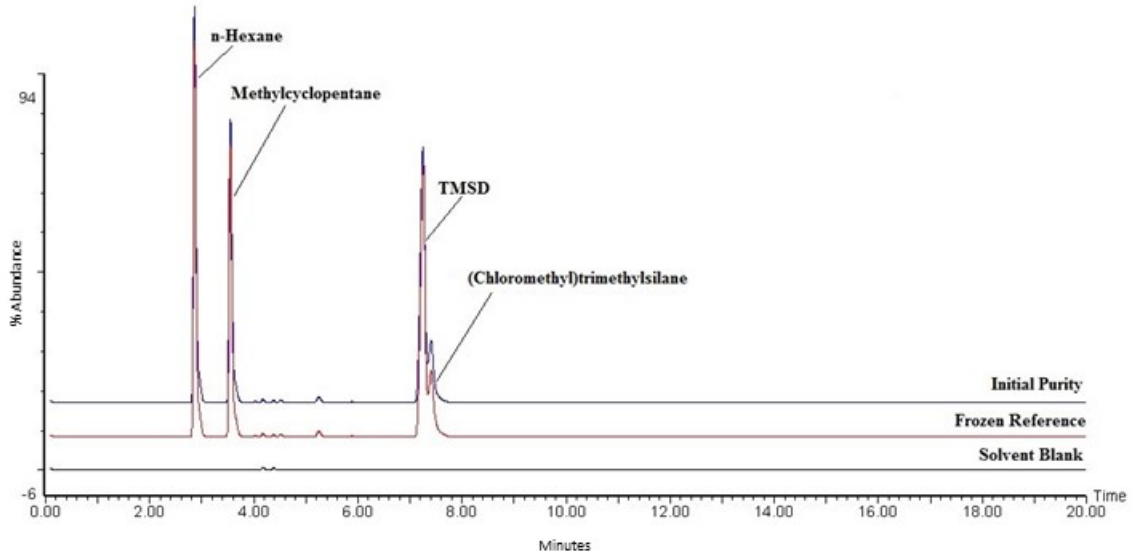

Figure A-6. Total Ion Chromatogram from Gas Chromatography/Mass Selective Detection Analysis of Trimethylsilyldiazomethane

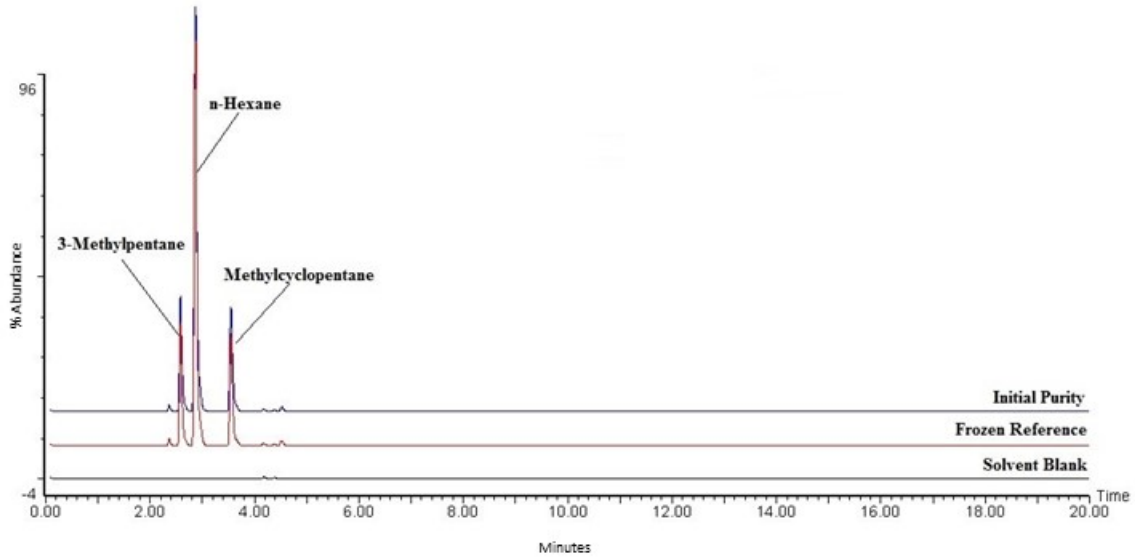

Figure A-7. Total Ion Chromatogram from Gas Chromatography/Mass Selective Detection Analysis of Mixed Hexanes

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

Tables

Table B-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soy Protein Concentrate	4.0
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

USP = United States Pharmacopeia.
[a]Rice hulls and limestone as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
α-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

