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Sensory Irritation Potential of Aviation-Related Chemical Contaminants in Normoxic and Hyperoxic Conditions.

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STATEMENTS

Disclaimer

The views expressed in this report are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

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Animal Care and Use statement

The study protocol was reviewed and approved by the Wright Patterson Air Force Base Institutional Animal Care and Use Committee in compliance with all federal regulations governing the protection of animals and research.

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List of Abbreviations

ASTM	American Society for Testing and Materials
CBA	Cyclobutylamine
FTIR	Fourier-Transform Infrared Spectrometer
IPA	Isopropanol
N	Naphthalene
NIST	National Institute of Standards and Technology
NO_2	Nitrogen Dioxide
NOEU	Nose Only Exposure Unit
OBOGS	On-Board Oxygen Generating System
PE	Physiological Episodes
RCCA	Root Cause and Corrective Action
RD_{50}	50 % Respiratory Depression
RH	Relative Humidity
RSD	Relative Standard Deviation
SD	Standard Deviation
TMB	1,2,4 – Trimethylbenzene

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Summary

Physiological Episodes (PEs) involving military aviators include a variety of both objective and subjective symptoms. Included among these are respiratory symptoms that resemble sensory irritancy. Sensory irritation in the respiratory system involves the response of nervous system reflexes that are perceived as a burning, stinging or itching in the upper airways. Chemical exposure has been suspected as a cause of these upper airway symptoms because of the presence of chemicals, fuels, fluids, and jet exhausts found in the aviation environment and within proximity to military aircraft (Mumy, 2019). A number of chemicals were identified from air sampling in and around tactical aircraft that may be capable of producing sensory irritancy for which either no experimental data exist, or data exists that does not fall within standard testing guidelines to determine the degree of sensory irritancy. Sensory irritation involves the response of nervous system reflexes to inhaled chemicals that typically act to lower the respiratory rate in mice. The concentration of an airborne contaminant that produces a 50% decrease in respiratory rate from the baseline respiratory rate is expressed as the Respiratory Depression 50% (RD_{50}) value. This report contains results for calculating the RD_{50} value for five chemicals presumed to be sensory irritants that were detected in air sampling in and around tactical aircraft. These five chemicals are isopropanol, 1,2,4-trimethylbenzene, cyclobutylamine, naphthalene and nitrogen dioxide. Four of the five chemicals produced respiratory depression consistent with sensory irritancy. Nitrogen dioxide exposure did not cause a respiratory depression. The RD_{50} values determined herein for these four chemicals in normoxic 21% oxygen are isopropanol at 77,545 mg/m^3 (31,547 ppm), 1,2,4-trimethylbenzene at 1,408 mg/m^3 (286 ppm), cyclobutylamine at 1,208 mg/m^3 (415 ppm) and naphthalene at 25.9 mg/m^3 (4.9 ppm). When repeated in 94% hyperoxic conditions, all RD_{50} values fell within the 95% confidence intervals established at 21% oxygen suggesting high oxygen environments do not further enhance sensory irritancy. These results will help determine if levels found in the aviation environment might be hazardous and/or contribute to sensory irritation like effects that have been experienced by Navy aircrews.

Introduction and Background

Physiological Episodes (PEs) involving military aviators include a variety of both objective and subjective symptoms. Included among these are respiratory symptoms that resemble sensory irritancy. Sensory irritation in the respiratory system involves the response of nervous system reflexes that are perceived as a burning, stinging or itching in the upper airways. Chemical exposure has been suspected as a cause of these upper airway symptoms because of the presence of numerous chemicals, fuels, fluids, and jet exhausts found in the aviation environment and within proximity to military aircraft (personal communication, K. Mumy, September 2019). A number of chemicals were identified from air sampling in and around tactical aircraft that may be capable of producing sensory irritancy, but for which either no experimental data exists, or data exists that does not fall within standard testing guidelines to determine the degree of sensory irritancy. Sensory irritation involves the response of nervous system reflexes to inhaled chemicals that typically act to lower the respiratory rate in mice. The concentration of an airborne contaminant that produces a 50% decrease in the respiratory rate from the baseline respiratory rate is expressed as the Respiratory Depression 50% (RD_{50}) value. The RD_{50} value is an indicator of the degree of sensory irritation experienced, and studies show a correlation between chemicals causing such a decrease in respiratory rate in mice and their sensory irritant properties in humans (Alarie, 1981). Thus, the American Society for Testing and Materials (ASTM) has standard guidelines to determine the RD_{50} value using a Swiss-Webster mouse model (2019).

A NAVAIR Root Cause and Corrective Action (RCCA) team investigating Physiological Episodes (PEs) in Naval aircrew has identified irritancy as a potential area of concern. A list of potential airborne irritants from chemical monitoring data in and around aircraft was generated by the RCCA team and toxicologists (personal communication, K. Mumy, September 2019). A number of these irritants do not have an established RD_{50} value. Cyclobutylamine, naphthalene, 1,2,4-trimethylbenzene, nitrogen dioxide and isopropanol are five of the identified contaminants (personal communication, K. Mumy, September 2019) for which there is either insufficient RD_{50} data in the literature, or that have known irritant effects that could be altered by co-exposure to hyperoxia (high levels of oxygen). Currently, there are no data to show how sensory irritancy is impacted by high oxygen levels (94% oxygen) of pilot breathing air supplied by the on-board oxygen generating system (OBOGS).

The RCCA team reported that cyclobutylamine was present at concentration of 1.75 mg/m^3 (0.6 ppm), naphthalene at 4.19 mg/m^3 (0.8 ppm), 1,2,4-trimethylbenzene at 2.95 mg/m^3 (0.6 ppm), nitrogen dioxide at 30.1 mg/m^3 (8.0 ppm) and isopropanol at 0.74 mg/m^3 (0.3 ppm) (personal communication, K. Mumy, September 2019). The nitrogen dioxide concentration is of particular interest because it exceeds the ceiling permissible exposure limit value of 5 ppm and the short-term exposure limit of 1 ppm for the Occupational Health and Safety Administration and the National Institute of Occupational Safety and Health, respectively. (OSHA, 2021). RD_{50} values for mice can start in the lower ppm levels for the most irritant compounds, with the uncertainty factor (UF) of 0.03 often applied to translate the irritant effects in mice to developing exposure limits for human exposures. For example, an exposure limit could be found that would be as low as 0.1 ppm for a chemical with a RD_{50} value of 3 ppm (UF x RD_{50}).

Each of the five chemicals identified by the RCCA team are either known or suspected irritants, but it is unclear from existing data if these chemicals are present at levels of exposure that are sufficient to cause irritancy in aircrew. Additionally, it is unknown if co-exposure to hyperoxia can modulate the irritant effects of any of these chemicals. Cyclobutylamine is an amine structurally similar to other aliphatic amines that have been classified as highly irritating (Nielsen and Yamagiwa, 1989). Cyclobutylamine can cause skin corrosion and irritation, but has not been characterized as a sensory irritant, thus an RD_{50} value has not been calculated (Ruth, 1986). Naphthalene is a hydrocarbon found in coal and crude oil that is also used in the manufacturing of plastics, resins and fuels. Naphthalene has been characterized as a sensory irritant and hazardous to human health, however an RD_{50} has not been calculated (Bruning et al., 2014). 1,2,4-trimethylbenzene is also a hydrocarbon that is naturally occurring in coal tar and petroleum and is also a constituent of jet fuel (Ritchie et al., 2001). 1,2,4-trimethylbenzene is classified as a respiratory sensory irritant (Korsak et al., 1995). An RD_{50} of 2844 mg/m^3 (578 ppm) has been calculated for 1,2,4-trimethylbenzene, however, it was determined using BALB/c mice following shorter exposure periods that might produce a higher RD_{50} value than if calculated using the ASTM method (Korsak et al., 1995). Nitrogen dioxide has also been identified as a respiratory sensory irritant (Lindvall, 1985). An RD_{50} has been calculated for nitrogen dioxide, 349 ppm, in anesthetized mice via tracheotomy that bypasses the upper respiratory tract (Alarie, 1981), which does not adhere to the current ASTM method. The source of each of these four chemicals is most likely exhaust from other aircraft and vehicles on the flight line. Isopropanol is an alcohol primarily used as a disinfectant and is also identified as an irritant (Smeets and Dalton, 2002). Although an RD_{50} has been calculated for isopropanol, 17,693 ppm, it is unknown if co-exposure to higher oxygen concentrations will impact the RD_{50} value.

The objective of this study is to assess the sensory irritation potential of cyclobutylamine, naphthalene, 1,2,4-trimethylbenzene, isopropanol and nitrogen dioxide when administered via inhalation to mice under both normal oxygen and high oxygen conditions. To our knowledge, no known RD_{50} values have been calculated in hyperoxic conditions. The testing method that was used followed the American Society for Testing and Materials guideline: Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals (ASTM, 2019). Separate cohorts of Swiss-Webster mice were exposed nose-only to vapors of the five concentrations of each of the chemicals for 30 minutes. Respiratory rates were measured with a plethysmograph prior to, during and following the exposure to determine the change in respiratory rates from pre-exposure baseline and calculate an RD_{50} value for each chemical.

Methods

The American Society for Testing and Materials

The testing method to determine RD_{50} used herein follows the American Society for Testing and Materials guideline: Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals (ASTM, 2019). Separate cohorts of Swiss-Webster mice were exposed nose-only to vapors of five concentrations of each of the chemicals for 30 minutes. Each mouse was exposed to a single chemical in a nose-only plethysmograph with no acclimation prior to the day of the study. After a brief acclimation period to the restraint of the plethysmography tubes, respiratory rates were measured prior to, during and following the exposure to determine the change in respiratory rates from pre-exposure baseline and calculate an RD_{50} value for each chemical (2019). Per ASTM standards, the mice used were of male sex with an intended narrow weight range of 22-28g to reduce variability and the need for larger numbers of animals (2019). However, quarantine requirements of the animal facility required the use of mice that were slightly larger than the recommended weight range by the time they were released from quarantine. Detailed exposure schedule is provided in Appendix 1.

Animal Husbandry

All animal procedures were performed in accordance with the policies of the Institutional Animal Care and Use Committee (Protocol F-WA-2020-0188) at Wright Patterson Air Force Base. A total of 202 male Swiss-Webster [Crl:CFW(SW)] mice (*Mus musculus*) were purchased from Charles River Laboratories (Wilmington, MA) at approximately 3-4 weeks old and 16 to 18g body weight. Mice were acclimated to the facility for seven days after arrival at the vivarium. Food and water were available *ad libitum* while mice were housed in the vivarium prior to exposure; neither were available during exposure. Animals were weighed upon receipt and again on the day of exposure. All mice were monitored daily by professional staff in the vivarium and exposure facilities at Wright Patterson Air Force Base. On exposure days, each animal was examined both pre- and post- exposure. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration and reactivity to handling or sensory stimuli. Average exposure groups and weights for each animal are listed in Table 1 and individual mouse details are listed in Appendix 2.

Table 1 Average Weights of Swiss-Webster Mice Per Exposure Group

Exposure Date	Chemical*	Concentration (mg/m^3)	Concentration (ppm)	O_2 Conditions	Exposure Group	# of mice	Weight (g) ± SD
20-Feb-20	Clean Air	0	0	Normoxia	Training	4	28.3 ± 1.4
3-March-20	IPA	6,700	2,723	Normoxia	1	4	29.5 ± 0.8

3-March-20	IPA	9,516	3,871	Normoxia	2	4	28.7 ± 0.6
3-March-20	IPA	12,856	5,230	Normoxia	3	4	28.1 ± 0.3
4-March-20	IPA	26,483	10,774	Normoxia	4	4	28.6 ± 0.3
4-March-20	IPA	45,488	18,506	Normoxia	5	4	27.9 ± 0.1
5-March-20	IPA	6,676	2,716	Hyperoxia	1	3	28.5 ± 0.2
5-March-20	IPA	9,478	3,856	Hyperoxia	2	4	27.9 ± 0.5
5-March-20	IPA	13,866	5,641	Hyperoxia	3	4	26.7 ± 0.3
6-March-20	IPA	27,417	11,154	Hyperoxia	4	4	25.9 ± 0.6
6-March-20	IPA	48,834	19,867	Hyperoxia	5	3	25.5 ± 0.9
10-March-20	TMB	1,398	284	Normoxia	1	4	28.3 ± 0.3
10-March-20	TMB	763	155	Normoxia	2	4	27.6 ± 0.5
10-March-20	TMB	295	60	Normoxia	3	4	28.2 ± 0.7
11-March-20	TMB	503	102	Normoxia	4	4	28.4 ± 0.7
11-March-20	TMB	164	33	Normoxia	5	4	27.7 ± 0.2
12-March-20	TMB	818	166	Hyperoxia	1	4	28.5 ± 0.7
12-March-20	TMB	463	94	Hyperoxia	2	4	27.9 ± 0.2
12-March-20	TMB	329	67	Hyperoxia	3	4	27.1 ± 0.1
13-March-20	TMB	1,306	266	Hyperoxia	4	4	27.3 ± 0.5
13-March-20	TMB	204	42	Hyperoxia	5	4	26.2 ± 1.3
14-March-20	CycloB	320	110	Normoxia	1	4	26.4 ± 0.4
14-March-20	CycloB	142	49	Normoxia	2	4	25.5 ± 0.4
14-March-20	CycloB	586	201	Normoxia	3	4	25.5 ± 0.3
14-March-20	CycloB	1,188	408	Normoxia	4	4	25.1 ± 0.5
14-March-20	CycloB	913	314	Normoxia	5	4	24.6 ± 0.2
14-March-20	CycloB	768	264	Hyperoxia	1	4	23.6 ± 0.5
14-March-20	CycloB	1,368	470	Hyperoxia	2	4	23.8 ± 0.8
14-March-20	CycloB	525	180	Hyperoxia	3	4	23.5 ± 0.7
14-March-20	CycloB	146	50	Hyperoxia	4	3	23.6 ± 0.2
14-March-20	CycloB	304	105	Hyperoxia	5	4	23.4 ± 0.8
9-June-20	Naph	41.4	7.9	Normoxia	1	4	31.5 ± 0.6
9-June-20	Naph	17.8	3.4	Normoxia	2	4	30.4 ± 0.1
9-June-20	Naph	9.4	1.8	Normoxia	3	4	29.5 ± 1.0
10-June-20	Naph	16.3	3.1	Normoxia	4	4	30.6 ± 0.5
10-June-20	Naph	7.37	1.5	Normoxia	5	4	30.0 ± 0.2
11-June-20	Naph	1.73	0.33	Hyperoxia	1	4	29.8 ± 0.02
11-June-20	Naph	0.00	0.0	Hyperoxia	2	4	29.2 ± 0.3
11-June-20	Naph	43.0	8.2	Hyperoxia	3	4	28.5 ± 0.4
12-June-20	Naph	1.8	0.35	Hyperoxia	4	4	28.7 ± 0.4
12-June-20	Naph	9.4	1.8	Hyperoxia	5	4	25.8 ± 1.2
21-July-20	NO_2	158.0	42	Normoxia	1	4	31.7 ± 0.3
21-July-20	NO_2	52.7	14	Normoxia	2	4	30.9 ± 0.1
21-July-20	NO_2	259.6	69	Normoxia	3	4	30.5 ± 0.2
22-July-20	NO_2	225.7	60	Hyperoxia	1	4	31.0 ± 0.3
22-July-20	NO_2	147	39	Hyperoxia	2	4	30.6 ± 0.2

23-July-20	NO_2	41.4	11	Hyperoxia	3	4	30.4 ± 0.4
24-July-20	NO_2	109.1	29	70% O_2	4	4	28.0 ± 1.0
23-July-20	n/a	94% O_2	94% O_2	Hyperoxia	1	4	29.6 ± 0.3
23-July-20	n/a	21% O_2	21% O_2	Normoxia	2	4	29.1 ± 0.04
24-July-20	n/a	70% O_2	70% O_2	70% O_2	3	4	28.7± 0.5

*Chemicals tested include isopropanol (IPA), 1,2,4-trimethylbenzene (TMB), cyclobutylamine (CBA), naphthalene (Naph) and nitrogen dioxide (NO_2).

Chemicals and Test Atmosphere Monitoring

Each of the five chemicals identified by the RCCA team analysis are either known or suspected irritants and commercially available. Chemicals selected for this study include 2-propanol (isopropanol) (item: 190764, CAS# 67-63-0), 1,2,4-trimethylbenzene (item: T73601, CAS# 95-63-6), and cyclobutylamine (item: 225185, CAS# 2516-34-9) and naphthalene (item: 147141, CAS# 91-20-3) were manufactured by Sigma-Aldrich (Natick, MA) and received by NAMRU-D in Jan-2020. Both 94%O_2 and NO_2 (CAS# 10544-72-6) were manufactured by Matheson Tri-Gas and was received in March 2020. Compressed air was generated in-house. Detailed chemical properties are shown in Appendix 5.

Isopropanol (IPA)

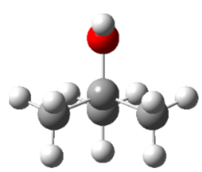

Figure 1 Isopropanol

Isopropanol (Figure 1) was characterized by the FTIR using liquid injections for a mass-to-volume value.

Trimethylbenzene (TMB)

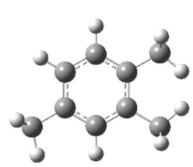

Figure 2 Trimethylbenzene

Trimethylbenzene (**Error! Reference source not found.** 2) was characterized by the FTIR using liquid injections for a mass-to-volume value.

Cyclobutylamine (CBA)

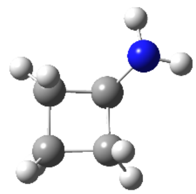

Figure 3 Cyclobutylamine

Cyclobutylamine (Figure 3) was characterized by the FTIR using liquid injections for a mass-to-volume value.

Naphthalene (N)

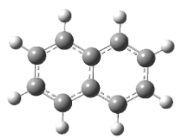

Figure 4 Naphthalene

Naphthalene (Figure 4) was characterized by the FTIR using a known mass of a solid sample that was placed into the gas bag for a mass-to-volume value. The bag was initially used as a blank then was required to incubate overnight for sublimation. The oven used for calibrations was also used for vapor generation, where the sample transfer line to the mixing chamber required a heated component. The unheated transfer line would deposit naphthalene onto the Teflon tubing, requiring a similar oven temperature.

Nitrogen Dioxide (NO_2)

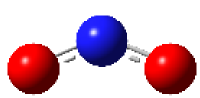

Figure 5 Nitrogen Dioxide

Nitrogen dioxide (Figure 5) was characterized by the FTIR using a known volume of gas from a standard cylinder and then diluted with laboratory-clean air for a volume-to-volume. The spectrum did not match the literature due to the dimerization of nitrogen dioxide to dinitrogen tetroxide in undried air, where NIST Webbook only showed a representative spectrum of the dimer. Based on bond vibrations, the analyzed spectrum was acceptable.

Fourier Transform Infrared Spectrometer (FTIR)

An FTIR instrument (Thermo Scientific model Nicolette IS10, Waltham MA) was used for the analysis of vapor concentrations. FTIR characterization began by analyzing a qualitative bag of test article in air. Gas bags (SKC, SamplePro PVDF Sample Bag, Cat No.: 248-25, Batch No.: 45268) were used with a known mass/volume of material and a known volume of compressed air. The spectrum that was produced gave prominent peaks at specific wavenumbers. These peaks were visually compared to the National Institute of Standards and Technology (NIST) standard spectra of the materials for verification of material identity (NIST Mass Spec Data Center). Prior to exposures, absorbance values from an initial characterization spectrum were defined at one known concentration of each chemical. All test articles were then linearly calibrated at multiple concentrations using FTIR. FTIR chemical concentration curves for each exposure are listed in Appendix 3 and chemical concentrations listed in Appendix 4.

Temperature, humidity and oxygen

A data acquisition device (NI 9207) recorded environmental conditions every 2 seconds in a NI cDAQ-9178 8-slot NI CompactDAQ USB chassis (National Instruments Corporation, Austin, TX). Temperature & relative humidity (%RH) (Rotronics Instruments Inc. model HF532WB6XD1XX & model HC2-S, Hauppauge, NY) was collected at two of the Nose Only Exposure Unit (NOEU) ports and also sent to LabVIEW (National Instruments LabVIEW Software v.12.0, National Instruments Corporation, Austin, TX) for data logging. Temperatures at the NOEU ports were not influenced during the course of the study and ranged from 73 to 81 °F (22.8 to 27.2°C). Relative humidity (RH) was not influenced during the course of the study and different gas supply sources were associated with different relative humidity levels. During the house-dried normoxic air exposures, the humidity was around 4.2 %RH. During the supplied gas exposures, the humidity was around 1.4 %RH. The oxygen concentration was recorded over the entire exposure period using infrared spectrometry. Detailed temperature, humidity and oxygen recordings for each exposure are listed in Appendix 6.

Static Pressure

A data acquisition device (NI 9207) recorded environmental conditions every 2 seconds in a NI cDAQ-9178 8-slot NI CompactDAQ USB chassis (National Instruments Corporation, Austin, TX). Static pressure (Building Automation Products Inc. model ZPS-05-SR09-EZ-ST-D, Gays Mills, WI) was collected at two of the NOEU ports and also sent to LabVIEW for data logging. The static pressure was recorded over the entire exposure period. During exposure group 1, run 1 (Appendix 6), a port cap was knocked off during subject placement and remained open during the study. The positive numbers listed as the statistic max for different days indicates a subject was moved off the tower, likely before acclimation or during an exposure. Detailed static pressure recordings for each exposure are listed in Appendix 6.

Generation System

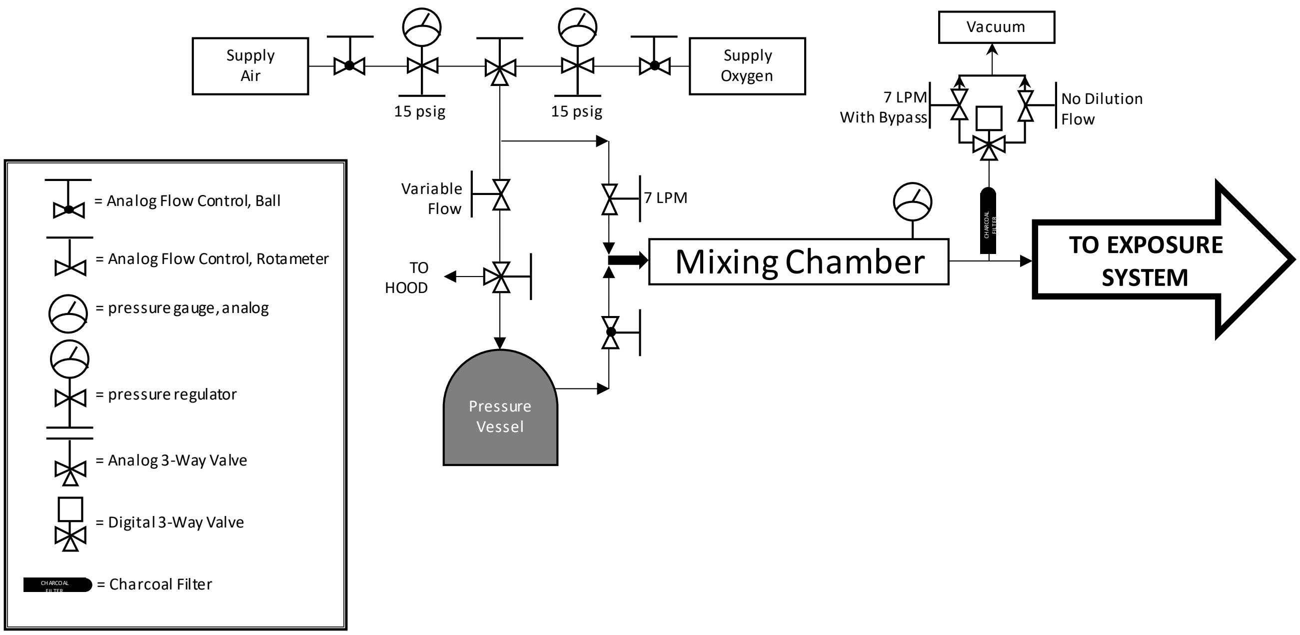

Figure 6 Inhalation Generation System

The generation system used a supply gas, either compressed air or oxygen, to be split off into either dilution gas or generation gas of the test article (Figure 6). For IPA, CBA, and TMB the headspace of the vapor in a 5-gallon pressure vessel was used. For naphthalene, a midget impinger was used and was heated by an oven during exposures. For NO_2, straight gas was used. Gas flows were restricted through rotameters to ensure that the total flow remained constant. The recombined gases were diluted in a nebulizer and then mixed in a large tube towards the exposure system. Depending on which part of the exposure was occurring, the bypass would either flow the diluted gas directly to vacuum or into the exposure system.

The flows were mixed in a 4” internal diameter (ID) stainless steel generator for 3’, then funneled down to ½” ID over another foot. The generation system could be split during bypass with the digital valves from the exposure system by closing off the ½” ID ball valve on the main line. The bypass was via a three-way valve and allowed for a quick, easy switch between test article and clean air delivery. The two vacuum lines were setup so that when the bypass was on, there was vacuum flow, and when the bypass was off there was no dilution flow.

Exposures and Respiratory Rate Measurements

The generated atmosphere flowed into exposure system (Figure 7) which consisted of a 52-port NOEU (Lab Products, Seaford, DE), animal plethysmographs and analytical instruments. Exposure schedule details can be found in Appendix 1. The outer plenum of the NOEU carried the animal's exhaled breath and excess test atmosphere to an exhaust. The NOEU operated as a push-pull system where the air supply was positive and the exhaust flow was negative. The supply was set at the target flow rate and the exhaust was adjusted to maintain a static pressure in the range of 0.00 to -0.30 inches of water (targeted for -0.05 inches of water). The exposure atmosphere flowed at a total flow rate of approximately 7 L/minute through the central, inner plenum and out through the delivery nozzles into the breathing zone of each animal at approximately 0.27 L/minute per open port. NOEUs were switched out between test articles. Half of the ports were open at the inner plenum, while the other half were plugged with steel rods to deliver the necessary flow to each port using a lower total flow through the NOEU than the flow that would be required if all ports were open (Figure 8).