	Amount	Source
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table B-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.85 ± 0.495	14.5–15.2	2
Crude Fat (% by Weight)	8.15 ± 0.071	8.1–8.2	2
Crude Fiber (% by Weight)	9.96 ± 0.205	9.81–10.1	2
Ash (% by Weight)	5.09 ± 0.141	4.99–5.19	2
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.021	0.15–.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.100 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamins			
Vitamin A (IU/kg)	5,980 ± 416	3,040–8,920	2
α-Tocopherol (ppm)	2,456 ± 12,817	13.6–69,100	29
Thiamine (ppm)[a]	8.6 ± 0.990	7.9–9.3	2
Riboflavin (ppm)	8.17 ± 2.841	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[a]	9.75 ± 2.045	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
Vitamin B_{12} (ppb)	50.41 ± 34.89	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633.8	1,160–3,790	29
Minerals			
Calcium (%)	0.928 ± 0.091	0.863–0.992	2
Phosphorus (%)	0.571 ± 0.008	0.565–0.577	2
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.54	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.02 ± 1.04	0.33–3.97	28
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	27

[a]As hydrochloride.

Table B-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.253 ± 0.023	0.236–0.269	2
Cadmium (ppm)	0.049 ± 0.006	0.045–0.053	2
Lead (ppm)	0.0875 ± 0.032	0.065–0.11	2
Mercury (ppm)[a]	0.01 ± 0.0	0.01–0.01	2
Selenium (ppm)	0.1745 ± 0.006	0.17–0.179	2
Aflatoxins (ppb)[a]	<5.0	–	2
Nitrate Nitrogen (ppm)[b]	15.85 ± 0.354	15.6–16.1	2
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	2
BHA (ppm)[a,c]	<1.00	–	2
BHT (ppm)[a,c]	<1.00	–	2
Aerobic Plate Count (CFU/gm)	<10.0	–	2
Coliform (MPN/gm)	<3	–	2
Escherichia coli (MPN/gm)[a]	<3.0	–	2
Salmonella (MPN/gm)	Negative	–	2
Total Nitrosamines (ppb)[d]	10.6 ± 3.04	8.4–12.7	2
N,N-Dimethylamine (ppb)[d]	1.6 ± 2.26	0.0–3.2	2
N,N-Pyrrolidine (ppb)[d]	9.0 ± 0.78	8.4–9.5	2
Pesticides (ppm)			
α-BHC[a]	<0.01	–	2
β-BHC[a]	<0.02	–	2
γ-BHC[a]	<0.01	–	2
δ-BHC[a]	<0.01	–	2
Heptachlor[a]	<0.01	–	2
Aldrin[a]	<0.01	–	2
Heptachlor Epoxide[a]	<0.01	–	2
DDE[a]	<0.01	–	2
DDD[a]	<0.01	–	2
DDT[a]	<0.01	–	2
HCB[a]	<0.01	–	2
Mirex[a]	<0.01	–	2
Methoxychlor[a]	<0.05	–	2
Dieldrin[a]	<0.01	–	2
Endrin[a]	<0.01	–	2

	Mean ± Standard Deviation	Range	Number of Samples
Telodrin[a]	<0.01	–	2
Chlordane[a]	<0.05	–	2
Toxaphene[a]	<0.10	–	2
Estimated PCBs[a]	<0.20	–	2
Ronnel[a]	<0.01	–	2
Ethion[a]	<0.02	–	2
Trithion[a]	<0.05	–	2
Diazinon[a]	<0.10	–	2
Methyl Chlorpyrifos	0.092 ± 0.024	0.075–0.109	2
Methyl Parathion[a]	<0.02	–	2
Ethyl Parathion[a]	<0.02	–	2
Malathion	0.02 ± 0.0	0.02–0.02	2
Endosulfan I[a]	<0.01	–	2
Endosulfan II[a]	<0.01	–	2
Endosulfan Sulfate[a]	<0.03	–	2

BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]If all values were below the detection limit, the detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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Tables

C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed 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.

For these toxicity studies, blood samples were collected and allowed to clot, and the serum was separated. All samples were processed appropriately with serology testing sent to IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are listed below; the times at which samples were collected during the studies are also listed in Table C-1. and Table C-2.

Table C-1. Methods and Results for Sentinel Animal Testing in Rats

One-day and Five-day Exposure Studies	
Collection Time Point	**Quarantine**
Number Examined (Males)	10
Method/Test	
Multiplex Fluorescent Immunoassay	
Kilham rat virus (KRV)	–
Mycoplasma pulmonis	–
Parvo NS-1	–
Pneumonia virus of mice (PVM)	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–
Rat minute virus (RMV)	–
Rat parvo virus (RPV)	–
Rat theilovirus (RTV)	–
Sendai	–
Theiler's murine encephalomyelitis virus (TMEV)	–
Toolan’s H–1	–
In-house Evaluation	
Endoparasite evaluation (evaluation of cecal content)	–
Ectoparasite evaluation (evaluation of perianal surface)	–

– = negative result.