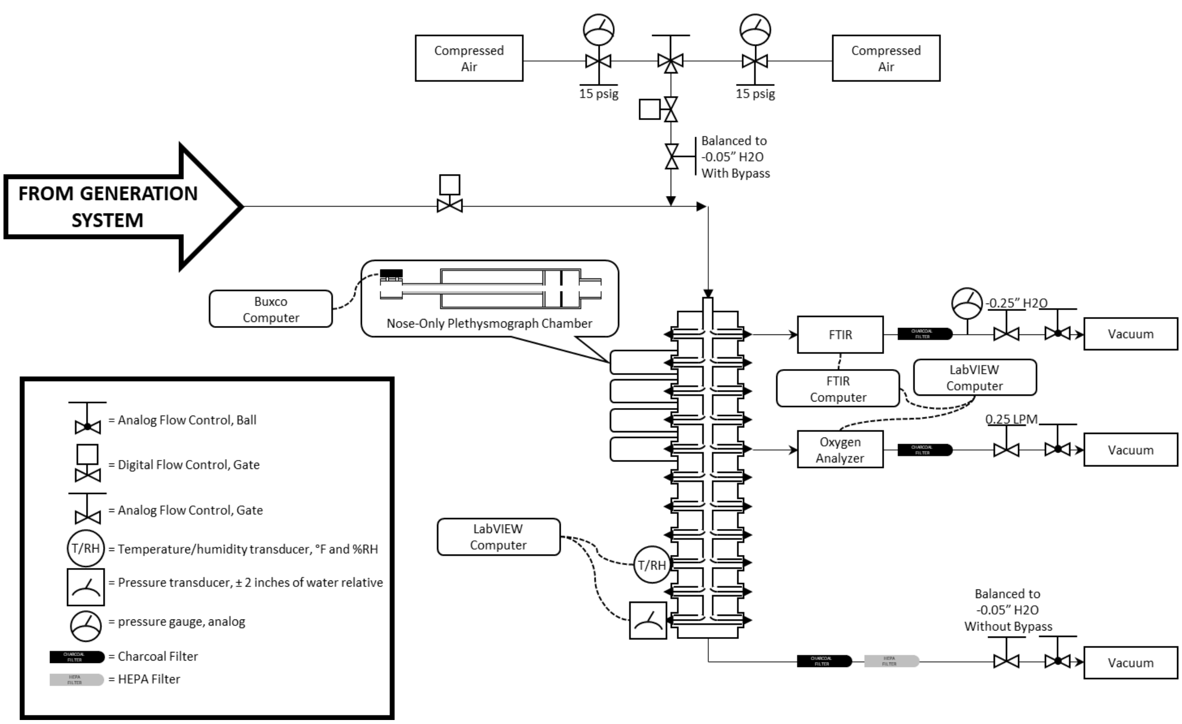

Figure 7 Exposure System

Vapor concentrations were taken by FTIR (Thermo Scientific model Nicolette IS10, Waltham MA) and processed on a computer which was networked to LabVIEW. Vapor concentrations were monitored and recorded throughout the exposure, where sampling was set to 235 mL/min and an internal FTIR pressure of -2.5 in H_20 (Appendix 3).

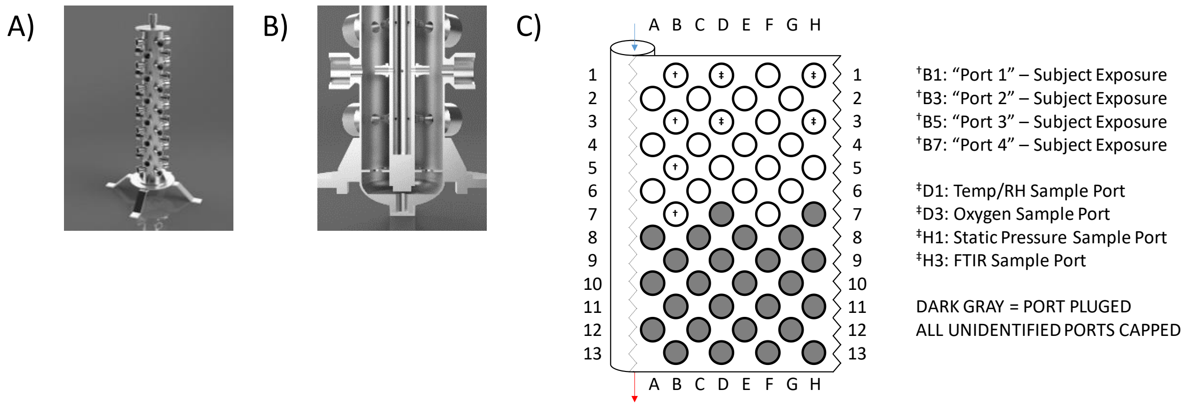

Figure 8 Nose-only Exposure Unit Tower. Photos in left two panels show (A) full tower and (B) close-up cross-section, Panel (C) is a diagram of open ports.

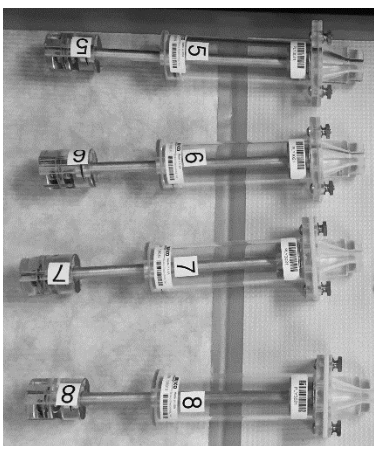

Figure 9 Nose-only Mice Plethysmographs.

The inhalation tower accommodated DSI (Data Sciences International, New Brighton, MN) Buxco nose-only plethysmography mouse chambers (PLY3021-M, Figure 9) and system to collect, amplify, digitize and analyze respiratory data. The nose-only plethysmographs provided a separate compartment for exposure to the nose and body to allow an analysis of the breathing rate by detecting the air displacement created by expansion (inhalation) and contraction (exhalation) of the body. The separation between the body and head chambers was created using a neck dam made out of latex and reinforced with 0.625” thick high-density polyethylene (HDPE). The latex was used with either a 0.313” or 0.375” hole for the neck dam. Mice were positioned into the chamber with a posterior plunger, which also exposed the body chamber to the open air through a pneumotachograph. The pneumotachograph contained a fine-mesh screen which created a pressure resistance between the compartment and outside conditions. The differential pressure across the screen was collected with a flow measurement transducer (TRD5700). The signal was then amplified by the strain gauge preamplifiers

(MAX2270) and digitized (MAX1500) and recorded by the Buxco FinePointe software (Data Sciences International, New Brighton, MN). The plethysmographs were cross-checked with each other by comparing simultaneous recording of a simulated mouse tidal volume generated by a rodent ventilator before each exposure to ensure precise readings of respiration.

Prior to the exposure, four animals were loaded into individual nose-only plethysmograph tubes and placed onto the nose-only exposure tower. Mice underwent a 10-minute acclimation period breathing HEPA-filtered clean air. Following the acclimation period, mice continued to breathe normoxic air for ten minutes to establish baseline respiratory rates. Following baseline collection, the respiratory rates were monitored and recorded for up to, but not exceeding, 30 minutes of the pre-determined concentration of the chemical (Appendix 3). The exposure was followed by a 10-minute post exposure recovery period during which the animals breathed clean air to determine if respiratory parameters returned to baseline levels. For hyperoxic exposures, mice were acclimated for ten minutes followed by a five-minute period of exposure each to 21% and 94% oxygen. After stabilization, the mice were exposed to one of the five test chemicals for up to 30 minutes, followed by a ten-minute recovery period stepping from 94% oxygen for five minutes to 21% oxygen for five minutes each. All data was collected and stored electronically by the Buxco plethysmography system using the Buxco FinePointe Software (Data Sciences International, New Brighton, MN)

Data Analysis

Following the ASTM guidelines, the baseline respiratory rate for each mouse was calculated as the average of six 15-second intervals immediately preceding the test agent exposure period. The average respiratory rate for each 15-second interval of the first five minutes of exposure and at three-minute intervals for the remainder of the 30-minute exposure period was calculated. Data for all files was processed by visually identifying and removing segments of high frequency, low periodicity noise that typically corresponded to movement artifacts of the animal within the plethysmograph tubes.

For each mouse, the average respiratory rate during the first five minutes and subsequent 25 minutes of exposure was calculated, with the 25-minute average being used for the development of the concentration-response curve. Respiratory rates were calculated at one-minute intervals for the 10-minute post-exposure period and the mean of the last five minutes of the recovery period represented the recovery response for each mouse.

The RD_{50} value was calculated by developing a concentration-response curve using the average respiratory rate decreases at each of the five exposure concentrations as the dependent variable, and the common logarithm of the exposure concentration as the independent variable for each chemical. The data sets were used to prepare the concentration-response regression to calculate an RD_{50} value (the concentration required to reduce the respiratory rate by 50%) and 95% confidence limits. The 95% confidence interval was generated in OriginPro 2021 Software (OriginLab Corporation, Northampton, MA, USA) using the mean respiratory rate change from baseline and standard deviation for each chemical concentration. Data fit was assessed using the method of least squares (Armitage, 1971; 2019).

Results

Isopropanol (IPA) Exposures

IPA Animals

Mice that underwent IPA exposures had an average weight of 27.8 g ranging from 24.5 g to 30.4 g (one animal weighed only 21.5 g and was removed from the study). Groups of IPA exposed animals under normoxic conditions had weights averaging 28.5 g with the highest being 30.4 g and the lowest at 27.7 g. Those exposed under hyperoxic conditions with IPA averaged 26.9 g (animals ranged 25.2 g - 28.8 g).

IPA Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted between runs at separate concentrations. Refer to Appendix 6 for detailed environmental conditions for each run. Furthermore, temperature was stable during vaporization and condensation of test articles.

FTIR concentration stability was acceptable during a low rate of air-changes in the pressure vessel that generated the concentrated vapor (Appendix 3 and 4). The IR absorbance spectra collected during exposure compared to published standards are shown in Figure 10.

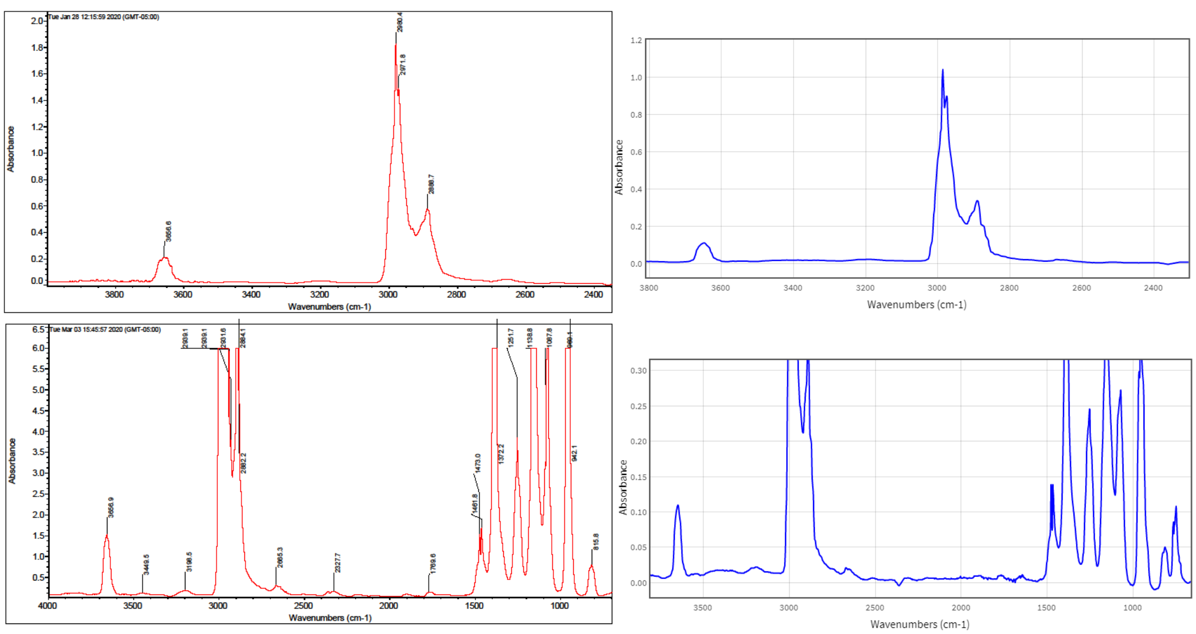

Figure 10 Isopropanol IR Spectra. Collected during experiments (LEFT) and NIST published spectra (RIGHT).The top panels display a narrow view of the spectra and the bottom panels display a wider range.

Normoxic IPA Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of IPA at 21% O_2 (Table 2, Figure 11). After an initial rapid decline in the first few minutes of exposure, respiratory rates generally plateaued with the exception the 45,488 mg/m^3 dose which had a dip towards the end of the 30-minute exposure period. At the 30-minute point and onset of recovery, all groups began to increase their respiratory rate to baseline levels with the exception of the 45,488 mg/m^3 exposure group which continued to decline. These animals were stable and appeared to be breathing normally after the completion of the study.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 12). The respiratory rate significantly decreased with increased IPA concentration. Consistent with this observation, the slope of the fit line is significantly different from zero (ANOVA, p=0.02).

Table 2 Normoxic Isopropanol Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR Stdev	1st 5 Min % RR Change	Last 25 Min % RR Change
126	6,700	2,723	184.4	20.8	2.3	3.1
135	6,700	2,723	212.7	5.3	-16.2	-11.1
139	6,700	2,723	226.5	15.5	-5.1	-6.2
138	6,700	2,723	206.6	6.2	-3.2	-13.5
136	9,516	3,871	241.1	23.4	-17.7	-16.0
131	9,516	3,871	210.5	14.7	1.7	-5.1
104	9,516	3,871	161.7	8.7	-0.8	2.8
101	9,516	3,871	219.7	4.4	4.6	1.3
105	12,856	5,230	207.5	16.2	-8.1	-8.7
121	12,856	5,230	170.4	3.2	-3.5	-0.2
130	12,856	5,230	244.1	14.6	-14.9	-15.8
126	12,856	5,230	184.4	20.8	2.3	3.1
117	26,483	10,774	268.8	7.7	-45.1	-53.7
114	26,483	10,774	230.3	4.5	-24.3	-22.6
103	26,483	10,774	239.8	7.5	-30.5	-22.3
124	26,483	10,774	212.1	4.4	-44.9	-14.4
118	45,488	18,506	190.1	6.5	-35.6	-31.9
112	45,488	18,506	277.4	5.6	-35.2	-53.0
120	45,488	18,506	207.2	6.0	-29.9	-42.9
125	45,488	18,506	190.9	8.8	-24.4	-43.9

Respiratory rate (RR) is listed as breaths per minute (BPM).

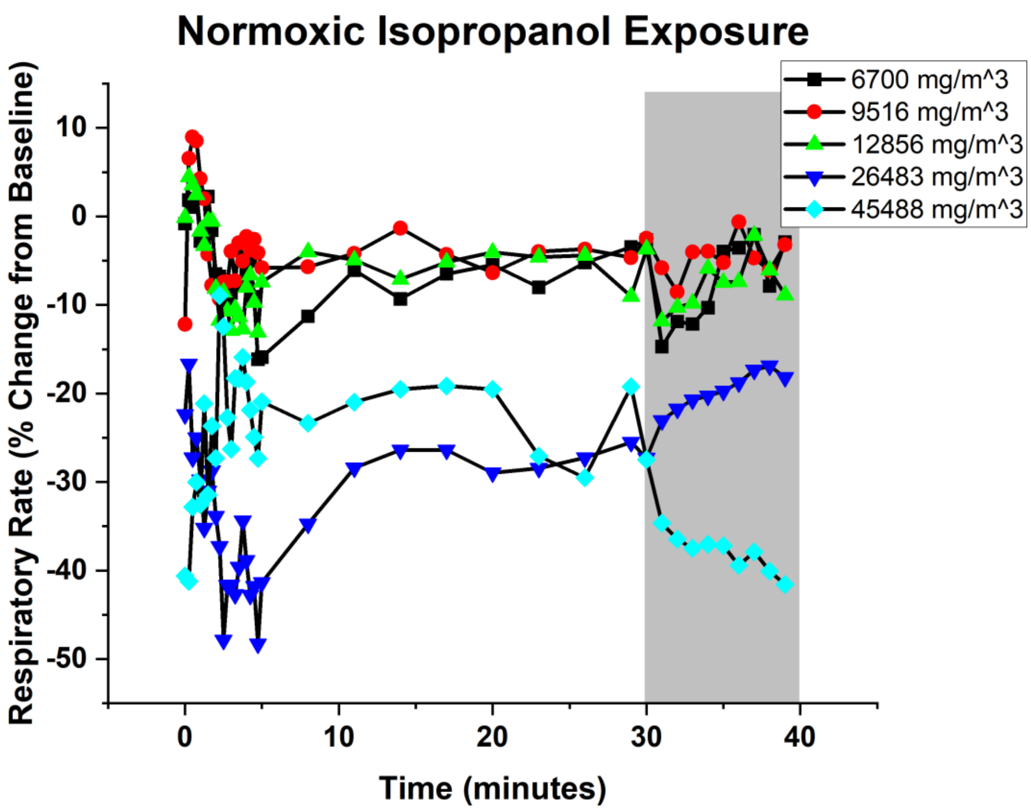

Figure 11 Average Respiratory Rate Response to Normoxic Isopropanol Inhalation Over Time. Data points are compounded means from n=4 mice at each of the 5 exposure concentrations. Inhalation exposures to isopropanol in 21% O_2 occurred for the first 30 minutes, with a 10-minute air-only recovery at the end indicated with the gray panel. Data points are compounded averages for every 25 seconds the first five minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

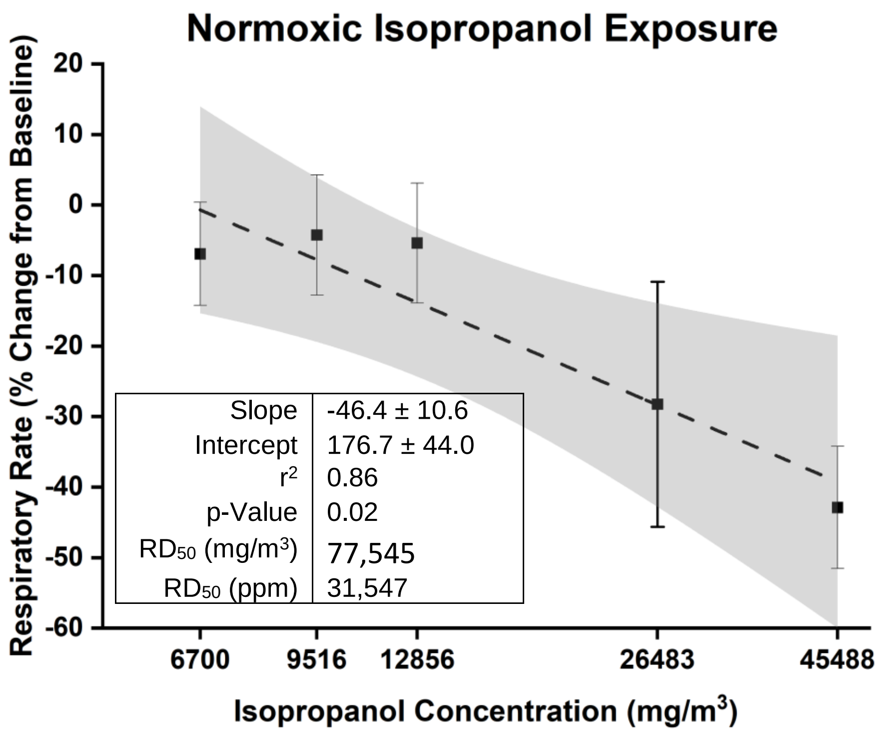

Figure 12 Concentration-Response Curve for Normoxic Isopropanol. Concentration (logarithmic) of inhalation to isopropanol in 21% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.02, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at each of the 5 exposure concentrations with error bars equal ± SD.

Hyperoxic IPA Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of IPA in 95% O_2 (Table 3, Figure 13). After an initial rapid decline in the first few minutes of exposure, respiratory rates plateaued through the duration of exposure. At the 30-minute point and onset of recovery, all groups began to increase their respiratory rate to baseline levels.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 14). The respiratory rate decreased with increased IPA concentration, however this relationship was not significant (ANOVA, p=0.08).

Table 3 Hyperoxic Isopropanol Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR Stdev	1st 5 Min % RR Change	Last 25 Min % RR Change
127	6,676	2,716	183.3	6.6	-1.2	-1.6
140	6,676	2,716	147.6	10.8	14.9	15.5
102	6,676	2,716	194.9	10.0	-1.6	-7.5
115	9,478	3,856	220.7	8.4	-1.1	-3.1
137	9,478	3,856	175.0	6.5	-2.0	-7.3
111	9,478	3,856	187.4	11.9	5.1	-11.3
109	9,478	3,856	184.9	24.2	-12.2	-12.8
108	13,866	5,641	194.3	6.0	5.2	-14.5
132	13,866	5,641	235.0	7.4	-14.4	-15.6
123	13,866	5,641	220.6	13.4	-3.6	-2.7
116	13,866	5,641	214.6	1.2	-10.3	-7.9
119	27,417	11,154	211.2	8.5	-36.1	-18.1
107	27,417	11,154	185.2	9.1	-25.6	-0.5
110	27,417	11,154	165.9	5.3	6.5	0.7
129	27,417	11,154	188.0	5.4	-3.0	-7.0
128	48,834	19,867	152.1	6.6	-50.0	-44.8
133	48,834	19,867	202.8	6.6	-27.6	-44.4
113	48,834	19,867	176.2	9.2	-55.3	-57.4

Respiratory rate (RR) is listed as breaths per minute (BPM).

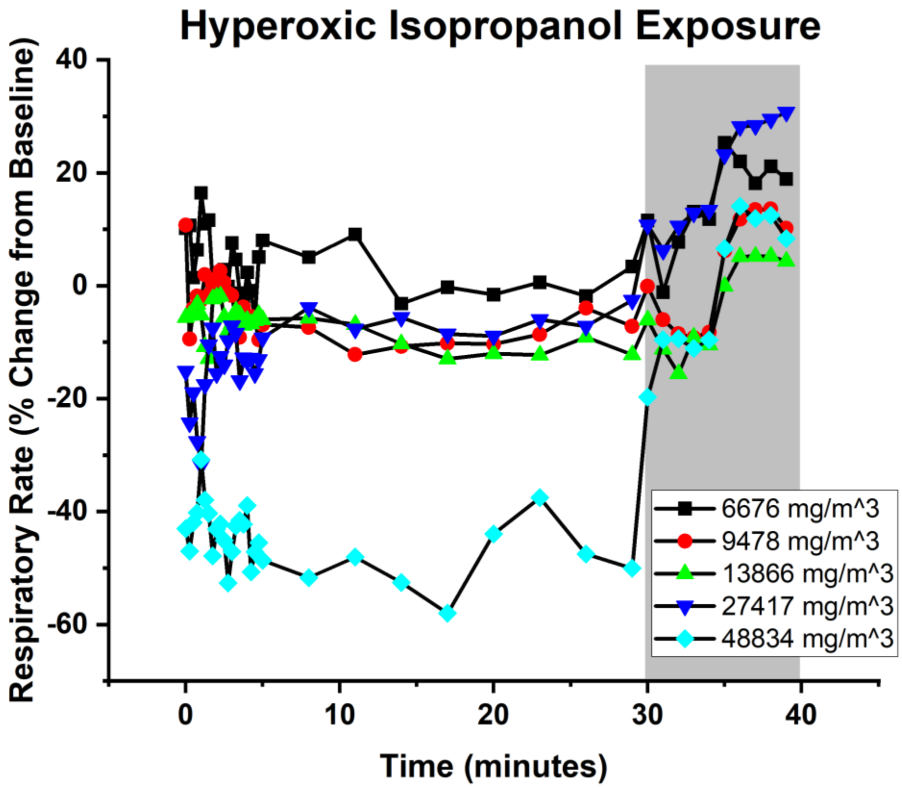

Figure 13 Average Respiratory Rate Response to Isopropanol Inhalation in 94% O_2 Over Time. Data points are compounded means from n=3 mice at the 6,676 mg/m^3 and 48,834 mg/m^3 concentrations and n= 4 mice at each of the other 3 exposure concentrations. Inhalation exposures in 94% O_2 occurred for the first 30 minutes, with a 5 recovery in 94% O_2 followed by a 5-minute air-only inhalation recovery at the end. The recovery period is indicated with the gray panel. Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the final 10 minutes of recovery.

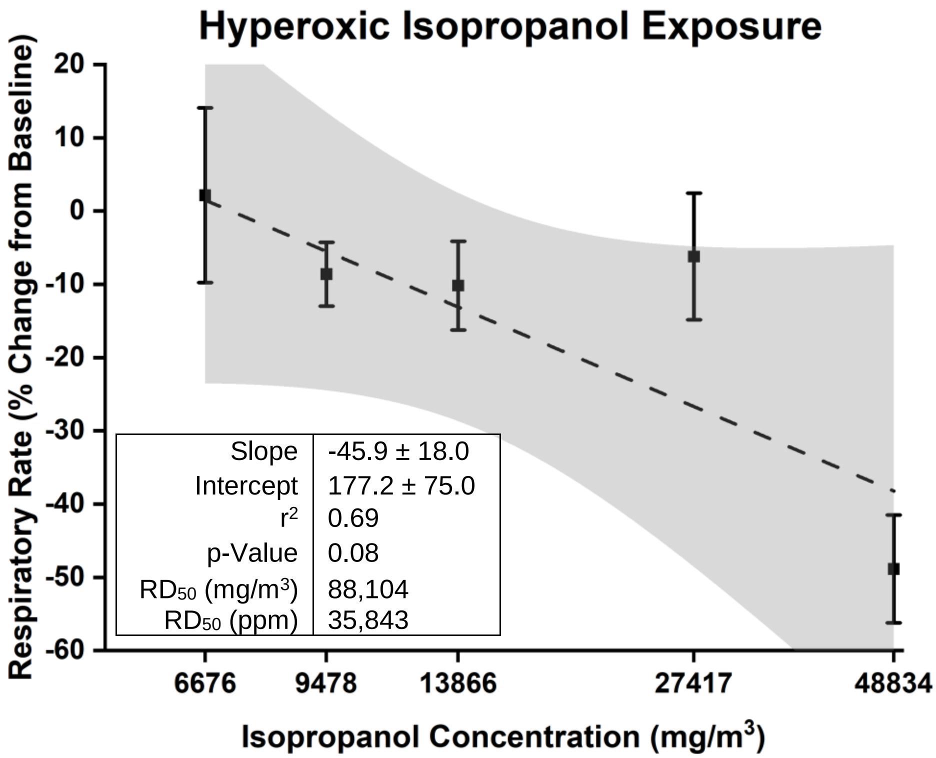

Figure 14 Concentration-Response Curve for Hyperoxic Isopropanol. Concentration (logarithmic) of inhalation isopropanol in 94% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is not significantly different (p= 0.08, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n=3 mice at the 6,676 mg/m^3 and 48,834 mg/m^3 concentrations and n=4 mice at 9,478 mg/m^3, 13,866 mg/m^3 and 27,417 mg/m^3 concentrations. Error bars equal ± SD.

1,2,4-Trimethylbenzene (TMB) Exposures

TMB Animals

Animals exposed to TMB had an average weight of 27.7 g on the day of exposure, with weights ranging from 24.6 g to 29.5 g. Those exposed to TMB under normoxic conditions averaged 28.0 g ranging from 27.3 g to 29.4 g. Animals, undergoing TMB exposures under hyperoxic conditions, ranged between 24.6 g and 29.5 g. Animal # 212 had a white spot of an unknown origin in its eye noted after euthanasia. Animal # 220 had swelling on its rear flank, however, it was not known if this was due to weight gain during its time prior to exposure. Animals # 203, 211, 223, and 232 had urine-soaked fur post exposure, and animal # 236 had noted urine dampened fur observed after euthanasia. Animal # 239 had a small cut at the tip of its tail noted prior to exposure and every subsequent observation.

TMB Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted. Refer to Appendix 6 for environmental condition details per each run. Furthermore, temperature was not a significant issue with vaporization and condensation of test articles.

FTIR concentration stability was acceptable during a low rate of air-changes in the pressure vessel that generated the concentrated vapor (Appendix 3 and 4). The IR absorbance spectrum collected during exposure compared to published standards are shown in Figure 15.

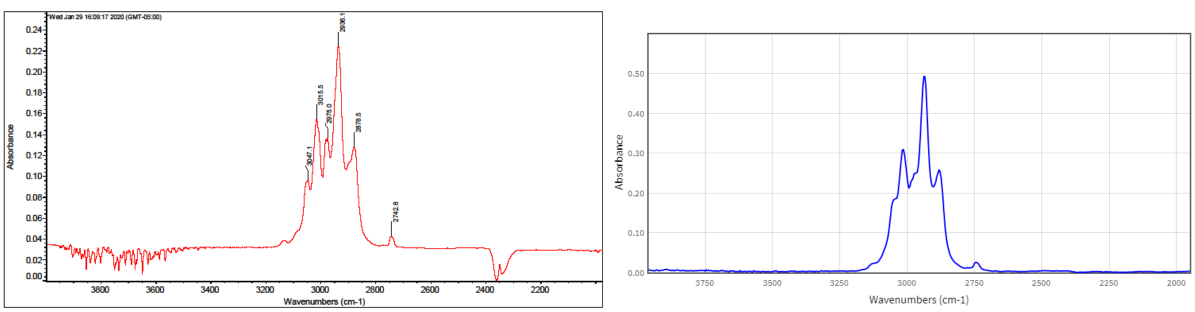

Figure 15 Trimethylbenzene IR Spectrum. IR spectrum as collected during exposures (left) and NIST published spectrum (right).

Normoxic TMB Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of TMB in 21% O_2 (Table 4, Figure 16). After an initial rapid decline in the first few minutes of exposure, respiratory rates generally plateaued through the duration of the exposure. At the 30-minute point, and onset of recovery, all exposure groups began to increase their respiratory rate to baseline levels.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 17). The respiratory rate significantly decreased with increased TMB concentration. Consistent with this observation, the slope of the fit line is significantly different from zero (ANOVA, p=0.001).

Table 4 Normoxic 1,2,4-Trimethylbenzene Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR Stdev	1st 5 Min % RR Change	Last 25 Min % RR Change
224	1,398	284	214.6	24.0	4.0	-47.1
209	1,398	284	221.4	6.1	-9.4	-52.7
218	1,398	284	218.6	14.3	-22.8	-54.6
226	1,398	284	213.1	8.7	-36.2	-47.7
214	763	155	197.9	8.4	-5.8	-38.0
205	763	155	212.7	3.3	-4.1	-25.8
216	763	155	268.9	19.2	-15.8	-55.2
215	763	155	221.2	5.4	2.4	-43.5
211	295	60	261.4	6.9	-3.0	-16.0
222	295	60	253.3	9.3	-9.8	-24.8
221	295	60	211.6	23.3	9.2	-2.5
213	295	60	246.3	24.0	1.5	-12.0
217	503	102	272.8	9.8	-4.9	-27.7
238	503	102	267.5	5.3	-5.2	-38.5
231	503	102	253.9	3.7	-10.5	-30.0
228	503	102	264.9	8.7	-12.3	-24.2
202	164	33	207.0	7.2	-4.0	-14.4
212	164	33	207.0	7.2	-4.2	-14.6
219	164	33	238.3	3.5	-6.6	-15.6
220	164	33	300.1	7.1	-6.0	-8.2

Respiratory rate (RR) is listed as breaths per minute (BPM).

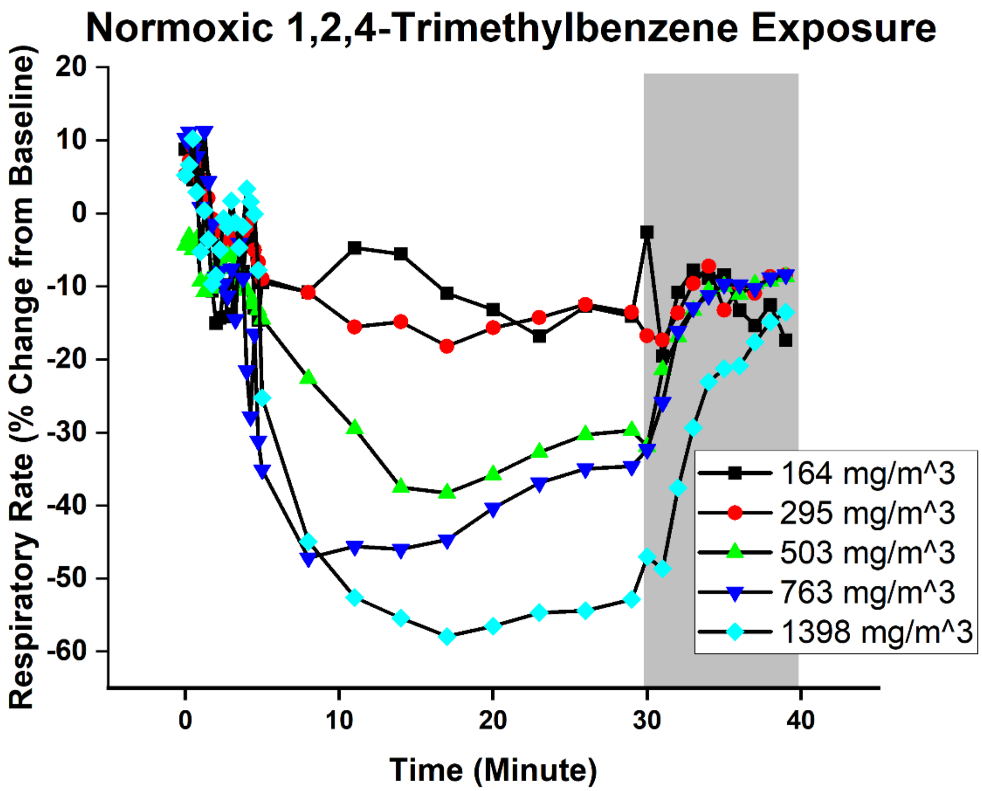

Figure 16 Average Respiratory Rate Response to Normoxic 1,2,4-Trimethylbenzene Inhalation Over Time. Data points are compounded means from n=4 mice at each of the 5 exposure concentrations. Inhalation exposures to 1,2,4-Trimethylbenzene in 21% O_2 occurred for the first 30 minutes, with a 10-minute air-only recovery at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first five minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

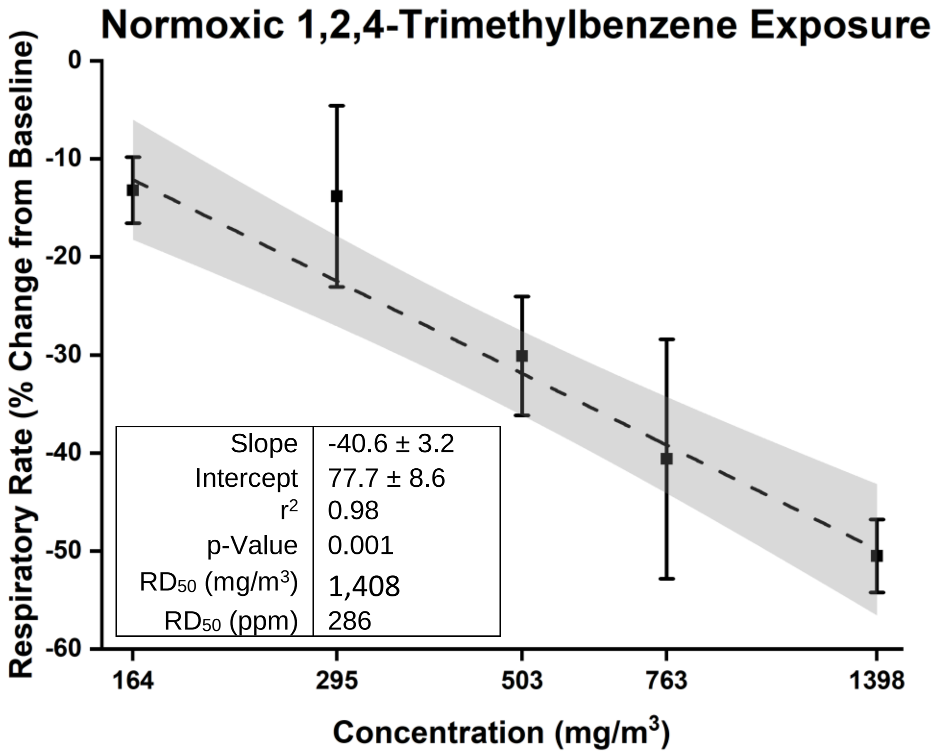

Figure 17 Concentration-Response Curve for Normoxic 1,2,4-Trimethylbenzene Exposure. Concentration (logarithmic) of inhalation to 1,2,4-Trimethylbenzene in 21% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.001, ANOVA) from zero. Shaded area represents 95% confidence interval of fitted curve. Data points are means from n= 4 mice at each of the 5 exposure concentrations with error bars equal ± SD.

Hyperoxic TMB Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of TMB in 94% O_2 (Table 5, Figure 18), however, the highest two concentrations produced similar impacts on the respiratory rate. After an initial rapid decline in the first few minutes of exposure, respiratory rates plateaued through the duration of exposure. At the 30-minute point and onset of recovery, all groups began to increase their respiratory rate to baseline levels.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 19). Despite similar impacts at the highest two dosages, the respiratory rate significantly decreased with increased TMB concentration (ANOVA, p=0.004).

Table 5 Hyperoxic Trimethylbenzene Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
204	818	166	199.9	6.2	-3.8	-43.2
208	818	166	228.7	8.8	-11.5	-47.0
230	818	166	200.2	5.4	-21.8	-47.6
235	818	166	256.3	4.4	-15.3	-47.7
201	463	94	244.6	3.3	-9.4	-21.7
210	463	94	221.6	10.4	-2.7	-16.4
227	463	94	233.6	11.9	-21.6	-44.1
240	463	94	211.6	2.2	-13.7	-40.7
203	329	67	186.7	10.0	-0.3	-9.3
229	329	67	203.6	5.9	1.9	-16.6
237	329	67	181.6	5.8	1.4	-6.0
239	329	67	268.1	5.2	-23.5	-42.3
223	1,306	266	157.1	10.7	-16.5	-44.7
225	1,306	266	210.1	3.3	-12.5	-34.7
232	1,306	266	190.7	5.4	-29.6	-43.3
234	1,306	266	167.9	2.2	-26.6	-60.6
236	204	42	252.8	12.2	-1.6	-6.0
233	204	42	195.3	16.2	3.4	-1.7
207	204	42	199.0	5.7	1.5	-3.2
206	204	42	197.9	26.1	-13.1	-16.2

Respiratory rate (RR) is listed as breaths per minute (BPM).

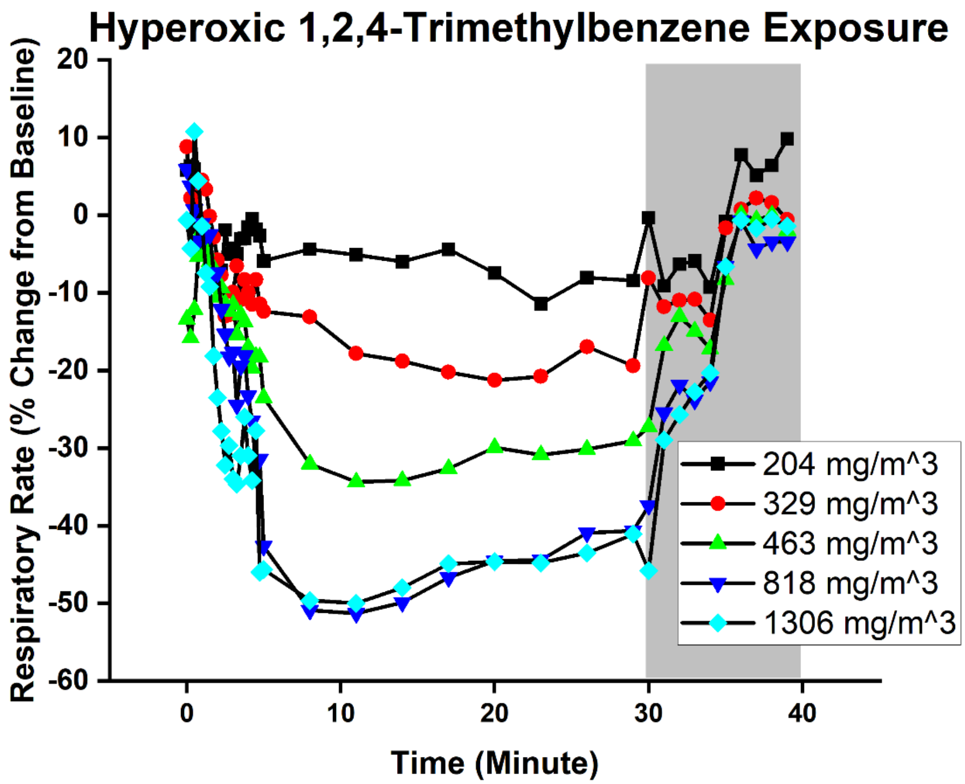

Figure 18 Average Respiratory Rate Response to 1,2,4-Trimethylbenzene in 94% O_2 Over Time. Data points are compounded means from n= 4 mice at each of the exposure concentrations. Inhalation exposures in 94% O_2 occurred for the first 30 minutes, with a 5 recovery in 94% O_2 followed by a 5-minute air-only inhalation recovery at the end. The recovery period is indicated with the gray panel. Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

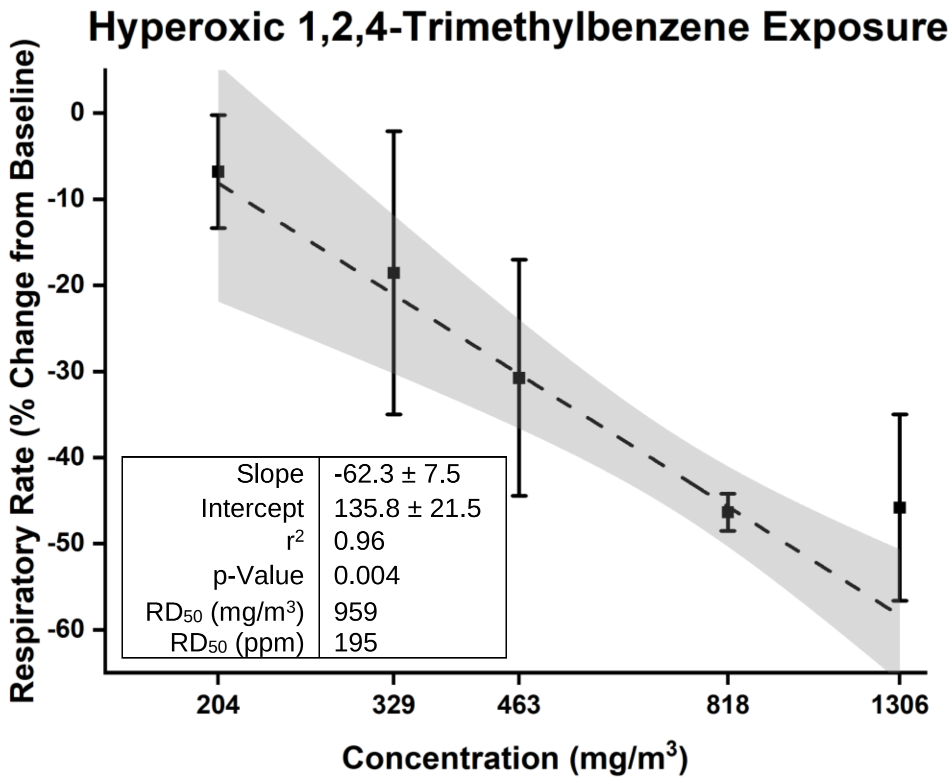

Figure 19 Concentration-Response Curve for Hyperoxic 1,2,4-Trimethylbenzene. Concentration (logarithmic) of inhalation of 1,2,4-Trimethylbenzene in 94% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.004, ANOVA) from zero. Shaded area represents 95% confidence interval of fitted curve. Data points are means from n=4 mice at all concentrations. Error bars equal ± SD.

Cyclobutylamine (CBA) Exposures

CBA Animals

Mice exposed to cyclobutylamine had an average weight of 24.5 g with a range from 22.6 g to 26.7 g. Mice exposed under normoxic conditions had an average weight of 25.4 g ranging from 24.3 g to 26.7 g. Groups of mice undergoing cyclobutylamine exposure under hyperoxic conditions had an average mass of 23.6 g ranging from 24.5 g to 22.6 g. One animal was removed from the study due to a low weight of 20.8 g which was outside the acceptable weight range specified in ASTM testing guidelines. Animal # 316 had its ear pinched in the plethysmography tube during the exposure which was noted in the post exposure observations but quickly resolved.

CBA Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted. Refer to Appendix 6 for environmental conditions per each run. Furthermore, temperature was not a significant issue with vaporization and condensation of test articles.

FTIR concentration stability was acceptable during a low rate of air-changes in the pressure vessel that generated the concentrated vapor (Appendix 3 and 4). The IR absorbance spectrum collected during exposure compared to published standards are shown in Figure 20.

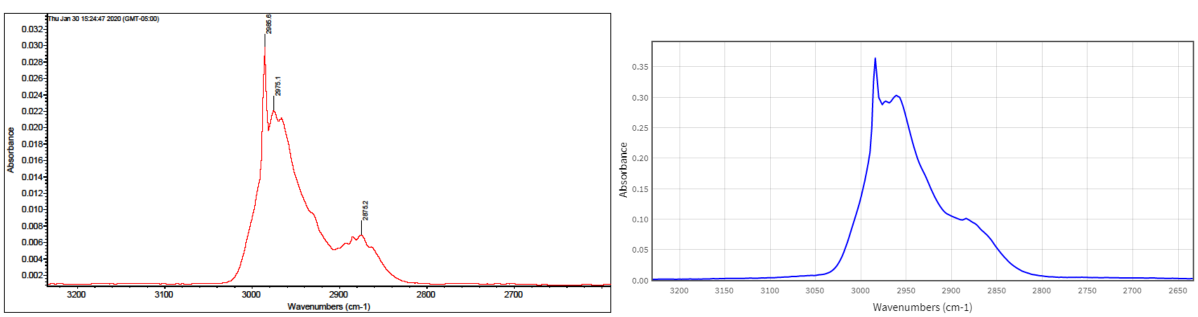

Figure 20 Cyclobutylamine IR Spectrum. IR spectrum as collected during exposures (Left) and NIST published (Right).

Normoxic CBA Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of CBA in 21% O_2 (Table 6, Figure 21). After an initial rapid decline in the first few minutes of exposure, respiratory rates generally plateaued through the duration of the exposure. At the 30-minute point, and onset of recovery, all exposure groups began to increase their respiratory rate to baseline levels.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 22). The respiratory rate significantly decreased with increased CBA concentration. Consistent with this observation, the slope of the fit line is significantly different from zero (ANOVA, $p=6.2\times10^{-5}$).

Table 6 Normoxic Cyclobutylamine Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
306	320	110	268.9	34.6	-22.7	-21.5
309	320	110	227.2	25.3	-4.9	-5.3
313	320	110	238.4	25.2	-9.7	-15.5
317	320	110	244.3	11.4	-21.1	-25.9
324	142	49	215.8	9.4	-1.2	0.2
327	142	49	254.5	16.2	-2.6	-6.5
338	142	49	236.3	5.9	-1.5	2.1
339	142	49	258.9	4.9	-2.6	3.7
325	586	202	254.5	19.2	-20.5	-27.7
303	586	202	265.0	8.9	-28.3	-39.4
305	586	202	228.1	12.8	-21.2	-29.4
310	586	202	247.3	6.6	-33.5	-41.1
314	1,188	408	229.5	19.2	-42.2	-50.9
316	1,188	408	288.6	4.3	-48.2	-62.3
340	1,188	408	208.2	5.8	-27.3	-45.3
312	1,188	408	189.7	11.2	-17.1	-35.4
315	913	314	223.2	7.7	-38.9	-39.4
319	913	314	252.1	13.5	-46.0	-58.8
304	913	314	210.2	22.1	-23.4	-30.5
330	913	314	226.7	7.4	-28.9	-45.0

Respiratory rate (RR) is listed as breaths per minute (BPM).

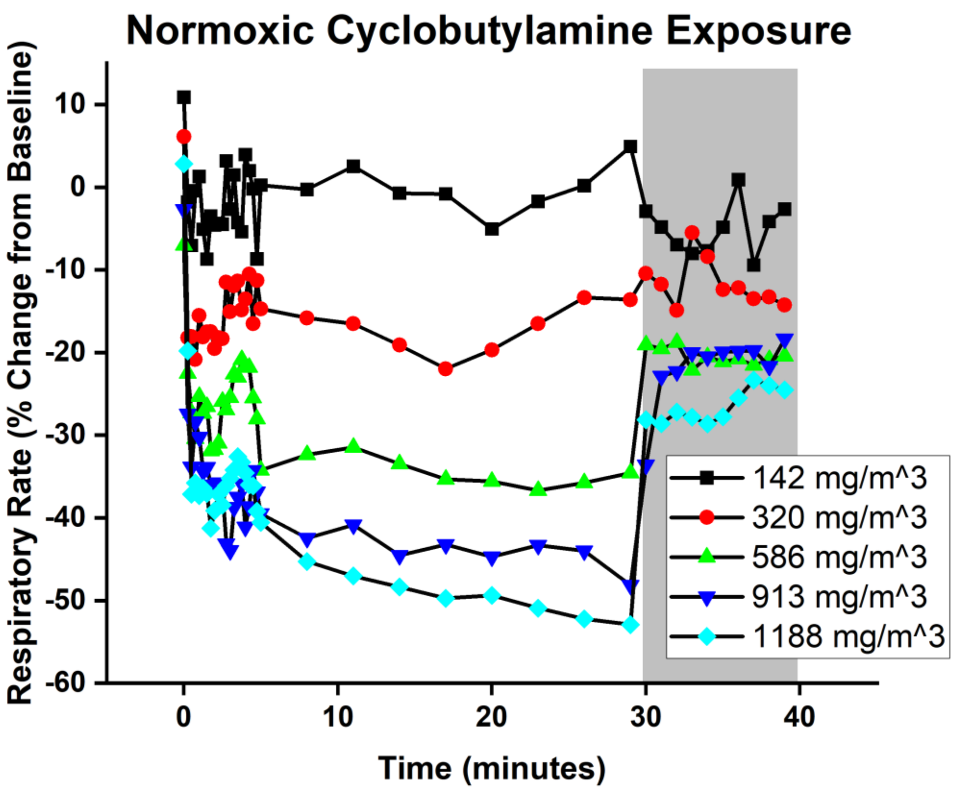

Figure 21 Average Respiratory Rate Response to Normoxic Cyclobutylamine Inhalation Over Time. Data points are compounded means from n=4 mice at each of the 5 exposure concentrations. Inhalation exposures to Cyclobutylamine in 21% O_2 occurred for the first 30 minutes, with a 10-minute air-only recovery at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first five minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

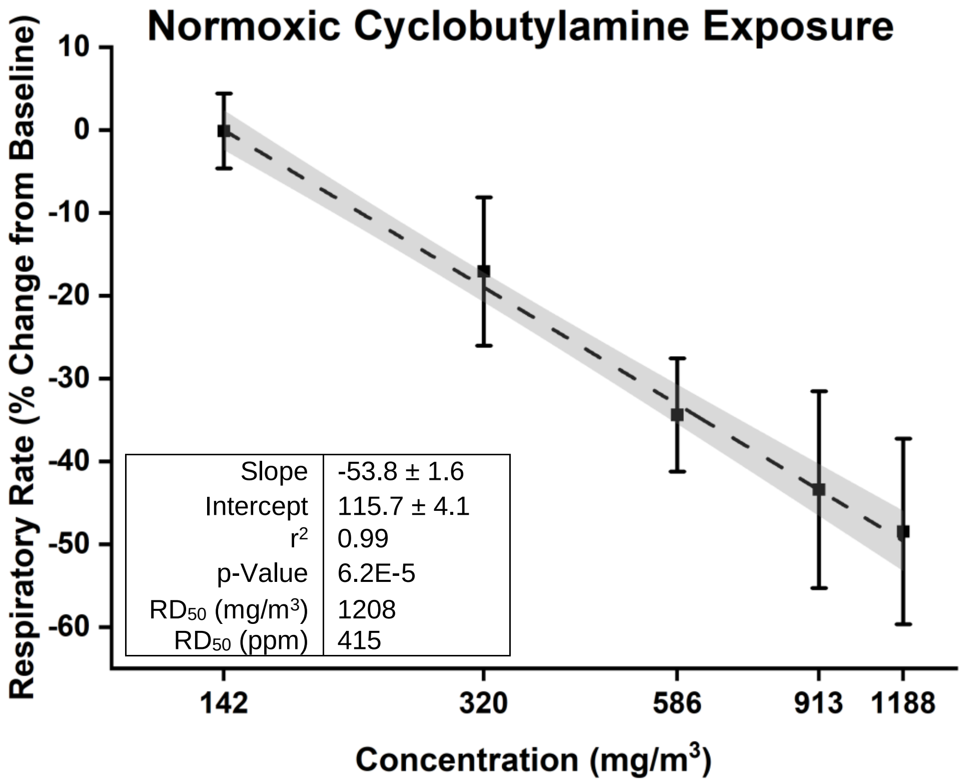

Figure 22 Concentration-Response Curve for Normoxic Cyclobutylamine Exposure. Concentration (logarithmic) of inhalation to Cyclobutylamine in 21% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different ($p= 6.2x10^{-5}$, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at each of the 5 exposure concentrations with error bars equal ± SD.