Table C-2. Methods and Results for Sentinel Animal Testing in Mice

One-day and Five-day Exposure Studies	
Collection Time Point	**Quarantine**
Number Examined (Males)	10
Method/Test	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	–
Epizootic diarrhea of infant mice (EDIM)	–
Lymphocytic choriomeningitis virus (LCMV)	–
Mycoplasma pulmonis	–
Mouse hepatitis virus (MHV)	–
Mouse norovirus (MNV)	–
Parvo NS-1	–
Mouse parvovirus (MPV)	–
Minute virus of mice (MVM)	–
Pneumonia virus of mice (PVM)	–
Rat theilovirus (RTV)	–
Reovirus (REO3)	–
Sendai	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–
In-house Evaluation	
Endoparasite evaluation (evaluation of cecal content)	–
Ectoparasite evaluation (evaluation of perianal surface)	–

– = negative result.

C.2. Results

All test results were negative.

Appendix D. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TOX-101.

D.1. Study Tables – Rats

I01 – Animal Removal Summary
C1104901_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C1104901_I02_Animal_Removals.pdf

I03 – Growth Curves
C1104901_I03_Growth_Curves.pdf

I04 – Mean Body Weight Summary
C1104901_I04_–_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain
C1104901_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary
C1104901_I05_Clinical_Observations_Summary.pdf

PA02 – Neoplastic Lesion Summary with Percent Incidence
C1104901_PA02_Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA03 – Non-Neoplastic Lesion Summary with Percent Incidence
C1104901_PA03_Non-Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA05 – Incidence Rates of Neoplastic Lesions with Systemic Lesions Abridged
C1104901_PA05_Incidence_Rates_of_Neoplastic_Lesions_with
_Systemic_Lesions_Abridged.pdf

PA06 – Organ Weights Summary
C1104901_PA06_Organ_Weights_Summary.pdf

PA08 – Statistical Analysis of Neoplastic Lesions
C1104901_PA08_Statistical_Analysis_of_Neoplastic_Lesions.pdf

PA10 – Statistical Analysis of Non-Neoplastic Lesions
C1104901_PA10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

PA14 – Individual Animal Pathology Data
C1104901_PA14_Individual_Animal_Pathology_Data.pdf

PA18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grade
C1104901_PA18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grade.pdf

PA46 – Summary of Gross Pathology
C1104901_PA46_Summary_of_Gross_Pathology.pdf

D.2. Individual Animal Data – Rats

Individual Animal Body Weight Data
C1104901_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Observations Data
C1104901_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Gross Pathology Data
C1104901_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Histopathology Data
C1104901_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Organ Weight Data
C1104901_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
C1104901_Individual_Animal_Removal_Reasons_Data.xlsx

D.3. Study Tables – Mice

I01 – Animal Removal Summary
C1104902_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C1104902_I02_Animal_Removals.pdf

I03 – Growth Curves
C1104902_I03_Growth_Curves.pdf

I04 – Mean Body Weight Summary
C1104902_I04_–_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain
C1104902_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary
C1104902_I05_Clinical_Observations_Summary.pdf

PA02 – Neoplastic Lesion Summary with Percent Incidence
C1104902_PA02_Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA03 – Non-Neoplastic Lesion Summary with Percent Incidence
C1104902_PA03_Non-Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA05 – Incidence Rates of Neoplastic Lesions with Systemic Lesions Abridged
C1104902_PA05_Incidence_Rates_of_Neoplastic_Lesions_with_Systemic_Lesions_Abridged.pdf

PA06 – Organ Weights Summary
C1104902_PA06_Organ_Weights_Summary.pdf

PA08 – Statistical Analysis of Neoplastic Lesions
C1104902_PA08_Statistical_Analysis_of_Neoplastic_Lesions.pdf

PA10 – Statistical Analysis of Non-Neoplastic Lesions
C1104902_PA10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

PA14 – Individual Animal Pathology Data
C1104902_PA14_Individual_Animal_Pathology_Data.pdf

PA18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grade
C1104902_PA18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grade.pdf

PA46 – Summary of Gross Pathology
C1104902_PA46_Summary_of_Gross_Pathology.pdf

D.4. Individual Animal Data – Mice

Individual Animal Body Weight Data
C1104902_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Observations Data
C1104902_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Gross Pathology Data
C1104902_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Histopathology Data
C1104902_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Organ Weight Data
C1104902_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
C1104902_Individual_Animal_Removal_Reasons_Data.xlsx

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