Hyperoxic CBA Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of CBA in 94% O_2 (Table 7, Figure 23). However, there was little separation of the respiratory effects between the 525 and 768 mg/m^3 concentrations. After an initial rapid decline in the first few minutes of exposure, respiratory rates plateaued through the duration of exposure. At the 30-minute point and onset of recovery, all groups began to increase their respiratory rate to baseline levels.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 24). Despite similar impacts at the highest two dosages, the respiratory rate significantly decreased with increased CBA concentration (ANOVA, p=0.03).

Table 7 Hyperoxic Cyclobutylamine Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
322	768	264	241.8	7.7	-20.5	-36.1
308	768	264	239.7	6.9	-18.8	-34.2
334	768	264	273.1	3.9	-21.9	-41.0
307	768	264	230.5	5.5	-23.5	-34.7
335	1,368	470	276.8	10.3	-43.6	-60.7
337	1,368	470	266.1	8.7	-53.4	-65.0
332	1,368	470	264.2	5.5	-35.0	-55.4
321	1,368	470	216.5	9.2	-40.0	-57.1
329	525	181	222.2	4.9	-19.7	-38.4
331	525	181	274.5	8.3	-21.0	-34.4
336	525	181	250.1	14.0	-18.1	-45.2
301	525	181	239.3	11.5	-33.0	-40.6
302	146	50	222.9	11.5	-6.0	-15.7
333	146	50	247.6	5.2	-0.5	-9.0
311	146	50	230.6	9.1	-10.8	-22.0
323	304	105	213.8	6.3	-18.5	-18.0
328	304	105	238.8	4.9	-24.1	-18.8
326	304	105	239.6	2.2	-13.8	-14.3
320	304	105	261.6	7.6	-23.9	-23.8

Respiratory rate (RR) is listed as breaths per minute (BPM).

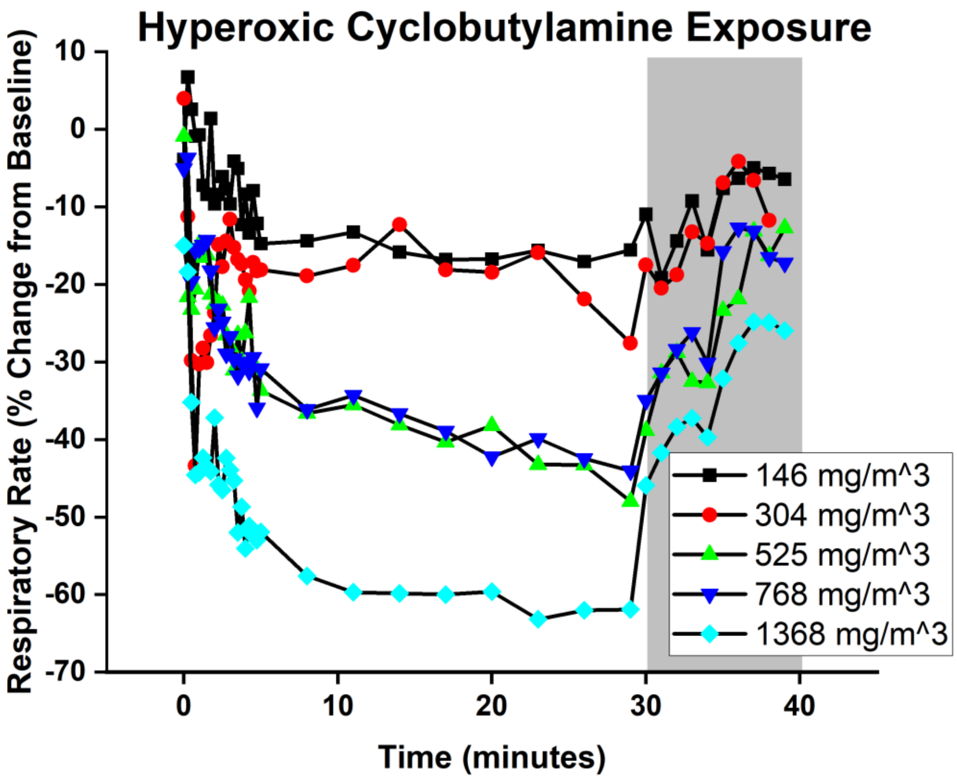

Figure 23 Average Respiratory Rate Response to Cyclobutylamine in 94% O_2 Over Time. Data points are compounded means from n=3 mice at the 146 mg/m^3 concentration and n= 4 mice at all other exposure concentrations. Inhalation exposures in 94% O_2 occurred for the first 30 minutes, with a 5 recovery in 94% O_2 followed by a 5-minute air-only inhalation recovery at the end. The recovery period is indicated with the gray panel. Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

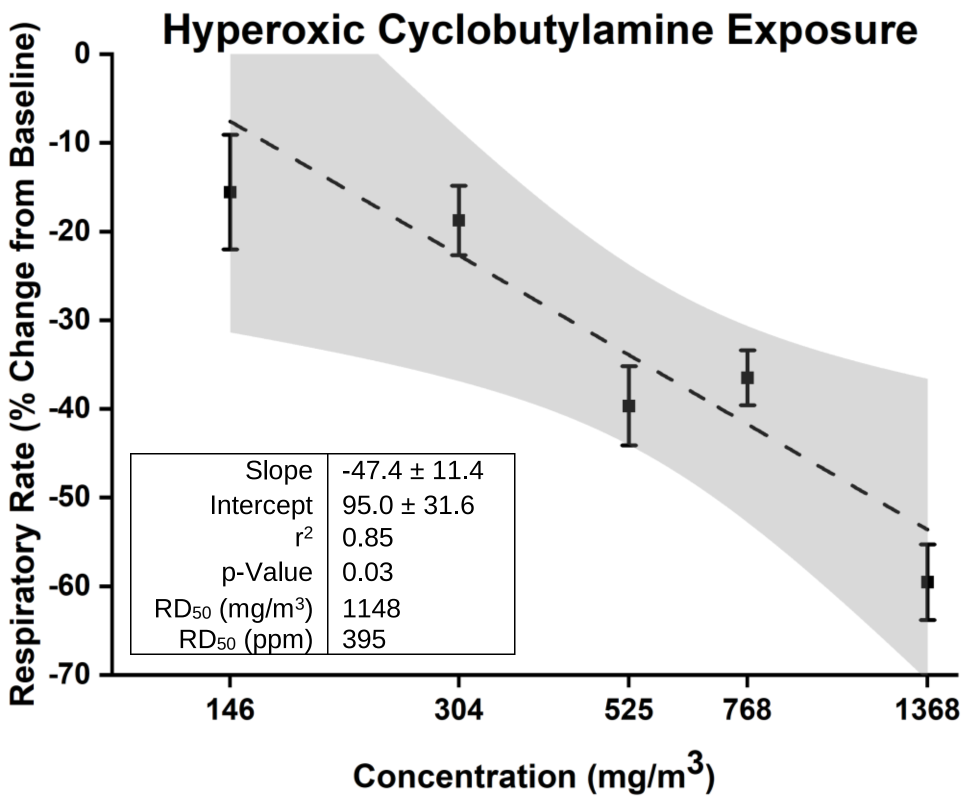

Figure 24 Concentration-Response Curve for Hyperoxic Cyclobutylamine. Concentration (logarithmic) of inhalation of cyclobutylamine in 94% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.03, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n=3 mice at the 146 mg/m^3 concentration and n= 4 mice at all other exposure concentrations. Error bars equal ± SD.

Naphthalene Exposures

Naphthalene Animals

Animals in groups exposed to naphthalene had an average weight of 29.4 g ranging from 24.6 g to 32.2 g. Exposure groups under normoxic conditions had an average weight of 30.4 g ranging from 28.0 g to 32.2 g. Mice exposed under hyperoxic conditions had an average weight of 28.4 g ranging from 24.6 g to 29.8 g. Animal # 408 had a laceration on mouth during insertion to the plethysmography tube which resolved prior to exposure. All other animals appeared healthy and not in distress upon completion of experiments.

Naphthalene Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted. Refer to Appendix 6 for environmental conditions per each run. Furthermore, temperature was not a significant issue with vaporization and condensation of test articles.

FTIR concentration stability was acceptable during a low rate of air-changes in the pressure vessel that generated the concentrated vapor (Appendix 3 and 4). The IR absorbance spectrum collected during exposure compared to published standards are shown in Figure 25.

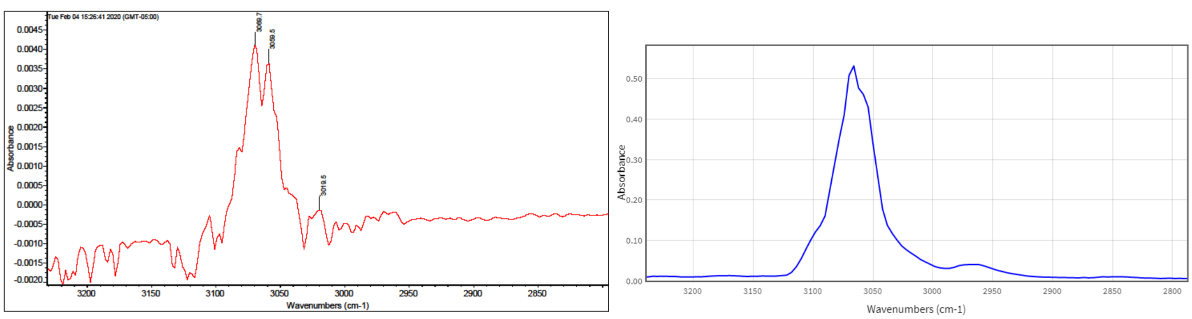

Figure 25 Naphthalene IR spectrum. IR spectrum as collected during exposures (left) and NIST published spectrum.

Normoxic Naphthalene Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations greater than 3.1 ppm of naphthalene in 21% O_2 (Table 8, Figure 26). In all exposure groups, after an initial rapid decline in the first few minutes of exposure, respiratory rates generally plateaued through the duration of the exposure. Unlike other chemical exposures shown in this report, naphthalene did not clear from the tower rapidly. Therefore, at the 30-minute point animals were removed from the tower, but kept in their plethysmography chambers, to breathe clean room air for recovery. All exposure groups increased their respiratory rates during recovery.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 27). The respiratory rate significantly decreased with increased naphthalene concentrations above 3.1 ppm. Consistent with this observation, the slope of the fit line is significantly different from zero (ANOVA, p=0.04).

Table 8 Normoxic Naphthalene Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
440	41.4	7.9	230.9	7.0	-40.9	-64.2
413	41.4	7.9	194.4	3.6	-53.2	-64.5
415	41.4	7.9	215.2	6.1	-51.0	-62.0
439	41.4	7.9	217.7	8.9	-14.3	-52.0
401	17.8	3.4	213.8	8.6	-3.8	-63.5
417	17.8	3.4	239.9	4.7	-40.5	-52.1
434	17.8	3.4	198.3	7.4	-10.3	-45.6
435	17.8	3.4	233.8	7.0	-23.3	-47.8
409	9.4	1.8	227.8	8.1	7.9	-21.9
427	9.4	1.8	211.0	3.2	-6.4	-22.7
428	9.4	1.8	204.0	5.9	-2.0	-42.8
437	9.4	1.8	257.0	5.0	-15.0	-38.1
403	16.3	3.1	194.7	22.5	2.9	-23.7
405	16.3	3.1	192.6	9.3	-16.4	-33.3
412	16.3	3.1	205.5	24.4	-31.6	-36.6
430	16.3	3.1	240.4	7.4	-8.9	-17.8
406	7.37	1.5	178.5	4.3	-6.5	-20.6
407	7.37	1.5	200.2	1.8	-5.1	-36.5
411	7.37	1.5	201.3	3.4	-9.1	-21.9
438	7.37	1.5	240.2	6.3	-13.8	-26.7

Respiratory rate (RR) is listed as breaths per minute (BPM).

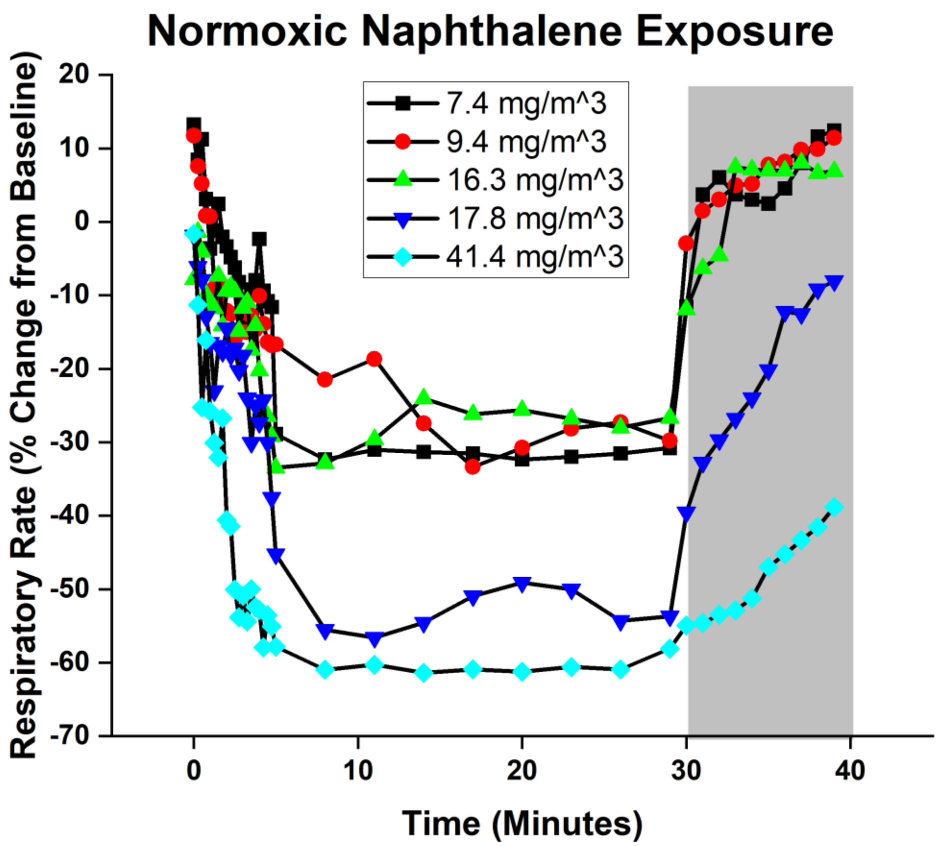

Figure 26 Average Respiratory Rate Response to Normoxic Naphthalene Inhalation Over Time. Data points are compounded means from n=4 mice at each of the 5 exposure concentrations. Inhalation exposures to naphthalene in 21% O_2 occurred for the first 30 minutes, with a 10-minute clean room air recovery at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first five minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

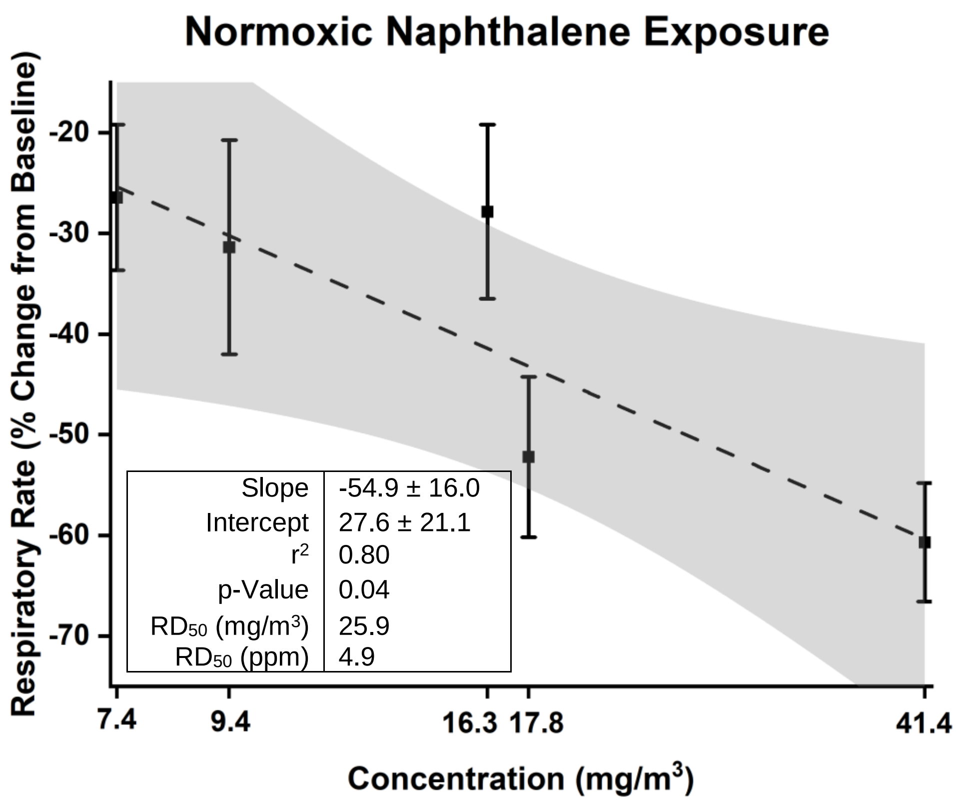

Figure 27 Concentration-Response Curve for Normoxic Naphthalene Exposure. Concentration (logarithmic) of inhalation to naphthalene in 21% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.04, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at each of the 5 exposure concentrations with error bars equal ± SD.

Hyperoxic Naphthalene Animal Response Data

The average respiratory rate of each exposure group trended lower with increasing concentrations of naphthalene in 94% O_2 (Table 9, Figure 28). After an initial rapid decline in the first few minutes of exposure, respiratory rates plateaued through the duration of exposure. Unlike other chemical exposures shown in this report, naphthalene did not clear from the tower rapidly. Therefore, at the 30-minute point animals were removed from the tower, but kept in the plethysmography chambers, to breath clean room air for recovery. All exposure groups increased their respiratory rates during recovery.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 29). The respiratory rate significantly decreased with increased naphthalene concentration (ANOVA, p=0.03).

Table 9 Hyperoxic Naphthalene Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
410	1.7	0.33	167.5	7.7	6.5	0.1
418	1.7	0.33	145.7	2.4	5.3	-3.2
433	1.7	0.33	211.2	5.5	-5.6	-11.8
436	1.7	0.33	231.4	10.6	-7.7	-17.0
420	0.0	0.0	149.4	3.0	12.9	10.0
422	0.0	0.0	214.9	13.9	3.6	1.6
429	0.0	0.0	207.1	10.4	12.4	-2.4
431	0.0	0.0	274.2	21.0	-29.8	-32.8
402	43.0	8.2	195.4	3.9	-33.3	-60.6
408	43.0	8.2	271.4	7.7	-49.4	-73.6
426	43.0	8.2	214.4	12.6	-29.3	-68.8
432	43.0	8.2	195.6	6.0	-15.5	-63.4
404	1.8	0.35	202.2	10.1	4.7	-15.3
423	1.8	0.35	197.8	10.6	-3.6	-35.8
424	1.8	0.35	175.9	4.4	2.4	-17.2
414	9.4	1.8	179.4	11.2	-5.2	-58.6
416	9.4	1.8	203.1	11.8	-14.2	-52.9
419	9.4	1.8	184.6	8.9	-40.8	-56.5
425	9.4	1.8	191.5	22.5	-13.5	-37.8

Respiratory rate (RR) is listed as breaths per minute (BPM).

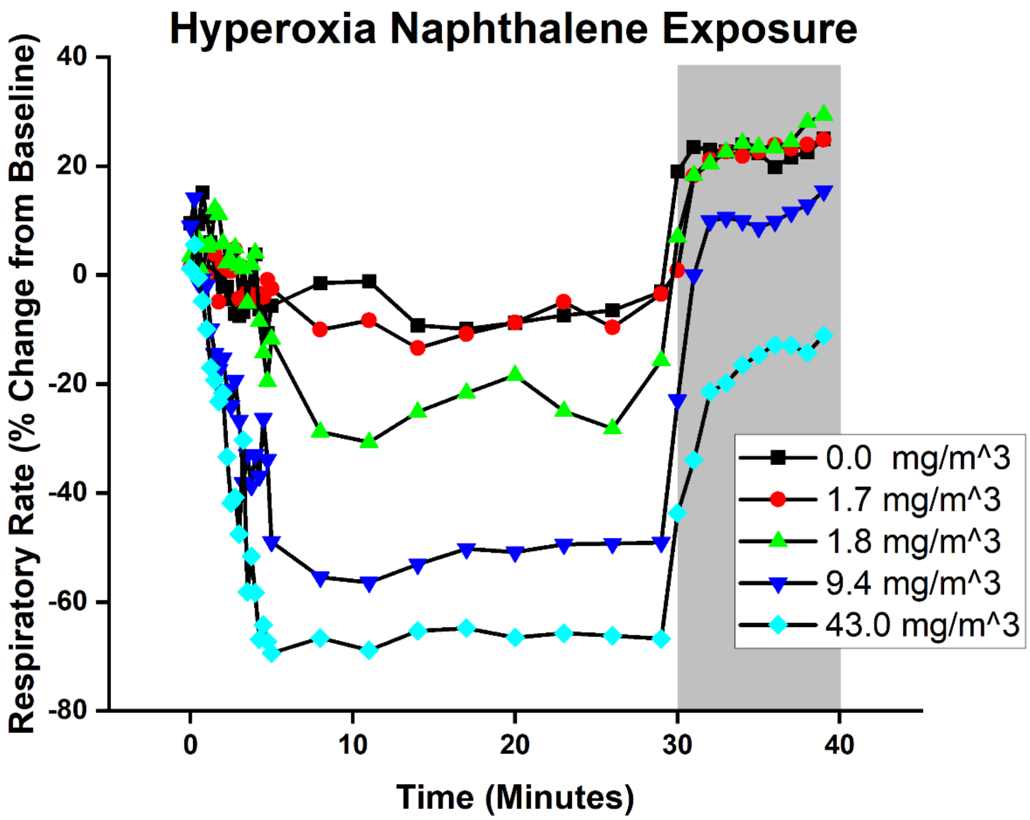

Figure 28 Average Respiratory Rate Response to Naphthalene in 94% O_2 Over Time. Data points are compounded means from n=3 mice at the 1.8 mg/m^3 concentration and n= 4 mice at all other exposure concentrations. Inhalation exposures in 94% O_2 occurred for the first 30 minutes, with a 10-minute recovery in clean room air at the end. Recovery period is indicated with the gray panel. Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

260

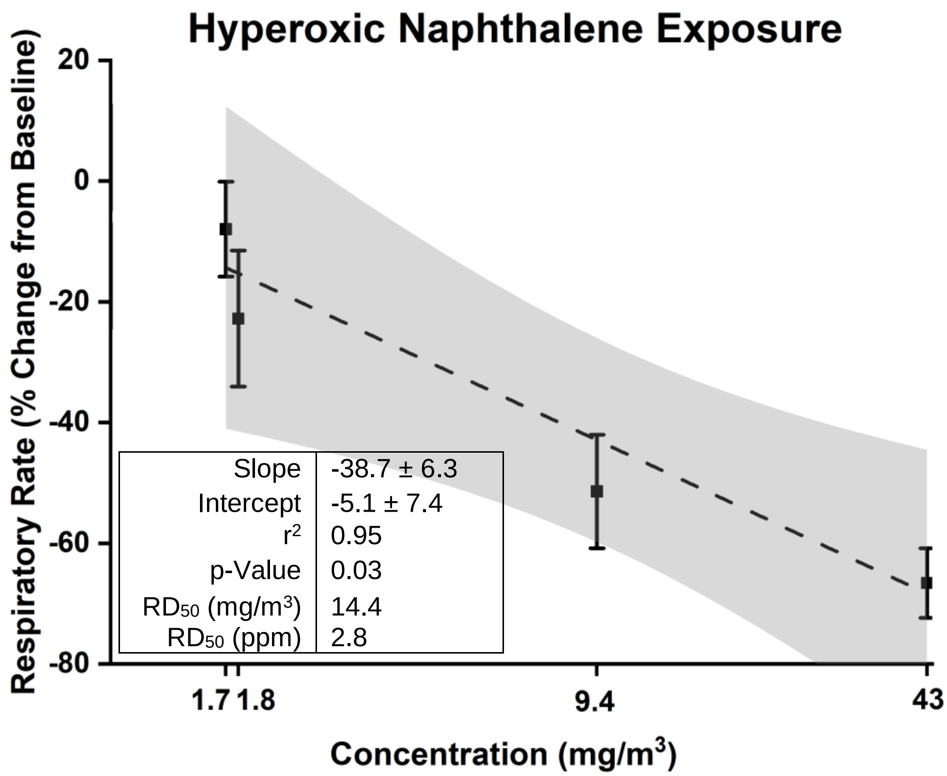

Figure 29 Concentration-Response Curve for Hyperoxic Naphthalene. Concentration (logarithmic) of inhalation of naphthalene in 94% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p=0.03 ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n=3 mice at the 1.8 mg/m^3 concentration and n= 4 mice at all other exposure concentrations. Error bars equal ± SD.

Nitrogen Dioxide (NO_2) Exposures

NO_2 Animals

NO_2 exposed mice under normoxic and hyperoxic conditions had a total average weight of 30.4 g ranging from 27.1 g and 32.1 g. Normoxic exposure animals had an average weight of 31.0 g with weights ranging between 30.3 g and 32.1 g. Hyperoxic exposure animals had an average weight of 30.0 g ranging between 27.1 g and 31.5 g. No notable observations of distress were recorded in these animal groups prior to and following exposure.

NO_2 Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted. Refer to Appendix 6 for environmental conditions per each run. Furthermore, temperature was not a significant issue with vaporization and condensation of test articles.

FTIR concentration stability was acceptable during a low rate of air-changes in the pressure vessel that generated the concentrated vapor (Appendix 3 and 4). The IR absorbance spectrum collected during exposure compared to published standards are shown in Figure 30.

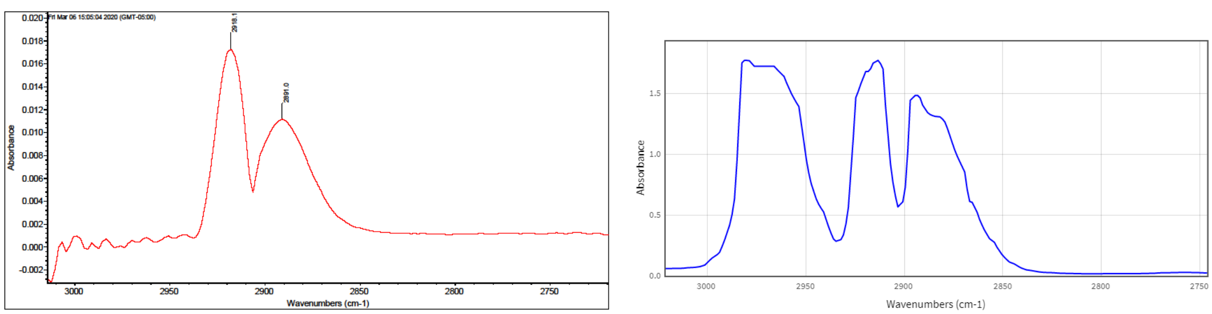

Figure 30 Nitrogen Dioxide IR spectrum. IR spectrum as collected during exposures (left) and Dinitrogen Tetroxide NIST published spectrum (right).

Normoxic NO_2 Animal Response Data

The average respiratory rate of each exposure group trended to increase with increasing concentrations of NO_2 in 21% O_2 (Table 10, Figure 31). This trend is not consistent with chemicals that are classified as sensory irritants resulting in respiratory depression. In all exposure groups, respiratory rates were variable during the first 5 minutes of exposure and steadied over the duration of exposure. At the 30-minute point, and the onset of recovery, respiration did not return to baseline. However, animals did steadily improve following experiments and exhibited no signs of distress.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression (Figure 32). The respiratory rate significantly increased with increased NO_2 concentrations. Consistent with this observation, the slope of the fit line is significantly different from zero (ANOVA, p=0.02). Because there was no depression of respiratory rates, an RD_{50} value cannot be calculated.

Table 10 Normoxic (21%) Nitrogen Dioxide Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
510	158	42	216.6	11.7	-4.9	7.1
519	158	42	190.5	7.2	0.9	12.8
527	158	42	209.3	27.1	-9.4	-1.2
501	158	42	219.2	14.0	0.1	13.4
512	53	14	209.4	7.7	4.4	0.1
506	53	14	200.5	7.8	-3.9	-4.2
511	53	14	275.9	21.0	-6.5	-12.2
514	53	14	234.2	55.7	3.8	-1.0
538	260	69	149.8	12.1	59.6	35.1
533	260	69	256.3	22.9	-15.5	-15.3
531	260	69	161.9	23.2	21.4	17.9
518	260	69	224.8	25.2	1.7	11.4

Respiratory rate (RR) is listed as breaths per minute (BPM).

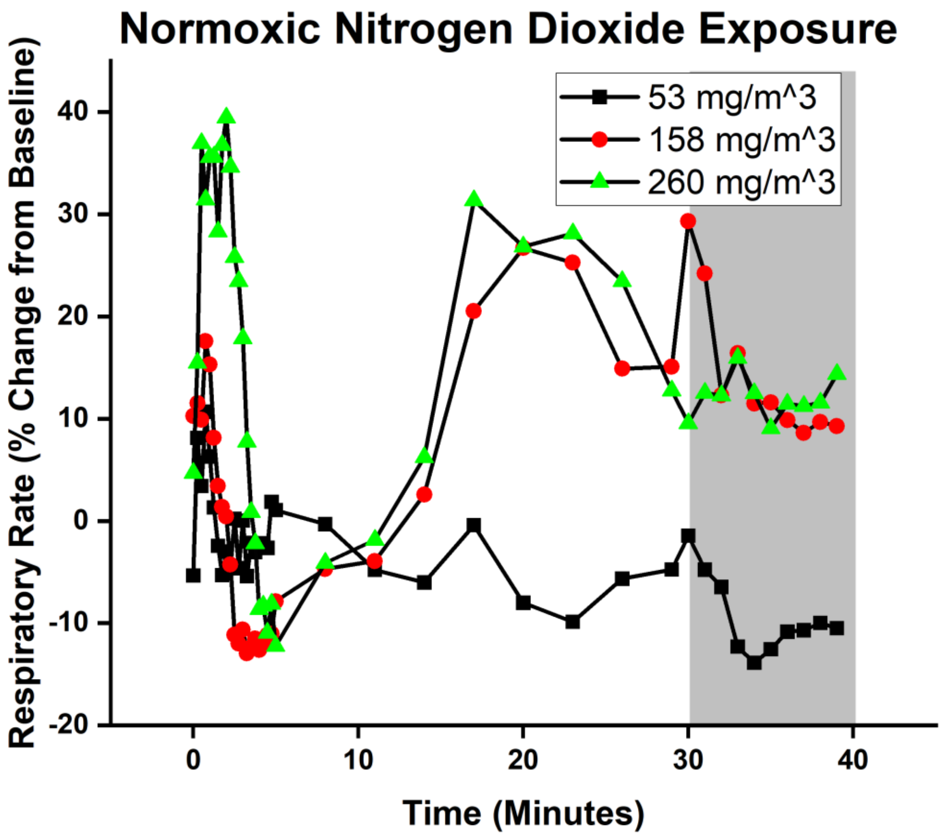

Figure 31 Average Respiratory Rate Response to Normoxic Nitrogen Dioxide Inhalation Over Time. Data points are compounded means from n=4 mice at each of the 3 exposure concentrations. Inhalation exposures to Nitrogen Dioxide in 21% O_2 occurred for the first 30 minutes, with a 10-minute clean room air recovery at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first five minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

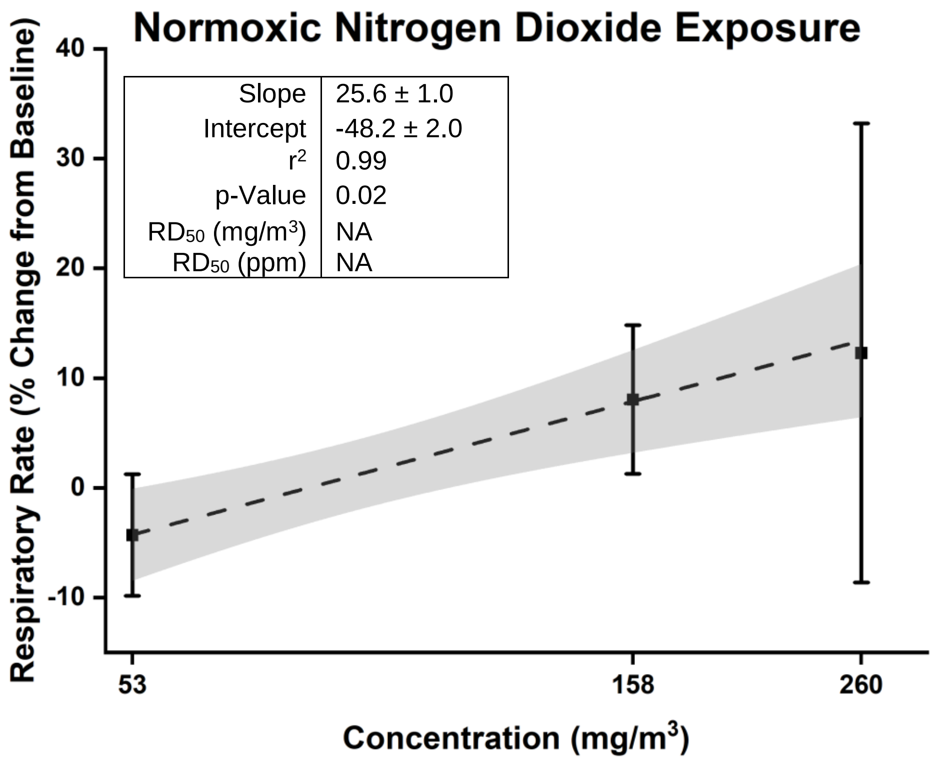

Figure 32 Concentration-Response Curve for Normoxic Nitrogen Dioxide Exposure. Concentration (logarithmic) of inhalation to Nitrogen Dioxide in 21% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.02, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at each of the 3 exposure concentrations with error bars equal ± SD.

Hyperoxic NO_2 Animal Response Data

The average respiratory rate of each exposure group was variable with increasing concentrations of NO_2 in 94% O_2 (Table 11, Figure 33). This trend is not consistent with chemicals that are classified as sensory irritants resulting in respiratory depression. In all exposure groups, respiratory rates were variable during the first 5 minutes of exposure and steadied over the duration of exposure. At the 30-minute point, and the onset of recovery, respiration rates remained variable. However, animals did steadily improve following experiments and exhibited no signs of distress.

Mean changes in respiratory rate from all animals at each exposure were used to generate a concentration-response regression to calculate an RD_{50} value (Figure 34). The slope of the fit line was not significantly different from zero (ANOVA, $p=0.85$) consistent with no trend relating respiratory rate and concentration. Because there is no significant depression of respiratory rates, an RD_{50} cannot be calculated.

Table 11 Hyperoxic (94%) Nitrogen Dioxide Animal Response Data

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
540	226	60	231.1	18.6	-18.8	-13.7
508	226	60	227.4	20.3	-4.5	3.7
515	226	60	225.2	16.7	-1.3	3.0
504	226	60	225.4	17.8	-16.5	-2.4
516	147	39	175.4	34.5	-14.6	-15.0
505	147	39	218.6	28.6	-21.3	-26.6
529	147	39	166.1	6.6	10.2	-8.3
530	147	39	192.0	26.5	-3.1	-1.4
517	41	11	221.4	5.7	3.9	-7.1
524	41	11	147.1	8.0	-8.2	-7.2
522	41	11	163.5	8.7	-2.4	-3.3
539	41	11	203.9	12.2	3.5	5.5

Respiratory rate (RR) is listed as breaths per minute (BPM).

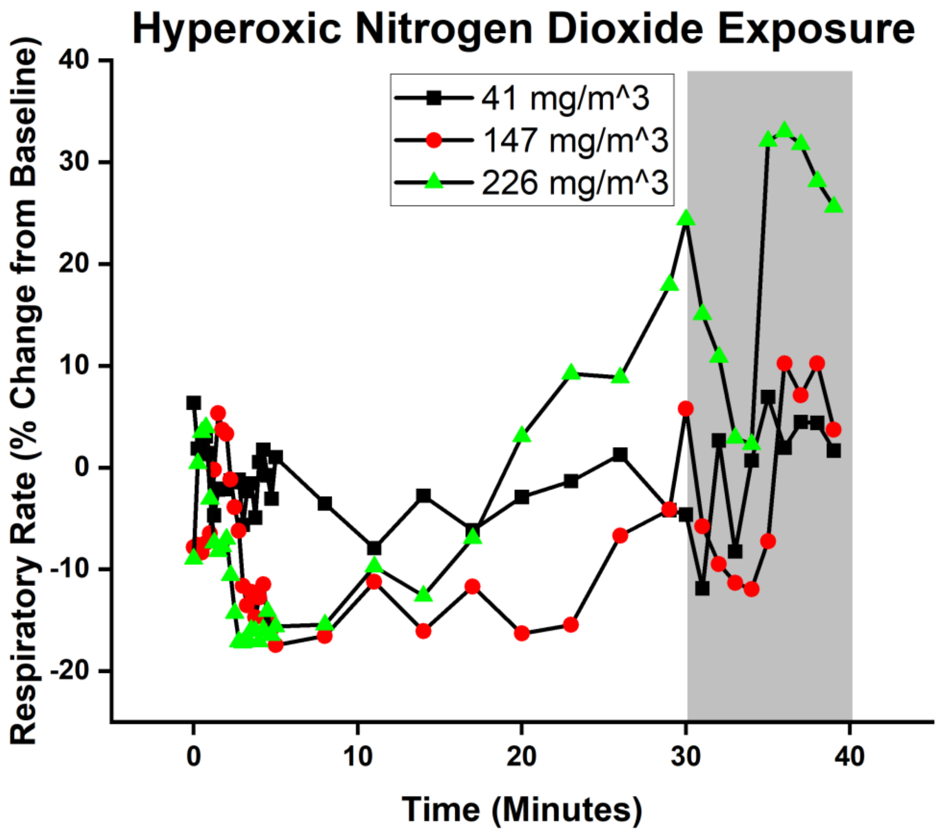

Figure 33 Average Respiratory Rate Response to Nitrogen Dioxide in 94% O_2 Over Time. Data points are compounded means from n=4 mice at all three exposure concentrations. Inhalation exposures in 94% O_2 occurred for the first 30 minutes, with a 10-minute recovery in clean air at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

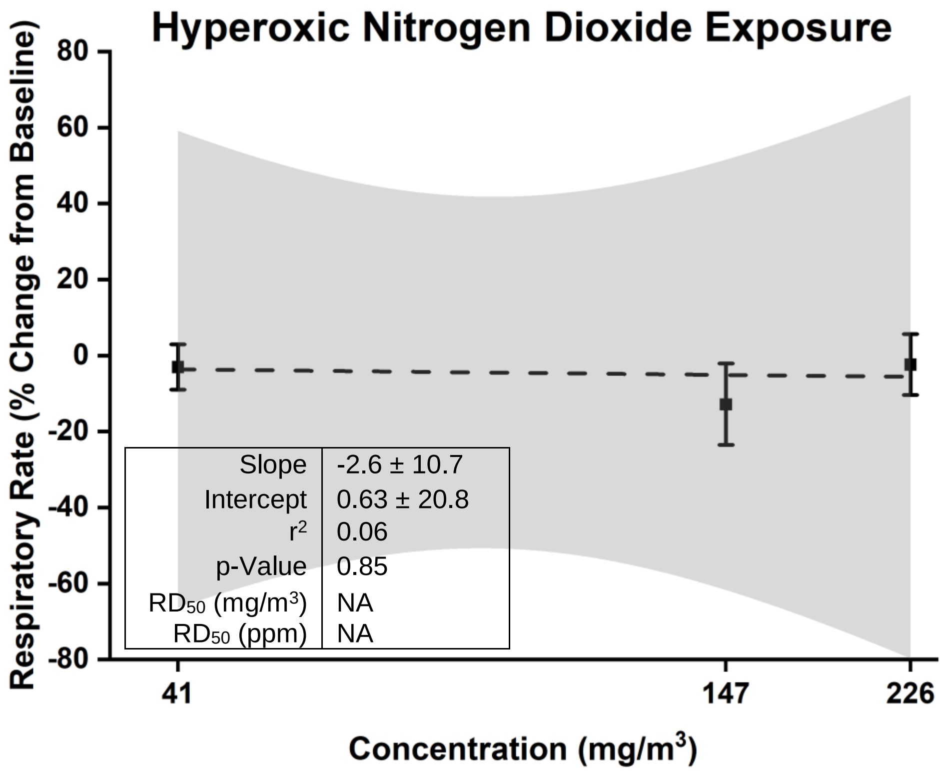

Figure 34 Concentration-Response Curve for Hyperoxic Nitrogen Dioxide. Concentration (logarithmic) of inhalation of nitrogen dioxide in 94% O_2 plotted against mean percent change of respiration rate from baseline. Fitted curve (dotted), with parameters ± SE listed in inset, is not significantly different (p= 0.85, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at all three exposure concentrations. Error bars equal ± SD.

138 mg/m^3 NO_2 at Varied Oxygen Concentrations Animal Response DataThe mean respiratory rate did not decrease when mice were exposed to increasing NO_2 suggesting NO_2 is not a sensory irritant. However, there appears to be an impact on increasing O_2 concentrations through increasing the latency to the onset of NO_2-induced respiratory increase (Table 10, Table 11, Table 12, and Figure 35). With NO_2 fixed at an approximate average of 138 mg/m^3 (ranging from 109.1 mg/m^3 to 158.0 mg/m^3), there is an initial decrease in respiration rate followed by a sharp increase at all O_2 concentrations tested (21%, 70% and 94%). At 21% O_2, respiration increases at approximately 15 minutes into exposure. At 70% O_2, respiration increases at approximately 20 minutes into exposure. Finally, at 94% O_2, respiration increases at approximately 25 minutes into exposure.

The net impact of the increased latency to NO_2 respiration increase results in a subtle decrease in the overall respiratory rate (Figure 36). The fitted regression line has an intercept of 14.06 ± 0.1 SE and slope of -0.29 ± 0.002 SE and the slope is significantly different from zero ($p = 0.004$, ANOVA).

Table 12 Nitrogen Dioxide Animal Response Data in 70% Oxygen

Animal	Concentration (mg/m^3)	Concentration (ppm)	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
502	109	29	242.9	11.5	-35.7	-27.6
513	109	29	203.4	10.5	-1.3	10.8
520	109	29	238.1	8.6	-11.2	-3.4
521	109	29	199.4	7.6	-5.5	-4.4

Respiratory rate (RR) is listed as breaths per minute (BPM).

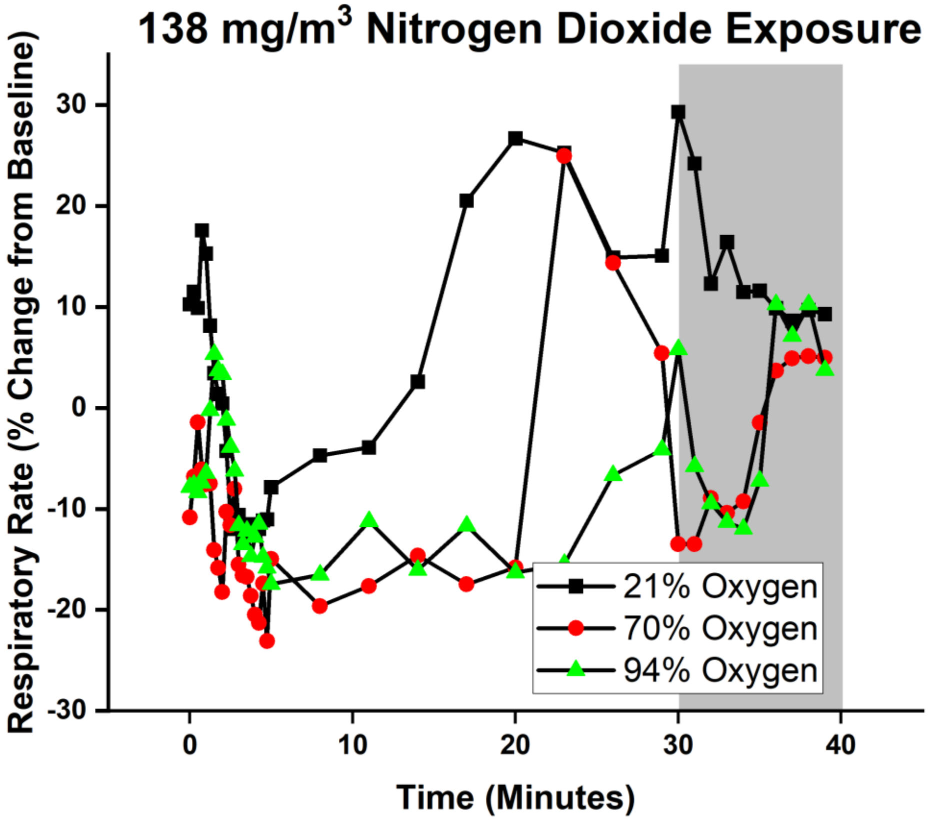

Figure 35 Average Respiratory Rate Response to 138 mg/m³ Nitrogen Dioxide in Various O_2 Concentrations Over Time. Data points are compounded means from n=4 mice at all three O_2 exposure concentrations. Inhalation exposures of an average of 138 mg/m³ NO_2 occurred for the first 30 minutes, with a 10-minute recovery in clean air at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

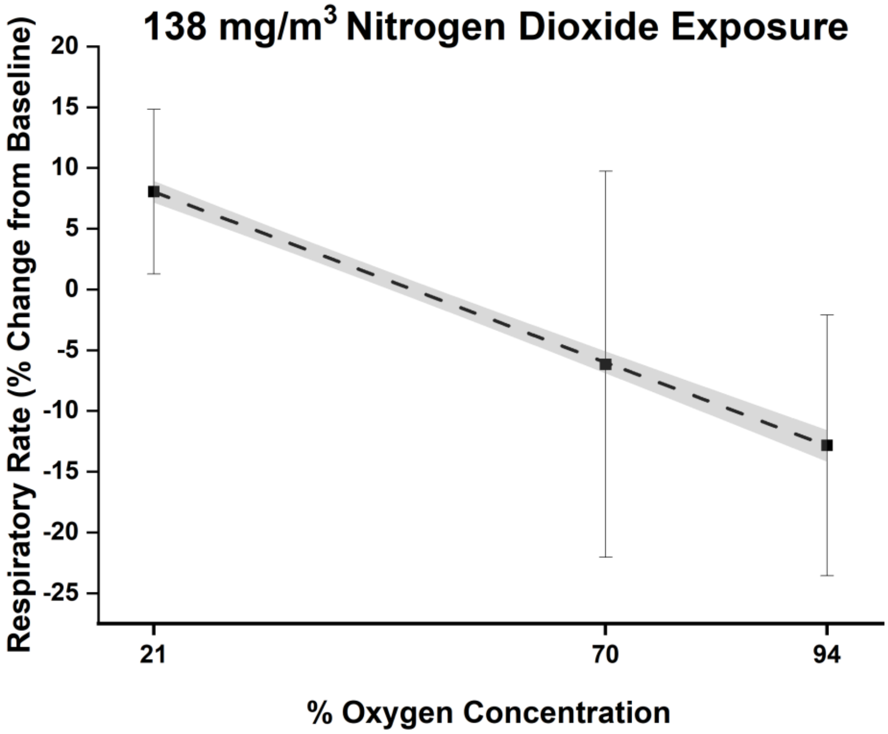

Figure 36 Concentration-Response Curve for 138 mg/m^3 Nitrogen Dioxide at Different Oxygen Concentrations. Concentration of O_2 plotted against mean percent change of respiration rate from baseline during exposure to approximately 138 mg/m^3 of NO_2. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different ($p = 0.004$, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at all three O_2 concentrations. Error bars equal ± SD.

Oxygen Exposures

Oxygen Animals

Mice exposed to various oxygen concentrations (without test chemicals) had an average weight of 29.1 g ranging between 28.1 g and 29.9 g. No notable observations were recorded for these animal groups prior to their exposure, during their exposure, or after their euthanasia.

Oxygen Exposure Conditions

No differences in temperature, relative humidity and static pressure were noted. Appendix 6 provides details for environmental conditions for each run.

Oxygen Exposures Animal Response Data

To determine the impact of different hyperoxia conditions on respiratory rate, mice were exposed to atmospheric normoxic concentrations (21%) and two concentrations of higher oxygen (70% and 94%). In all three exposures, respiratory rates were variable during the first 5 minutes of exposure and steadied over the duration of exposure (Table 13, Figure 37). There is a trend toward increasing respiration rates with increasing oxygen concentrations (Figure 38). The fitted regression line has an intercept of -3.8 ± 0.1 SE and slope of 0.1 ± 0.003 SE and the slope is significantly different from zero ($p = 0.02$, ANOVA). However, the trend toward increasing respiration rates with increasing oxygen concentrations is not significant ($p = 0.24$, ANOVA).

Table 13 Animal Response Data to Various Oxygen Concentrations

Animal	Oxygen	Baseline RR (BPM)	Baseline RR StDev	1st 5 Min % RR Change	Last 25 Min % RR Change
507	94%	160.5	9.8	0.7	0.6
535	94%	153.1	7.1	7.1	12.6
523	94%	179.3	3.8	9.7	10.0
526	94%	170.2	16.9	3.0	0.9
532	21%	183.4	4.4	4.6	-3.9
537	21%	181.2	9.1	4.7	3.1
503	21%	197.3	5.9	1.4	-1.9
509	21%	211.0	26.7	-9.4	-3.7
525	70%	187.9	3.5	8.5	6.7
528	70%	216.8	11.7	-3.2	-5.7
536	70%	225.0	13.1	0.1	10.2

Respiratory rate (RR) is listed as breaths per minute (BPM).

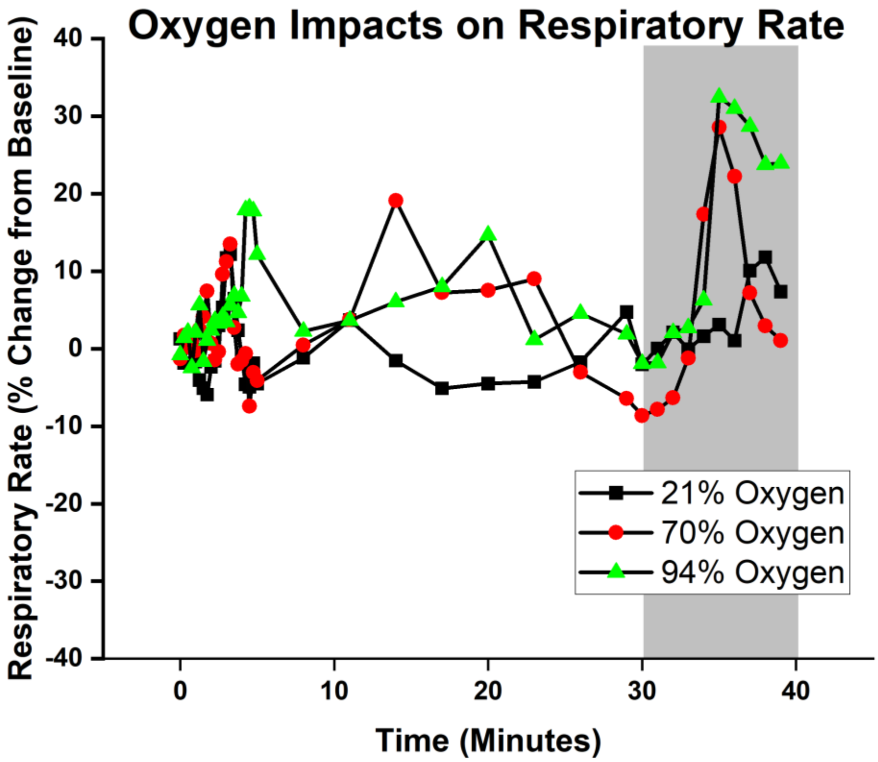

Figure 37 Average Respiratory Rate Response to Various O_2 Concentrations Over Time. Data points are compounded means from n=4 mice at 21% and 94% O_2 and n=3 mice at 70% O_2 concentrations. Mice were exposed to the experimental concentrations of O_2 for the first 30 minutes, with a 10-minute recovery in clean air at the end (indicated with the gray panel). Data points are compounded averages for every 25 seconds the first 5 minutes of exposure, compounded every 3 minutes from 5 to 30 minutes of exposure, and compounded every minute during the 10 minutes of recovery.

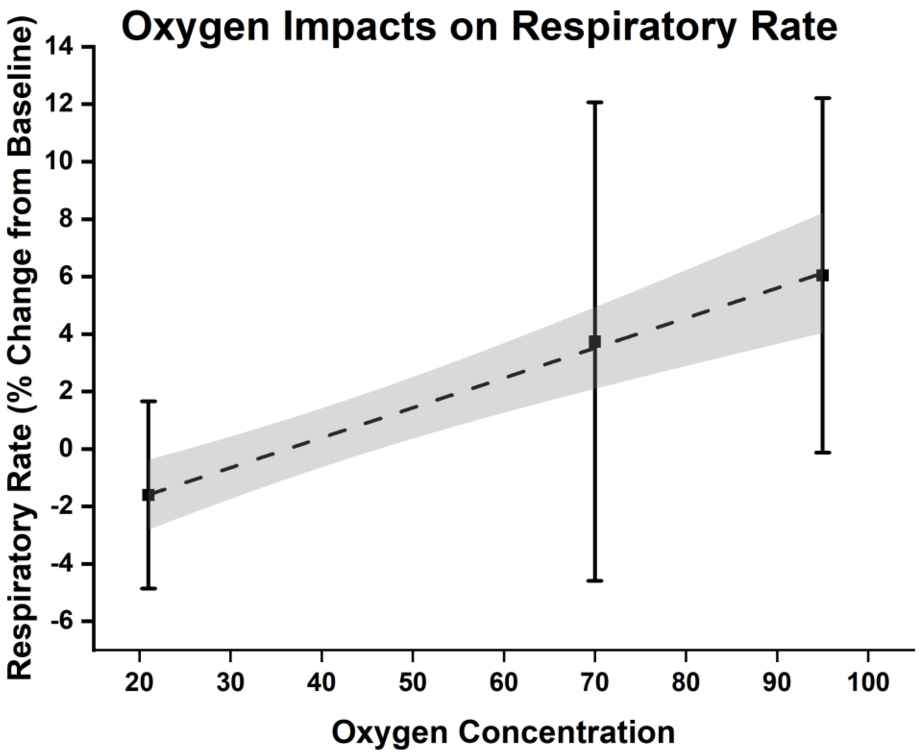

Figure 38 Concentration-Response Curve at Different Oxygen Concentrations. Concentration of O_2 plotted against mean percent change of respiration rate from baseline during exposure to three different O_2 concentrations. Fitted curve (dotted), with parameters ± SE listed in inset, is significantly different (p= 0.02, ANOVA) from zero. Shaded area represents the 95% confidence interval of fitted curve. Data points are means from n= 4 mice at 21% and 94% O_2 and n=3 mice at 70% O_2. Error bars equal ± SD.

Discussion

This objective of this study was to assess the sensory irritation potential of cyclobutylamine, naphthalene, 1,2,4-trimethylbenzene, isopropanol and nitrogen dioxide when administered via inhalation to mice under both normal oxygen and high oxygen conditions following ASTM standards. For all exposures, environmental conditions were on target and test chemical concentrations stable (Appendices 3 and 6). Prior to exposures, all concentration targets were tested with both air flows and pressures optimized to provide stable delivery of test chemicals to the exposure tower (Appendices 4 and 5).

All RD_{50} values determined in this study followed guidelines using ASTM standards (2019) are reported in Table 14. RD_{50} values were successfully calculated for four out of five test chemicals. For those test chemicals with RD_{50} values, 95% confidence intervals were successfully calculated. Because cyclobutylamine was not previously characterized as a sensory irritant, an RD_{50} value is not available (Ruth, 1986). In this study cyclobutylamine exposure did produced a significant trigeminal reflex-induced decrease in the respiration rate in a concentration-dependent fashion. Thus, these results support that cyclobutylamine is a sensory irritant and the data collected was used to generate an RD_{50} value for cyclobutylamine for the first time (Table 14). Despite naphthalene being characterized as a sensory irritant, an RD_{50} has not previously been calculated (Bruning et al., 2014). Data presenting in this report also confirms that naphthalene is a sensory irritant and the data collected was used to generate an RD_{50} value for naphthalene (Table 14). 1,2,4-trimethylbenzene is classified as a respiratory sensory irritant and has a reported RD_{50} of 2844 mg/m^3 (578 ppm) that was calculated using shorter exposure periods than recommended in the ASTM guidelines (Korsak et al., 1995). Using ASTM guidelines, 1,2,4-trimethylbenzene exposure reported in this study did cause a decrease in respiratory rate supporting that the chemical is a sensory irritant. However, the data generated using ASTM methods in this study resulted in RD_{50} value that was significantly lower at 1408 mg/m^3 (286 ppm) (Table 14). The RD_{50} calculated in this study for isopropanol exposure is consistent with previous reports, with the previously reported value of 17,693 ppm within the 95% confidence interval (Table 14).

Table 14 Summary of RD_{50} Results

Chemical	Normoxic RD_{50} (mg/m^3)	95% Confidence	Hyperoxic RD_{50} (mg/m^3)	95% Confidence	RCCA Reported (mg/m^3)*
Isopropanol	77,545	19,512 – 308,174	88,104	8,331 – 931,756	0.74
trimethylbenzene	1,408	961 – 2,063	959	785 – 1,171	2.95
cyclobutylamine	1,208	1,032 – 1,414	1,148	557 – 2,364	1.75
naphthalene	25.9	14.4 - 46.7	14.4	5.1 – 40.7	4.19
nitrogen dioxide	n/a		n/a		30.1
Chemical	**Normoxic RD_{50} (ppm)**	**95% Confidence**	**Hyperoxic RD_{50} (ppm)**	**95% Confidence**	**RCCA Reported (ppm)***
Isopropanol	31,547	7,938 – 125,372	35,843	3,389 – 379,059	0.3
trimethylbenzene	286	195 - 420	195	160 – 238	0.6
cyclobutylamine	415	355 - 486	395	191 – 813	0.6
naphthalene	4.9	2.7 – 8.9	2.8	1.0 – 7.7	0.8
nitrogen dioxide	n/a		n/a		8.0

*Values reported by the RCCA are from samples collected in and around aircraft. RD_{50} calculated from mice often have a 0.03 uncertainty factor applied to translate irritant effects to develop exposure limits for human exposures.

Despite that an RD_{50} was calculated in nitrogen dioxide in anesthetized cannulated mice, which bypass the nerve endings of the upper respiratory tract (Alarie, 1981), we found different results in unanesthetized, non-cannulated mice. Specifically, we show initial bradypnea that is characteristic of sensory irritancy followed by an increase in respiration in a dose-response relationship with nitrogen dioxide concentrations. The increase in respiration was not observed in the anesthetized, cannulated mice which suggests that the mechanisms underlying this effect may be sensitive to anesthetics, or there is a differential effect on respiratory rate resulting from stimulation of neural receptors in the lower respiratory tract compared to a combination of the upper and lower respiratory tracts. In addition to sensory irritation, there are physiological responses to pulmonary irritants that also impact respiration but do not cause the bradypnea characteristic of sensory irritancy (Muller and Schaeffer, 1996). While more research is needed in this area, it is likely that nitrogen dioxide is acting upon multiple mechanisms underlying respiratory regulation. We also found evidence that suggest increasing oxygen concentrations may increase the duration from bradypnea onset to tachypnea onset perhaps offering a tool to separate the mechanisms for future studies.

We did not find significant changes in respiratory rates in control animals exposed to different concentrations of oxygen following exposure guidelines for establishing RD_{50} guidelines. Thus, normoxic (21% O_2) and hyperoxic (94% O_2) conditions were not likely to contribute to baseline changes in respiration over the duration of chemical exposures tested herein. Although differences in the RD_{50} when comparing normoxic and hyperoxic conditions were found, the hyperoxic values always fell within the confidence interval of the normoxic values. Thus, these results do not support that hyperoxic conditions profoundly impact RD_{50} values in all the chemicals tested. The values reported from samples collected in and around aircraft by the NAVAIR Root Cause and Corrective Action (RCCA) team are well below the RD_{50} values reported herein for isopropanol, trimethylbenzene and cyclobutylamine suggesting that these chemicals individually are not likely to contribute to the sensory irritancy experienced by Naval aircrew (Table 14). However, the naphthalene levels detected by the RCCA team are just outside the RD_{50} 95% confidence interval (Table 14). Cytochrome P450 enzymes expressed in the airway metabolizes naphthalene and the sensory irritancy response of naphthalene is dependent upon this process (Lanosa et al., 2010). Moreover, there are species-specific expression profiles of different Cytochrome P450 isoforms and these isoforms have a variable ability to metabolize naphthalene (Plopper et al., 1992; Buckpitt et al., 2002; Baldwin et al., 2005; Li et al., 2017). Mouse (CYP2F2), rat (CYP2F4) and human (CYP2F1) isoforms have all demonstrated the ability to metabolize naphthalene, but the human form is less sensitive and metabolizes at a lower rate (Baldwin et al., 2005; Lewis et al., 2009; Li et al., 2017). Thus, compared to humans, mouse airways are likely more susceptible to naphthalene relative to other chemicals and the 0.03 x RD_{50} uncertainty factor typically used to translate irritant effects to humans may not be appropriate (Plopper et al., 1992; Baldwin et al., 2004). More studies will need to be performed to determine the difference in sensitivity to naphthalene in humans and rodents to accurately predict the sensory irritancy potential in humans.

Conclusions

RD_{50} values were successfully determined in four out of five test chemicals sampled herein. The results of these experiments will be examined in the context of prior sensory irritation studies on irritant chemicals and contribute to the development of recommendations of exposure levels that minimize the risk of causing sensory irritancy in the presence of hyperoxia.

Appendices

Appendix 1 Exposure Schedule

Date	Run	Chemical	Chemical Level	Oxygen Level	Number of Animals
03-Mar-2020	1	Isopropanol	1-Bottom	Normoxia	4
03-Mar-2020	2	Isopropanol	2-Low	Normoxia	4
03-Mar-2020	3	Isopropanol	3-Middle	Normoxia	4
04-Mar-2020	4	Isopropanol	4-High	Normoxia	4
04-Mar-2020	5	Isopropanol	5-Top	Normoxia	4
05-Mar-2020	6	Isopropanol	1-Bottom	Hyperoxia	4
05-Mar-2020	7	Isopropanol	2-Low	Hyperoxia	4
05-Mar-2020	8	Isopropanol	3-Middle	Hyperoxia	4
06-Mar-2020	9	Isopropanol	4-High	Hyperoxia	4
06-Mar-2020	10	Isopropanol	5-Top	Hyperoxia	4
10-Mar-2020	11	1,2,4-Trimethylbenzene	5-Top	Normoxia	4
10-Mar-2020	12	1,2,4-Trimethylbenzene	4-High	Normoxia	4
10-Mar-2020	13	1,2,4-Trimethylbenzene	2-Low	Normoxia	4
11-Mar-2020	14	1,2,4-Trimethylbenzene	3-Middle	Normoxia	4
11-Mar-2020	15	1,2,4-Trimethylbenzene	1-Bottom	Normoxia	4
12-Mar-2020	16	1,2,4-Trimethylbenzene	4-High	Hyperoxia	4
12-Mar-2020	17	1,2,4-Trimethylbenzene	3-Middle	Hyperoxia	4
12-Mar-2020	18	1,2,4-Trimethylbenzene	2-Low	Hyperoxia	4
13-Mar-2020	19	1,2,4-Trimethylbenzene	5-Top	Hyperoxia	4
13-Mar-2020	20	1,2,4-Trimethylbenzene	1-Bottom	Hyperoxia	4
14-Mar-2020	21	Cyclobutylamine	2-Low	Normoxia	4
14-Mar-2020	22	Cyclobutylamine	1-Bottom	Normoxia	4
14-Mar-2020	23	Cyclobutylamine	3-Middle	Normoxia	4
14-Mar-2020	24	Cyclobutylamine	5-Top	Normoxia	4
14-Mar-2020	25	Cyclobutylamine	4-High	Normoxia	4
15-Mar-2020	26	Cyclobutylamine	4-High	Hyperoxia	4
15-Mar-2020	27	Cyclobutylamine	5-Top	Hyperoxia	4
15-Mar-2020	28	Cyclobutylamine	3-Middle	Hyperoxia	4
15-Mar-2020	29	Cyclobutylamine	1-Bottom	Hyperoxia	4
15-Mar-2020	30	Cyclobutylamine	2-Low	Hyperoxia	3
09-Jun-2020	31	Naphthalene	5-Top	Normoxia	4
09-Jun-2020	32	Naphthalene	4-High	Normoxia	4
09-Jun-2020	33	Naphthalene	2-Low	Normoxia	4
10-Jun-2020	34	Naphthalene	3-Middle	Normoxia	4
10-Jun-2020	35	Naphthalene	1-Bottom	Normoxia	4
11-Jun-2020	36	Naphthalene	2-Low	Hyperoxia	4
11-Jun-2020	37	Naphthalene	1-Bottom	Hyperoxia	4
11-Jun-2020	38	Naphthalene	5-Top	Hyperoxia	4
12-Jun-2020	39	Naphthalene	3-Middle	Hyperoxia	4
12-Jun-2020	40	Naphthalene	4-High	Hyperoxia	4
21-Jul-2020	41	Nitrogen Dioxide		Normoxia	4

21-Jul-2020	42	Nitrogen Dioxide	Normoxia	4
21-Jul-2020	43	Nitrogen Dioxide	Normoxia	4
22-Jul-2020	44	Nitrogen Dioxide	Hyperoxia	4
22-Jul-2020	45	Nitrogen Dioxide	Hyperoxia	4
23-Jul-2020	46	Nitrogen Dioxide	Hyperoxia	4
23-Jul-2020	47	NONE	Hyperoxia	4
23-Jul-2020	48	NONE	Normoxia	4
24-Jul-2020	49	Nitrogen Dioxide	In-Between	4
24-Jul-2020	50	NONE	In-Between	4
TOTAL				**199**

Appendix 2 Experimental Animal Details

Animal ID#	Date Exposed	Exposure Chemical	Concentration (mg/m³)	O_2 Conditions	Exposure Group	Weight on Receipt (g)	Weight (g)
03T	20-Feb-2020	Clean Air	0	Normoxia	Training	-	26.70
04T	20-Feb-2020	Clean Air	0	Normoxia	Training	-	29.68
01T	20-Feb-2020	Clean Air	0	Normoxia	Training	-	29.27
02T	20-Feb-2020	Clean Air	0	Normoxia	Training	-	27.41
126	3-Mar-2020	IPA	6,700	Normoxia	1	21.24	30.43
135	3-Mar-2020	IPA	6,700	Normoxia	1	18.69	29.83
139	3-Mar-2020	IPA	6,700	Normoxia	1	19.55	28.87
138	3-Mar-2020	IPA	6,700	Normoxia	1	18.71	28.86
136	3-Mar-2020	IPA	9,516	Normoxia	2	17.13	28.58
131	3-Mar-2020	IPA	9,516	Normoxia	2	19.45	28.40
104	3-Mar-2020	IPA	9,516	Normoxia	2	18.04	29.46
101	3-Mar-2020	IPA	9,516	Normoxia	2	19.79	28.22
121	3-Mar-2020	IPA	12,856	Normoxia	3	19.11	28.05
130	3-Mar-2020	IPA	12,856	Normoxia	3	18.04	28.04
106	3-Mar-2020	IPA	12,856	Normoxia	3	18.03	27.80
105	3-Mar-2020	IPA	12,856	Normoxia	3	17.52	28.50
117	4-Mar-2020	IPA	26,483	Normoxia	4	15.99	28.69
114	4-Mar-2020	IPA	26,483	Normoxia	4	17.28	28.85
103	4-Mar-2020	IPA	26,483	Normoxia	4	17.70	28.06
124	4-Mar-2020	IPA	26,483	Normoxia	4	19.21	28.64
118	4-Mar-2020	IPA	45,488	Normoxia	5	18.85	27.70
112	4-Mar-2020	IPA	45,488	Normoxia	5	20.10	27.95

120	4-Mar-2020	IPA	45,488	Normoxia	5	19.85	27.94
125	4-Mar-2020	IPA	45,488	Normoxia	5	20.36	27.83
127	5-Mar-2020	IPA	6,676	Hyperoxia	1	18.07	28.37
140	5-Mar-2020	IPA	6,676	Hyperoxia	1	19.94	28.44
102	5-Mar-2020	IPA	6,676	Hyperoxia	1	19.92	28.76
115	5-Mar-2020	IPA	9,478	Hyperoxia	2	19.76	28.04
137	5-Mar-2020	IPA	9,478	Hyperoxia	2	18.31	28.46
111	5-Mar-2020	IPA	9,478	Hyperoxia	2	18.62	27.96
109	5-Mar-2020	IPA	9,478	Hyperoxia	2	17.35	27.21
108	5-Mar-2020	IPA	13,866	Hyperoxia	3	18.11	26.72
132	5-Mar-2020	IPA	13,866	Hyperoxia	3	17.62	26.91
123	5-Mar-2020	IPA	13,866	Hyperoxia	3	19.44	26.25
116	5-Mar-2020	IPA	13,866	Hyperoxia	3	18.36	26.81
134	6-Mar-2020	-	-	-	5	18.02	21.53
119	6-Mar-2020	IPA	27,417	Hyperoxia	4	16.51	25.59
107	6-Mar-2020	IPA	27,417	Hyperoxia	4	16.87	26.46
110	6-Mar-2020	IPA	27,417	Hyperoxia	4	18.35	25.22
129	6-Mar-2020	IPA	27,417	Hyperoxia	4	19.31	26.18
128	6-Mar-2020	IPA	48,834	Hyperoxia	5	17.93	26.48
133	6-Mar-2020	IPA	48,834	Hyperoxia	5	16.32	24.68
113	6-Mar-2020	IPA	48,834	Hyperoxia	5	19.14	25.24
224	10-Mar-2020	1,2,4-TMB	1,398	Normoxia	1	17.20	28.50
209	10-Mar-2020	1,2,4-TMB	1,398	Normoxia	1	17.13	28.41
218	10-Mar-2020	1,2,4-TMB	1,398	Normoxia	1	18.82	27.75
226	10-Mar-2020	1,2,4-TMB	1,398	Normoxia	1	16.68	28.39

214	10-Mar-2020	1,2,4-TMB	763	Normoxia	2	16.53	26.89
205	10-Mar-2020	1,2,4-TMB	763	Normoxia	2	16.73	27.66
216	10-Mar-2020	1,2,4-TMB	763	Normoxia	2	16.09	28.20
215	10-Mar-2020	1,2,4-TMB	763	Normoxia	2	16.09	27.72
211	10-Mar-2020	1,2,4-TMB	295	Normoxia	3	16.74	28.20
222	10-Mar-2020	1,2,4-TMB	295	Normoxia	3	16.80	28.77
221	10-Mar-2020	1,2,4-TMB	295	Normoxia	3	15.21	27.29
213	10-Mar-2020	1,2,4-TMB	295	Normoxia	3	17.60	28.57
217	11-Mar-2020	1,2,4-TMB	503	Normoxia	4	16.07	28.43
238	11-Mar-2020	1,2,4-TMB	503	Normoxia	4	16.03	27.95
231	11-Mar-2020	1,2,4-TMB	503	Normoxia	4	15.36	29.38
228	11-Mar-2020	1,2,4-TMB	503	Normoxia	4	15.68	27.97
202	11-Mar-2020	1,2,4-TMB	164	Normoxia	5	16.36	27.78
212	11-Mar-2020	1,2,4-TMB	164	Normoxia	5	16.11	27.86
219	11-Mar-2020	1,2,4-TMB	164	Normoxia	5	15.96	27.72
220	11-Mar-2020	1,2,4-TMB	164	Normoxia	5	15.72	27.41
204	12-Mar-2020	1,2,4-TMB	818	Hyperoxia	1	15.91	28.03

208	12-Mar-2020	1,2,4-TMB	818	Hyperoxia	1	17.15	28.39
230	12-Mar-2020	1,2,4-TMB	818	Hyperoxia	1	15.43	28.14
235	12-Mar-2020	1,2,4-TMB	818	Hyperoxia	1	15.69	29.53
201	12-Mar-2020	1,2,4-TMB	463	Hyperoxia	2	14.66	27.85
210	12-Mar-2020	1,2,4-TMB	463	Hyperoxia	2	15.05	27.86
227	12-Mar-2020	1,2,4-TMB	463	Hyperoxia	2	14.79	27.38
240	12-Mar-2020	1,2,4-TMB	463	Hyperoxia	2	16.25	28.60
203	12-Mar-2020	1,2,4-TMB	329	Hyperoxia	3	15.54	27.08
229	12-Mar-2020	1,2,4-TMB	329	Hyperoxia	3	15.17	26.98
237	12-Mar-2020	1,2,4-TMB	329	Hyperoxia	3	16.61	27.08
239	12-Mar-2020	1,2,4-TMB	329	Hyperoxia	3	16.08	27.18
223	13-Mar-2020	1,2,4-TMB	1306	Hyperoxia	4	15.66	28.11
225	13-Mar-2020	1,2,4-TMB	1306	Hyperoxia	4	14.94	27.17
232	13-Mar-2020	1,2,4-TMB	1306	Hyperoxia	4	15.43	27.10
234	13-Mar-2020	1,2,4-TMB	1306	Hyperoxia	4	15.17	26.87
236	13-Mar-2020	1,2,4-TMB	204	Hyperoxia	5	16.00	26.63
233	13-Mar-2020	1,2,4-TMB	204	Hyperoxia	5	15.85	27.63

207	13-Mar-2020	1,2,4-TMB	204	Hyperoxia	5	16.83	25.85
206	13-Mar-2020	1,2,4-TMB	204	Hyperoxia	5	17.78	24.60
306	14-Mar-2020	CycloB	320	Normoxia	1	19.84	26.74
309	14-Mar-2020	CycloB	320	Normoxia	1	19.74	26.69
313	14-Mar-2020	CycloB	320	Normoxia	1	19.42	25.92
317	14-Mar-2020	CycloB	320	Normoxia	1	20.11	26.42
324	14-Mar-2020	CycloB	142	Normoxia	2	18.44	25.96
327	14-Mar-2020	CycloB	142	Normoxia	2	16.55	25.71
338	14-Mar-2020	CycloB	142	Normoxia	2	18.19	25.17
339	14-Mar-2020	CycloB	142	Normoxia	2	17.36	25.13
325	14-Mar-2020	CycloB	586	Normoxia	3	20.00	25.78
303	14-Mar-2020	CycloB	586	Normoxia	3	17.69	25.56
305	14-Mar-2020	CycloB	586	Normoxia	3	18.22	25.29
310	14-Mar-2020	CycloB	586	Normoxia	3	19.26	25.24
314	14-Mar-2020	CycloB	1188	Normoxia	4	18.38	25.30
316	14-Mar-2020	CycloB	1188	Normoxia	4	18.29	25.66
340	14-Mar-2020	CycloB	1188	Normoxia	4	18.43	24.52

312	14-Mar-2020	CycloB	1188	Normoxia	4	19.28	25.10
315	14-Mar-2020	CycloB	913	Normoxia	5	17.62	24.26
319	14-Mar-2020	CycloB	913	Normoxia	5	18.28	24.79
304	14-Mar-2020	CycloB	913	Normoxia	5	18.86	24.63
330	14-Mar-2020	CycloB	913	Normoxia	5	19.78	24.79
318	14-Mar-2020	CycloB	-	-	-	17.08	20.75
322	15-Mar-2020	CycloB	768	Hyperoxia	1	16.14	23.95
308	15-Mar-2020	CycloB	768	Hyperoxia	1	17.65	22.99
334	15-Mar-2020	CycloB	768	Hyperoxia	1	17.93	24.03
307	15-Mar-2020	CycloB	768	Hyperoxia	1	16.67	23.34
335	15-Mar-2020	CycloB	1368	Hyperoxia	2	18.71	24.50
337	15-Mar-2020	CycloB	1368	Hyperoxia	2	18.17	24.33
332	15-Mar-2020	CycloB	1368	Hyperoxia	2	15.56	23.28
321	15-Mar-2020	CycloB	1368	Hyperoxia	2	16.10	22.94
329	15-Mar-2020	CycloB	525	Hyperoxia	3	18.54	23.70
331	15-Mar-2020	CycloB	525	Hyperoxia	3	16.13	23.51
336	15-Mar-2020	CycloB	525	Hyperoxia	3	15.88	24.37

301	15-Mar-2020	CycloB	525	Hyperoxia	3	16.21	22.57
302	15-Mar-2020	CycloB	146	Hyperoxia	4	18.28	23.47
333	15-Mar-2020	CycloB	146	Hyperoxia	4	15.82	23.62
311	15-Mar-2020	CycloB	146	Hyperoxia	4	15.55	23.84
323	15-Mar-2020	CycloB	304	Hyperoxia	5	16.39	24.35
328	15-Mar-2020	CycloB	304	Hyperoxia	5	14.20	22.99
326	15-Mar-2020	CycloB	304	Hyperoxia	5	14.96	23.49
320	15-Mar-2020	CycloB	304	Hyperoxia	5	18.15	22.59
440	9-Jun-2020	Napthalene	41.41	Normoxia	1	21.48	32.17
413	9-Jun-2020	Napthalene	41.41	Normoxia	1	19.62	31.55
415	9-Jun-2020	Napthalene	41.41	Normoxia	1	19.43	31.41
439	9-Jun-2020	Napthalene	41.41	Normoxia	1	20.80	30.78
401	9-Jun-2020	Napthalene	17.82	Normoxia	2	19.79	30.30
417	9-Jun-2020	Napthalene	17.82	Normoxia	2	17.74	30.47
434	9-Jun-2020	Napthalene	17.82	Normoxia	2	20.13	30.53
435	9-Jun-2020	Napthalene	17.82	Normoxia	2	20.67	30.32
409	9-Jun-2020	Napthalene	9.44	Normoxia	3	18.43	30.05
427	9-Jun-2020	Napthalene	9.44	Normoxia	3	17.95	28.02
428	9-Jun-2020	Napthalene	9.44	Normoxia	3	21.54	29.91
437	9-Jun-2020	Napthalene	9.44	Normoxia	3	18.42	30.09
403	10-Jun-2020	Napthalene	16.25	Normoxia	4	19.46	30.25
405	10-Jun-2020	Napthalene	16.25	Normoxia	4	19.86	31.25

412	10-Jun-2020	Napthalene	16.25	Normoxia	4	19.69	30.57
430	10-Jun-2020	Napthalene	16.25	Normoxia	4	18.92	30.23
406	10-Jun-2020	Napthalene	7.37	Normoxia	5	18.30	30.03
407	10-Jun-2020	Napthalene	7.37	Normoxia	5	19.73	30.07
411	10-Jun-2020	Napthalene	7.37	Normoxia	5	19.52	30.19
438	10-Jun-2020	Napthalene	7.37	Normoxia	5	19.10	29.80
410	11-Jun-2020	Napthalene	1.73	Hyperoxia	1	18.96	29.77
418	11-Jun-2020	Napthalene	1.73	Hyperoxia	1	21.91	29.78
433	11-Jun-2020	Napthalene	1.73	Hyperoxia	1	18.99	29.73
436	11-Jun-2020	Napthalene	1.73	Hyperoxia	1	18.61	29.76
420	11-Jun-2020	Napthalene	0.0	Hyperoxia	2	20.86	29.17
422	11-Jun-2020	Napthalene	0.0	Hyperoxia	2	18.03	29.03
429	11-Jun-2020	Napthalene	0.0	Hyperoxia	2	18.85	29.01
431	11-Jun-2020	Napthalene	0.0	Hyperoxia	2	18.92	29.58
402	11-Jun-2020	Napthalene	42.99	Hyperoxia	3	17.73	28.70
408	11-Jun-2020	Napthalene	42.99	Hyperoxia	3	15.61	28.97
426	11-Jun-2020	Napthalene	42.99	Hyperoxia	3	16.47	28.22

432	11-Jun-2020	Napthalene	42.99	Hyperoxia	3	16.94	28.21
404	11-Jun-2020	Napthalene	1.83	Hyperoxia	4	19.00	28.77
421	11-Jun-2020	Napthalene	1.83	Hyperoxia	4	17.14	28.42
423	11-Jun-2020	Napthalene	1.83	Hyperoxia	4	16.31	29.23
424	11-Jun-2020	Napthalene	1.83	Hyperoxia	4	17.35	28.21
414	12-Jun-2020	Napthalene	9.44	Hyperoxia	5	17.59	26.96
416	12-Jun-2020	Napthalene	9.44	Hyperoxia	5	16.55	26.54
419	12-Jun-2020	Napthalene	9.44	Hyperoxia	5	14.60	24.98
425	12-Jun-2020	Napthalene	9.44	Hyperoxia	5	14.61	24.61
510	21-Jul-2020	NO_2	158.01	Normoxia	1	22.87	32.11
519	21-Jul-2020	NO_2	158.01	Normoxia	1	16.40	31.84
527	21-Jul-2020	NO_2	158.01	Normoxia	1	19.69	31.61
501	21-Jul-2020	NO_2	158.01	Normoxia	1	22.72	31.42
512	21-Jul-2020	NO_2	52.67	Normoxia	2	17.19	30.93
506	21-Jul-2020	NO_2	52.67	Normoxia	2	21.98	30.92
511	21-Jul-2020	NO_2	52.67	Normoxia	2	23.80	30.87
514	21-Jul-2020	NO_2	52.67	Normoxia	2	22.66	30.79
538	21-Jul-2020	NO_2	259.59	Normoxia	3	21.28	30.75
533	21-Jul-2020	NO_2	259.59	Normoxia	3	21.08	30.45
531	21-Jul-2020	NO_2	259.59	Normoxia	3	-	30.39
518	21-Jul-2020	NO_2	259.59	Normoxia	3	19.21	30.29
540	22-Jul-2020	NO_2	225.73	Hyperoxia	1	20.59	31.48
508	22-Jul-2020	NO_2	225.73	Hyperoxia	1	18.20	30.81

515	22-Jul-2020	NO_2	225.73	Hyperoxia	1	16.89	30.78
504	22-Jul-2020	NO_2	225.73	Hyperoxia	1	22.56	30.77
516	22-Jul-2020	NO_2	147.00	Hyperoxia	2	20.13	30.76
505	22-Jul-2020	NO_2	147.00	Hyperoxia	2	23.04	30.72
529	22-Jul-2020	NO_2	147.00	Hyperoxia	2	21.31	30.48
530	22-Jul-2020	NO_2	147.00	Hyperoxia	2	20.96	30.31
507	23-Jul-2020	n/a	-	Hyperoxia	1	21.91	29.87
535	23-Jul-2020	n/a	-	Hyperoxia	1	21.15	29.77
523	23-Jul-2020	n/a	-	Hyperoxia	1	20.68	29.32
526	23-Jul-2020	n/a	-	Hyperoxia	1	23.53	29.31
517	23-Jul-2020	NO_2	41.38	Hyperoxia	3	21.60	30.74
524	23-Jul-2020	NO_2	41.38	Hyperoxia	3	23.11	30.62
522	23-Jul-2020	NO_2	41.38	Hyperoxia	3	23.64	30.09
539	23-Jul-2020	NO_2	41.38	Hyperoxia	3	17.02	29.97
532	23-Jul-2020	n/a	-	21 % O_2	1	19.64	29.11
537	23-Jul-2020	n/a	-	21 % O_2	1	19.98	29.07
503	23-Jul-2020	n/a	-	21 % O_2	1	21.49	29.02
509	23-Jul-2020	n/a	-	21 % O_2	1	22.37	29.02
525	24-Jul-2020	n/a	-	70 % O_2	1	18.98	28.99
528	24-Jul-2020	n/a	-	70 % O_2	1	21.53	28.08
534	24-Jul-2020	n/a	-	70 % O_2	1	11.16	29.12
536	24-Jul-2020	n/a	-	70 % O_2	1	17.10	28.50
502	24-Jul-2020	NO_2	109.1	70 % O_2	4	16.99	28.68
513	24-Jul-2020	NO_2	109.1	70 % O_2	4	17.19	27.31
520	24-Jul-2020	NO_2	109.1	70 % O_2	4	22.58	27.07
521	24-Jul-2020	NO_2	109.1	70 % O_2	4	20.71	29.01

Appendix 3 Chemical Concentration Profiles During Exposures

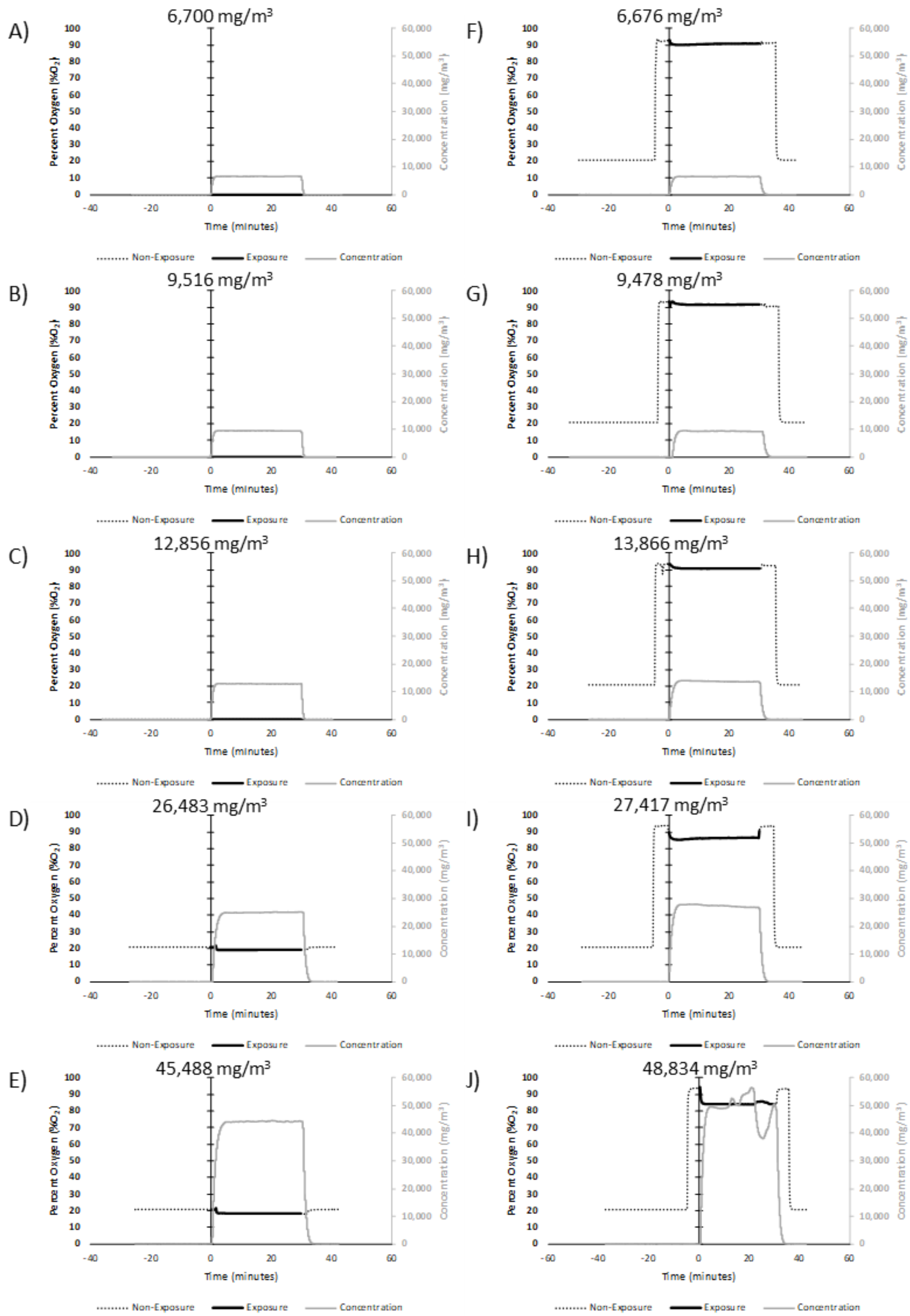

Figure 53: Graphs of IPA and O_2 concentrations over time for runs 1 to 10 (A to J respectively). The non-exposure and exposure lines tracks the oxygen concentrations (left axis) while the concentration line tracks the test chemical (right axis). A-E normoxic runs and F-J are hyperoxic runs. Mean concentration for each test article is listed above the medial y-axis.

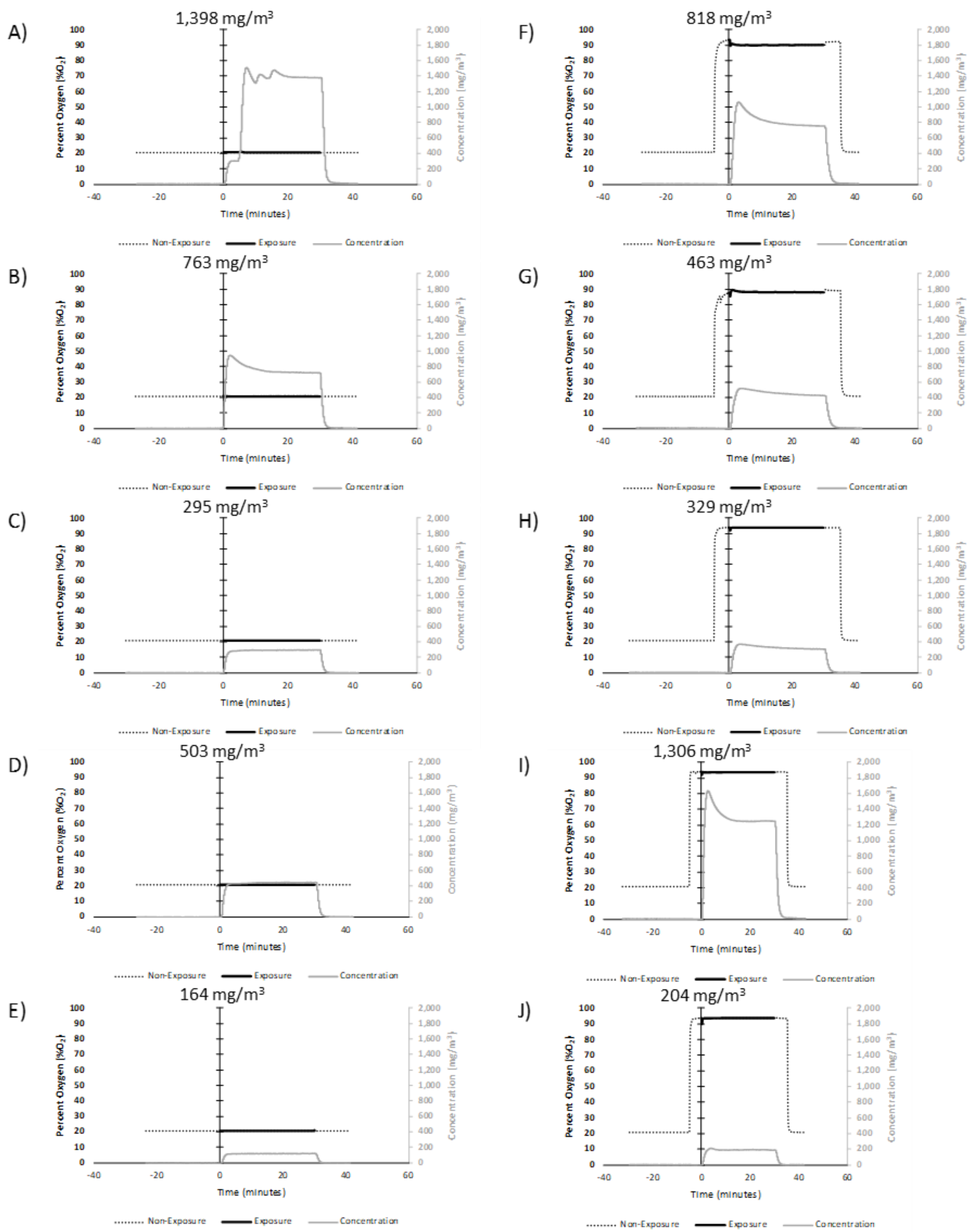

Figure 54: Graphs of TMB and O_2 concentrations over time for runs 11 to 20 (A to J respectively).

The non-exposure and exposure lines tracks the oxygen concentrations (left axis) while the concentration line tracks the test chemical (right axis). A-E normoxic runs and F-J are hyperoxic runs. Mean concentration for each test article is listed above the medial y-axis.

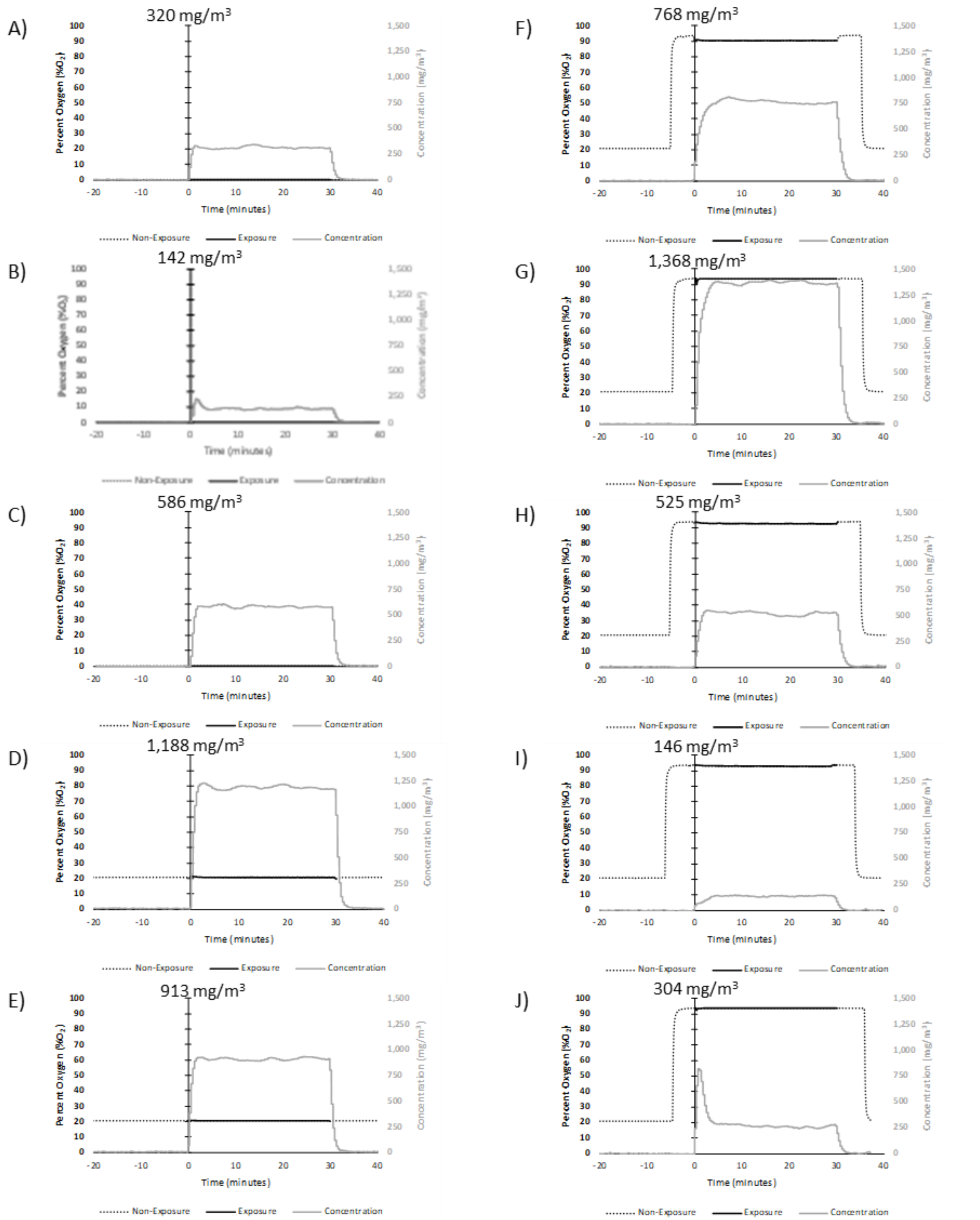

Figure 55: Graphs of CBA and O_2 concentrations over time for runs 21 to 30 (A to J respectively). The non-exposure and exposure lines tracks the oxygen concentrations (left axis) while the concentration line tracks the test chemical (right axis). A-E normoxic runs and F-J are hyperoxic runs. Mean concentration for each test article is listed above the medial y-axis.

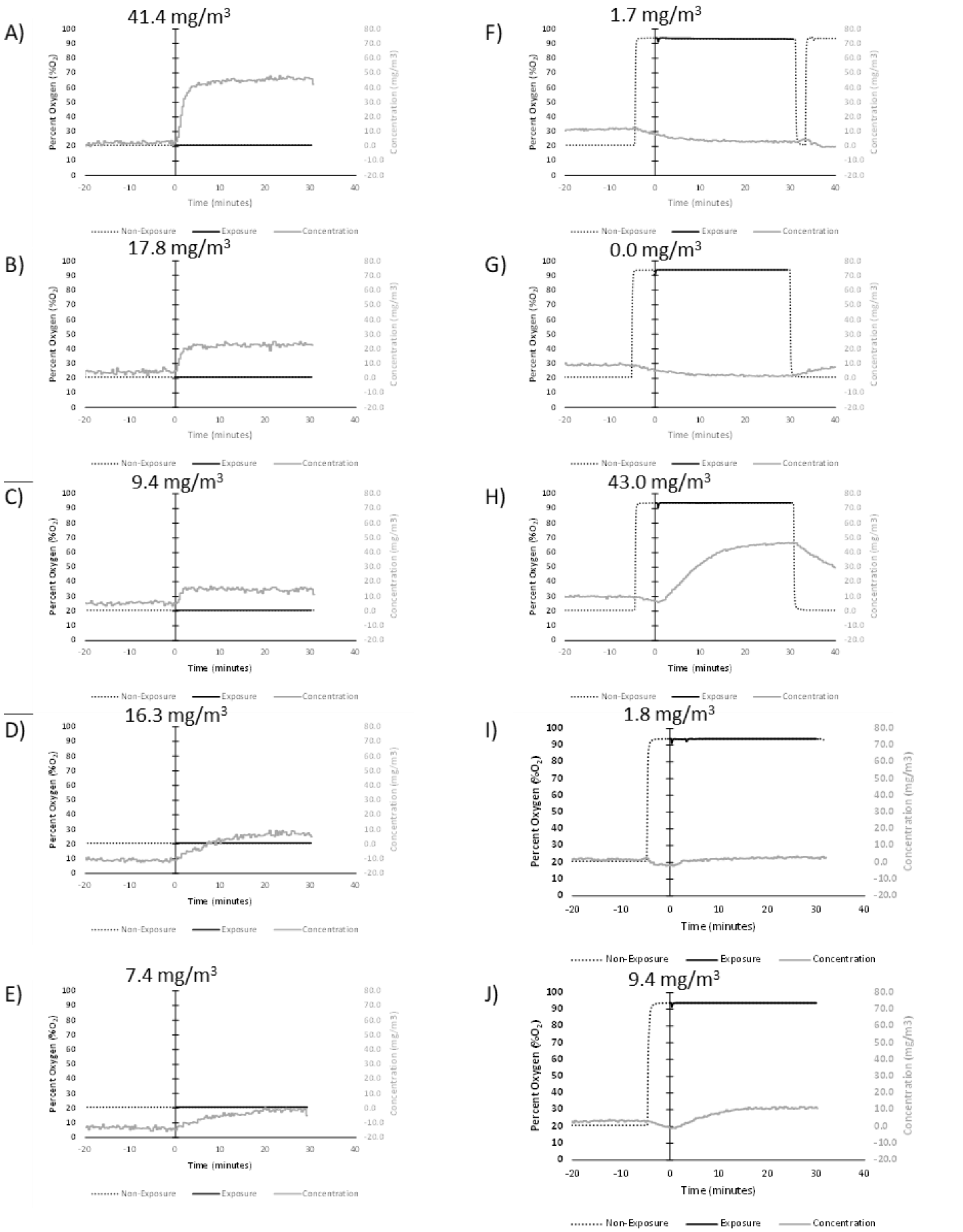

Figure 56: Graphs of naphthalene and O_2 concentrations over time for runs 31 to 40 (A to J respectively). The non-exposure and exposure lines tracks the oxygen concentrations (left axis) while the concentration line tracks the test chemical (right axis). A-E normoxic runs and F-J are hyperoxic runs. Mean concentration for each test article is listed above the medial y-axis.

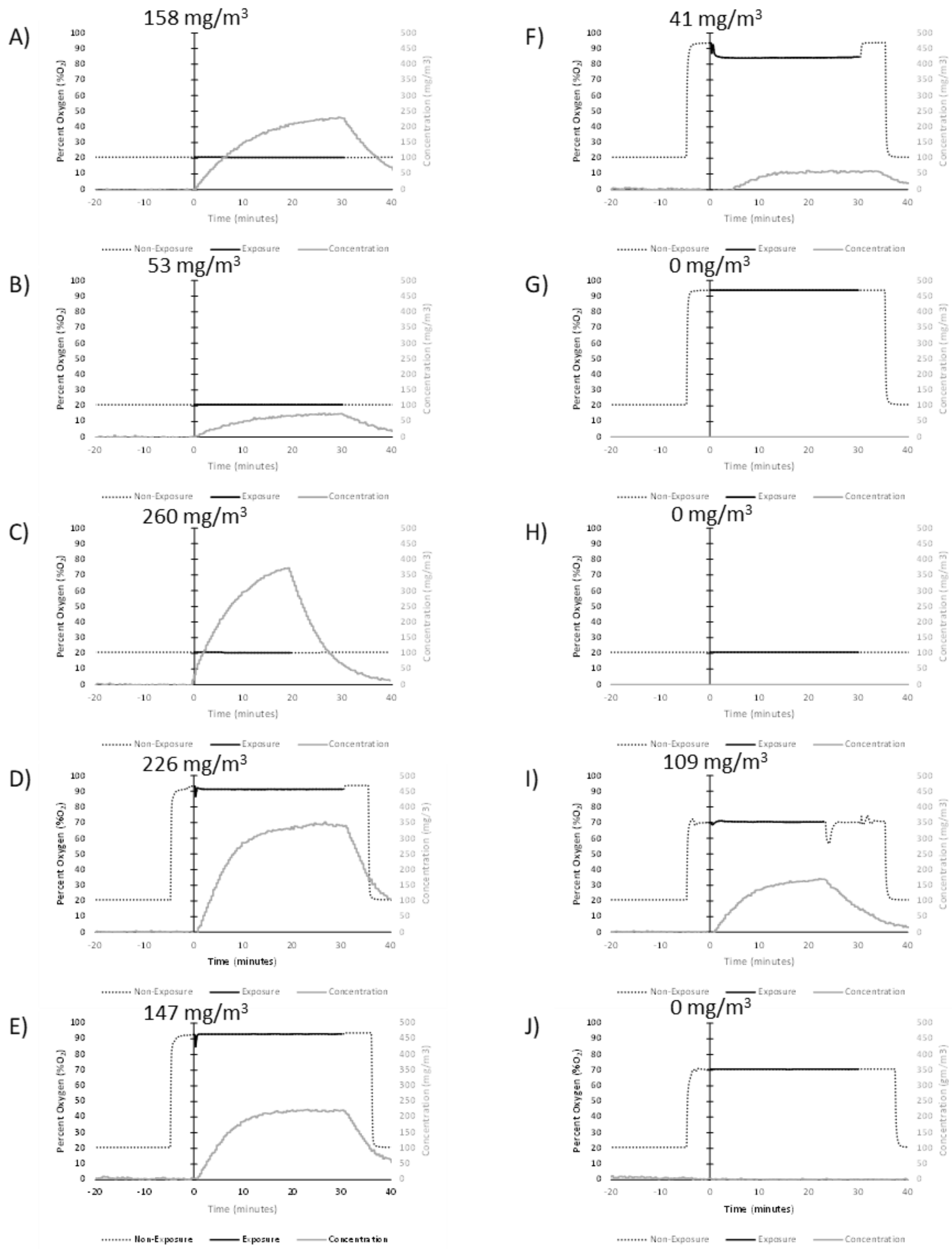

Figure 57: Graphs of NO_2 and O_2 concentrations over time for runs 41 to 50 (A to J respectively). The non-exposure and exposure lines tracks the oxygen concentrations (left axis) while the concentration line tracks the test chemical (right axis). Mean concentration for each test article is listed above the medial y-axis.

Appendix 4 FTIR Chemical Concentrations for Exposures

Table 15 FTIR Concentrations for Isopropanol Exposures

Mean (mg/m^3)	Min (mg/m^3)	Max (mg/m^3)	±SD	%RSD	Count (N)	$\%O_2$	Run#
6700	6,637	6,810	38	0.56%	72	21%	1
9516	9,443	9,567	29	0.30%	73	21%	2
12856	12,748	12,944	43	0.34%	76	21%	3
26483	24,864	26,680	248	0.94%	78	21%	4
45488	42,380	45,916	495	1.09%	76	21%	5
6676	6,594	6,814	47	0.71%	77	94%	6
9478	9,321	9,656	78	0.82%	77	94%	7
13866	13,288	14,131	152	1.09%	76	94%	8
27417	26,125	27,985	392	1.43%	77	94%	9
48834	38,074	56,410	4,795	9.82%	78	94%	10

Table 16 FTIR Concentrations for 1,2,4-Trimethylbenzene Exposures

Mean (mg/m^3)	Min (mg/m^3)	Max (mg/m^3)	±SD	%RSD	Count (N)	$\%O_2$	Run#
164	136	201	11.7	7.15%	75	21%	15
295	284	299	3.25	1.10%	77	21%	13
503	464	529	13.5	2.69%	76	21%	14
763	722	934	53.5	7.01%	75	21%	12
1398	1311	1492	34.34	2.46%	64	21%	11
204	199	221	4.3	2.12%	77	94%	20
329	306	369	17.9	5.44%	76	94%	18
463	430	520	26.5	5.72%	73	94%	17
818	757	1038	70	8.55%	72	94%	16
1306	1253	1619	87.2	6.67%	75	94%	19

Table 17 FTIR Concentrations for Cyclobutylamine Exposures

Mean (mg/m^3)	Min (mg/m^3)	Max (mg/m^3)	±SD	%RSD	Count (N)	$\%O_2$	Run#
142	97	234	18.7	13.1%	85	21%	22
320	279	352	12.1	3.8%	83	21%	21
586	456	614	17.6	3.0%	80	21%	23
913	893	936	12.2	1.3%	76	21%	25
1188	922	1233	34.2	2.9%	80	21%	24
146	128	154	5	3.4%	75	94%	29
304	249	821	109	36.0%	85	94%	30
525	493	552	17	3.3%	77	94%	28
768	697	816	22	2.8%	76	94%	26
1368	1274	1394	19	1.4%	76	94%	27

Table 18 FTIR Concentrations for Naphthalene Exposures

Mean (mg/m^3)	Min (mg/m^3)	Max (mg/m^3)	±SD	%RSD	Count (N)	$\%O_2$	Run#
7.4	3.7	11.0	8.9	15.0%	63	21%	35
9.4	6.3	11.5	4.9	3.0%	79	21%	33
16.3	10.0	19.9	9.4	9.7%	63	21%	34
17.8	13.6	19.9	5.0	2.5%	78	21%	32
41.4	36.7	44.6	6.8	2.4%	72	21%	31
1.7	0.8	2.9	0.4	23.0%	49	94%	36
1.8	0.1	3.0	0.12	35.0%	82	94%	39
9.4	5.2	11.5	0.63	15.0%	83	94%	40
43.0	38.8	45.1	1.7	4.0%	46	94%	38
-	-	-	-	-	-	94%	37*

*Run #37 contained no exposure gas during run

Table 19 FTIR Concentrations for Nitrogen Dioxide Exposures

Mean (mg/m^3)	Min (mg/m^3)	Max (mg/m^3)	±SD	%RSD	Count (N)	$\%O_2$	Run#
-	-	-	-	-	-	21%	48
53	45.2	60.2	3.8	7.0%	12	21%	42
158	135.4	173.1	10.9	7.0%	36	21%	41
260	233.3	274.6	11.7	4.4%	62	21%	43
109	94.1	116.6	6.0	5.7%	42	70%	49
-	-	-	-	-	-	70%	50
41	35.0	45.2	2.2	5.3%	69	94%	46
147	135.4	154.3	3.4	2.3%	51	94%	45
226	203.2	237.0	8.3	3.6%	53	94%	44
-	-	-	-	-	-	94%	47

*Runs #47, #48 and #50 contained no exposure gas during runs

Appendix 5 Chemical Properties

Isopropanol Properties

Substance Name:	Propan-2-ol
Other Names:	2-Propanol
	Isopropyl alcohol
	IPA
CAS #:	67-63-0
Linear Formula:	$(CH_3)_2CHOH$
Molecular Formula:	C_3H_8O
Molecular Weight:	60.1 g/mol
Description:	Colorless liquid
Test Article Category:	Volatile liquid
	(Boils at 82.3 °C)
	(45.4 mmHg at 25 °C)
Supplier:	Sigma-Aldrich
Additional Information:	Vapor density: 2.07 (Air = 1)

Trimethylbenzene Properties

Substance Name:	1,2,4-Trimethylbenzene
Other Names:	Trimethylbenzene
	TMB
	Psuedocumene
CAS #:	95-63-6
Linear Formula:	$C_6H_3(CH_3)_3$
Molecular Formula:	C_9H_{12}
Molecular Weight:	120.19 g/mol
Description:	Clear, colorless liquid
Test Article Category:	Semi-volatile liquid
	(Boils at 169.3 °C)
	(2.10 mmHg at 25 °C)
Supplier:	Sigma-Aldrich
Additional Information:	Vapor density: 0.8718 (Air = 1)

Cyclobutylamine

Substance Name:	Cyclobutylamine
Other Names:	CBA
	Aminocyclobutane
CAS #:	2516-34-9
Linear Formula:	$C_4H_7NH_2$
Molecular Formula:	C_4H_9N
Molecular Weight:	71.12 g/mol
Description:	Clear, colorless liquid
Test Article Category:	Volatile liquid
	(Boils at 81.5 °C)
	(61.3 mmHg at 20 °C)
Supplier:	Sigma-Aldrich
Additional Information:	Vapor density: 2.456 (Air = 1)

Naphthalene

Substance Name:	Naphthalene
Other Names:	Bicyclo[4.4.0]deca-1,3,5,7,9-pentaene
	Camphor tar
	N
CAS #:	91-20-3
Linear Formula:	$C_{10}H_8$
Molecular Formula:	$C_{10}H_8$
Molecular Weight:	128.174
Description:	White crystalline flakes
Test Article Category:	Solid, sublime
	(Boils at 218 °C)
	(Melts at 80.2 °C)
	(0.085 mmHg at 25 °C)
Supplier:	Sigma-Aldrich
Additional Information:	Vapor density: 4.42 (Air = 1)
	Odor: Aromatic

Nitrogen dioxide

Substance Name:	Nitrogen Dioxide
Other Names:	Dinitrogen tetroxide
	Nitrogen tetroxide
	NO2
CAS #:	10544-72-6
Linear Formula:	NO_2
Molecular Formula:	NO_2
Molecular Weight:	91.985806
Description:	Colorless gas, yellow/brown liquid below 22 °C
	(Boils at 21.15 °C)
	(Melts at -9.3 °C)
	(720 mmHg at 20 °C)
Test Article Category:	Gas, condensing
Supplier:	Matheson Gas
Additional Information:	Vapor density: 1.58 (Air = 1)

Appendix 6 Environmental Conditions During Exposures

Temperatures

Test Chemical	Concentration *(mg/m³)*	Min *(°F)*	Mean *(°F)*	Max *(°F)*	SD *(°F)*	*Count (N)*	*Run #*	O_2
IPA	2,726	76.7	77.1	77.5	0.2	4194	1	21%
IPA	3,870	77.5	78.0	78.3	0.2	4438	2	21%
IPA	5,230	77.8	78.1	78.3	0.1	4438	3	21%
IPA	10,773	75.1	75.6	76.1	0.3	4122	4	21%
IPA	18,505	76.7	77.0	77.2	0.1	4037	5	21%
IPA	2,715	73.9	74.5	74.9	0.3	4311	6	94%
IPA	3,855	75.1	75.5	75.8	0.2	4703	7	94%
IPA	5,640	75.7	76.0	76.2	0.1	4235	8	94%
IPA	11,153	74.0	74.6	75.1	0.3	4369	9	94%
IPA	19,878	75.0	75.2	75.4	0.1	4785	10	94%
TMB	164	76.4	76.7	77.1	0.2	3897	15	21%
TMB	295	77.6	78.0	78.3	0.2	4314	13	21%
TMB	503	75.8	76.2	76.7	0.3	4112	14	21%
TMB	763	77.6	77.9	78.3	0.2	4107	12	21%
TMB	1398	76.3	77.0	77.5	0.3	4099	11	21%
TMB	204	77.0	77.2	77.4	0.1	4291	20	94%
TMB	329	76.5	77.0	77.5	0.3	4384	18	94%
TMB	463	76.0	76.4	76.7	0.2	4288	17	94%
TMB	818	74.8	75.4	76.0	0.4	4115	16	94%
TMB	1306	76.4	77.0	77.3	0.2	4502	19	94%
CBA	142	75.8	75.9	76.1	0.1	3897	22	21%
CBA	320	74.2	74.6	75.1	0.3	3783	21	21%
CBA	586	76.2	76.3	76.4	0.0	3936	23	21%
CBA	913	76.4	76.5	76.7	0.1	4280	25	21%
CBA	1188	76.2	76.4	76.5	0.1	4084	24	21%
CBA	146	74.7	74.9	75.2	0.1	3880	29	94%
CBA	304	75.0	75.1	75.3	0.1	3746	30	94%
CBA	525	74.4	74.6	74.9	0.1	4157	28	94%
CBA	768	73.1	73.5	73.9	0.2	4055	26	94%
CBA	1368	74.0	74.3	74.6	0.1	3809	27	94%
Naph	9.4	78.0	78.5	78.8	0.2	3522	33	21%
Naph	7.4	79.4	79.5	79.6	0.0	3172	35	21%
Naph	16.3	77.9	78.3	78.6	0.1	3314	34	21%
Naph	17.8	77.1	77.6	78.0	0.3	3350	32	21%
Naph	41.4	75.1	75.7	76.3	0.3	3437	31	21%
Naph	0	77.4	77.7	78.0	0.2	4041	37	94%
Naph	1.73	76.7	77.1	77.5	0.2	3714	36	94%
Naph	1.8	76.0	76.5	76.9	0.3	3564	39	94%
Naph	9.4	76.9	77.3	77.6	0.2	3375	40	94%
Naph	43	77.7	78.0	78.3	0.2	4246	38	94%
NO_2	53	78.1	78.5	78.9	0.2	4272	42	21%
NO_2	158	77.1	77.6	78.0	0.3	3747	41	21%
NO_2	260	79.1	79.6	80.2	0.3	3742	43	21%
NO_2	41	77.2	77.8	78.3	0.3	4064	46	94%
NO_2	147	79.2	79.6	79.9	0.2	3787	45	94%
NO_2	226	78.1	78.6	79.2	0.3	3977	44	94%
O_2	21%	79.6	80.1	80.6	0.3	5289	48	21%
O_2	94%	78.5	78.9	79.5	0.3	4123	47	94%
NO_2	109	76.3	77.0	77.6	0.4	4648	49	70%
O_2	70%	77.5	77.8	78.3	0.2	4246	50	70%

Relative Humidity

Test Chemical	Concentration *(mg/m³)*	*Min (%RH)*	*Mean (%RH)*	*Max (%RH)*	*SD (%RH)*	*(N)*	*Run #*	*O_2*
IPA	6700	1.7	1.8	2.0	0.1	4,194	1	21%
IPA	9516	1.7	1.8	1.9	0.1	4,438	2	21%
IPA	12856	1.6	1.8	1.9	0.1	4,438	3	21%
IPA	26483	1.6	2.1	2.5	0.3	4,122	4	21%
IPA	45488	1.5	1.9	2.4	0.3	4,037	5	21%
IPA	6676	0.0	1.3	3.1	1.4	4,311	6	94%
IPA	9478	0.0	1.1	2.8	1.2	4,703	7	94%
IPA	13866	0.0	1.0	2.9	1.1	4,235	8	94%
IPA	27417	0.0	1.2	2.8	1.2	4,369	9	94%
IPA	48834	0.0	1.1	2.4	1.1	4,785	10	94%
TMB	164	1.8	1.9	2.0	0.02	3897	15	21%
TMB	295	1.9	1.9	2.0	0.03	4314	13	21%
TMB	503	1.9	2.0	2.0	0.03	4112	14	21%
TMB	763	1.9	1.9	2.0	0.03	4107	12	21%
TMB	1398	1.9	2.0	2.1	0.04	4099	11	21%
TMB	818	0.0	1.2	3.3	1.4	4115	16	94%
TMB	204	0.0	1.0	2.6	1.2	4291	20	94%
TMB	329	0.0	1.1	2.6	1.2	4384	18	94%
TMB	463	0.0	1.1	2.8	1.3	4288	17	94%
TMB	1306	0.0	1.1	2.7	1.2	4502	19	94%
CBA	142	2.2	2.5	2.9	0.1	3897	22	21%
CBA	320	2.6	2.9	4.8	0.2	3783	21	21%
CBA	586	2.0	2.3	2.4	0.1	3936	23	21%
CBA	913	1.9	2.1	2.2	0.1	4280	25	21%
CBA	1188	2.0	2.1	2.2	0.1	4084	24	21%
CBA	146	0.0	0.7	2.2	0.9	3880	29	94%
CBA	304	0.0	0.7	2.2	1.0	3746	30	94%
CBA	525	0.0	0.8	2.2	1.0	4157	28	94%
CBA	768	0.0	1.0	2.6	1.1	4055	26	94%
CBA	1368	0.0	0.7	2.2	1.0	3809	27	94%
Naph	9.4	2.8	7.0	11.5	3.9	3522	33	21%
Naph	7.4	2.9	3.5	5.3	0.7	3172	35	21%
Naph	16.3	3.3	4.6	16.8	1.9	3314	34	21%
Naph	17.8	2.8	6.8	10.8	3.5	3350	32	21%
Naph	41.4	3.2	3.6	4.3	0.3	3437	31	21%
Naph	0	0.0	1.3	3.0	1.4	4041	37	94%
Naph	1.73	0.0	1.0	3.3	1.4	3714	36	94%
Naph	1.8	0.0	1.3	3.3	1.5	3564	39	94%
Naph	9.4	0.0	1.1	3.0	1.4	3375	40	94%
Naph	43	0.0	1.4	3.0	1.4	4246	38	94%
NO_2	53	2.5	4.9	7.8	2.2	4272	42	21%
NO_2	158	2.5	6.3	10.6	3.7	3747	41	21%
NO_2	260	3.8	5.2	8.0	1.7	3742	43	21%
NO_2	41	0.6	4.4	6.8	2.0	4064	46	94%
NO_2	147	0.0	2.1	6.0	2.7	3787	45	94%
NO_2	226	0.1	3.4	9.8	3.0	3977	44	94%
O_2	94%	0.0	1.3	3.3	1.5	4123	47	94%
O_2	21%	2.0	2.5	2.9	0.2	5289	48	21%
NO_2	109	0.9	3.0	10.9	2.5	4648	49	70%
O_2	70%	0.6	1.6	3.2	1.0	4246	50	70%

Static Pressure

Test Chemical	Concentration *(mg/m³)*	Max *("H₂O)*	Mean *("H₂O)*	Min *("H₂O)*	SD *("H₂O)*	*(N)*	*Run #*	*O₂*
IPA	6700	-0.14	0.01	0.09	0.01	4,194	1	21%
IPA	9516	-0.19	-0.03	0.24	0.02	4,438	2	21%
IPA	12856	-0.14	-0.04	0.24	0.03	4,438	3	21%
IPA	26483	-0.09	-0.03	0.08	0.02	4,122	4	21%
IPA	45488	-0.09	-0.04	0.08	0.01	4,037	5	21%
IPA	6676	-0.31	-0.06	0.25	0.02	4,311	6	94%
IPA	9478	-0.46	-0.05	0.39	0.06	4,703	7	94%
IPA	13866	-1.61	-0.05	0.64	0.11	4,235	8	94%
IPA	27417	-0.49	-0.09	0.25	0.08	4,369	9	94%
IPA	48834	-2.48	-0.04	0.46	0.07	4,785	10	94%
TMB	164	-0.15	-0.06	0.12	0.01	3,897	15	21%
TMB	295	-0.12	-0.05	0.12	0.01	4,314	13	21%
TMB	503	-0.12	-0.06	0.20	0.01	4,112	14	21%
TMB	763	-0.09	-0.07	0.20	0.01	4,107	12	21%
TMB	1,398	-0.82	-0.05	0.17	0.04	4,099	11	21%
TMB	818	-0.28	-0.07	0.06	0.03	4,115	16	94%
TMB	204	-0.19	-0.05	0.35	0.04	4,291	20	94%
TMB	329	-0.17	-0.06	0.35	0.05	4,384	18	94%
TMB	463	-0.33	-0.07	0.22	0.03	4,288	17	94%
TMB	1,306	-0.57	-0.07	0.25	0.04	4,502	19	94%
CBA	142	-2.49	-0.32	1.64	0.67	3,897	22	21%
CBA	320	-2.50	-0.07	2.50	0.24	3,783	21	21%
CBA	586	-2.40	-0.14	2.04	1.03	3,936	23	21%
CBA	913	-0.14	-0.04	0.85	0.03	4,280	25	21%
CBA	1,188	-0.16	-0.05	0.75	0.03	4,084	24	21%
CBA	146	-0.47	-0.06	2.49	0.13	3,880	29	94%
CBA	304	-0.38	-0.06	1.24	0.08	3,746	30	94%
CBA	525	-0.75	-0.09	1.70	0.13	4,157	28	94%
CBA	768	-0.85	-0.07	1.33	0.21	4,055	26	94%
CBA	1,368	-1.03	-0.08	2.50	0.16	3,809	27	94%
Naph	9.4	-0.11	-0.04	0.23	0.04	3,522	33	21%
Naph	7.4	-0.57	-0.08	1.07	0.07	3,172	35	21%
Naph	16.3	-2.50	-0.33	0.89	0.38	3,314	34	21%
Naph	17.8	-0.15	-0.06	0.22	0.02	3,350	32	21%
Naph	41.4	-0.10	-0.03	0.26	0.02	3,437	31	21%
Naph	0.0	-0.88	-0.08	0.77	0.15	4,041	37	94%
Naph	1.7	-2.50	-0.08	1.02	0.17	3,714	36	94%
Naph	1.8	-0.31	-0.03	1.39	0.10	3,564	39	94%
Naph	9.4	-0.85	-0.07	0.79	0.07	3,375	40	94%
Naph	43.0	-1.18	-0.19	1.02	0.16	4,246	38	94%
NO_2	53	-0.47	-0.23	0.51	0.07	4,272	42	21%
NO_2	158	-0.61	-0.40	0.49	0.05	3,747	41	21%
NO_2	260	-0.60	-0.10	0.70	0.10	3,742	43	21%
NO_2	41	-0.70	-0.10	1.20	0.16	4,064	46	94%
NO_2	147	-2.50	-0.15	1.51	0.27	3,787	45	94%
NO_2	226	-2.50	-0.10	1.65	0.69	3,977	44	94%
O_2	94%	-1.23	-0.09	1.23	0.19	4,123	47	94%
O_2	21%	-0.27	-0.22	0.01	0.06	5,289	48	21%
NO_2	109	-2.50	-0.16	0.88	0.28	4,648	49	70%
O_2	70%	-1.03	-0.17	0.56	0.07	4,246	50	70%

Oxygen Concentration

Test Chemical	Concentration *(mg/m³)*	Min *(%O_2)*	Mean *(%O_2)*	Max *(%O_2)*	SD *(%O_2)*	*(N)*	*Run #*	*O_2*
IPA	6700	-	-	-	-	-	1	21%
IPA	9516	-	-	-	-	-	2	21%
IPA	12856	-	-	-	-	-	3	21%
IPA	26483	19.2	20.2	21.7	0.81	4,122	4	21%
IPA	45488	18.4	19.8	21.8	1.18	4,037	5	21%
IPA	6676	90.0	90.5	92.7	0.37	1,800	6	94%
IPA	9478	90.3	91.7	93.3	0.37	1,800	7	94%
IPA	13866	90.5	90.7	93.3	0.44	1,800	8	94%
IPA	27417	85.3	86.2	90.6	0.52	1,800	9	94%
IPA	48834	84.0	84.6	94.3	1.37	1,800	10	94%
TMB	164	20.7	20.8	20.9	0.007	3,897	15	21%
TMB	295	20.7	20.8	20.8	0.010	4,314	13	21%
TMB	503	20.7	20.8	20.8	0.019	4,112	14	21%
TMB	763	20.7	20.8	21.0	0.048	4,107	12	21%
TMB	1,398	20.6	20.8	21.1	0.060	4,099	11	21%
TMB	818	89.4	89.9	93.2	0.410	1,800	16	94%
TMB	204	87.9	93.5	93.8	0.504	2,380	20	94%
TMB	329	87.8	93.5	93.7	0.525	2,382	18	94%
TMB	463	78.4	87.9	89.7	1.3	2,363	17	94%
TMB	1,306	91.9	93.6	93.9	0.163	2,379	19	94%
CBA	142	-	-	-	-	-	22	21%
CBA	320	-	-	-	-	-	21	21%
CBA	586	-	-	-	-	-	23	21%
CBA	913	20.2	20.8	21.1	0.07	4,280	25	21%
CBA	1,188	19.9	20.8	21.3	0.11	4,084	24	21%
CBA	146	92.7	93.0	94.0	0.30	1,800	29	94%
CBA	304	92.3	93.7	93.8	0.01	1,800	30	94%
CBA	525	92.2	92.5	93.6	0.20	1,800	28	94%
CBA	768	90.0	90.3	92.2	0.12	1,800	26	94%
CBA	1,368	90.0	93.4	93.7	0.23	1,800	27	94%
Naph	9.4	20.7	20.7	20.7	-	3,522	33	21%
Naph	7.4	20.7	20.7	20.8	0.01	3,172	35	21%
Naph	16.3	20.5	20.7	20.8	0.01	3,314	34	21%
Naph	17.8	20.7	20.7	20.7	-	3,350	32	21%
Naph	41.4	20.7	20.7	20.7	-	3,437	31	21%
Naph	0.0	90.5	93.8	93.8	0.16	1,740	37	94%
Naph	1.7	93.1	93.3	93.5	0.11	1,440	36	94%
Naph	1.8	91.2	93.7	93.8	0.23	1,740	39	94%
Naph	9.4	91.4	93.8	93.8	0.16	1,799	40	94%
Naph	43.0	90.6	93.8	93.8	0.22	1,739	38	94%
NO_2	53	20.6	20.7	20.7	0.02	1,740	42	21%
NO_2	158	20.5	20.5	20.7	0.01	1,799	41	21%
NO_2	260	20.4	20.5	20.7	0.13	1,167	43	21%
NO_2	41	84.0	84.5	93.6	1.28	1,799	46	94%
NO_2	147	84.9	93.0	93.1	0.55	1,799	45	94%
NO_2	226	86.6	91.3	93.3	0.35	1,808	44	94%
O_2	94%	93.7	93.8	93.8	0.01	1,799	47	94%
O_2	21%	20.7	20.7	20.7	-	1,799	48	21%
NO_2	109	68.6	70.4	71.1	0.30	1,380	49	70%
O_2	70%	70.5	70.6	70.7	0.06	1,500	50	70%

References

(2019) ASTM International Designation: E981-19. West Conshohocken, PA.

Alarie Y (1981) Toxicological evaluation of airborne cemical irritants and allergens using respiratory reflex reactions. Ann Arbor, MI: Ann Arbor Science Publications.

Armitage P (1971) Statistical methods in medical research. New York,: Wiley.

Baldwin RM, Shultz MA, Buckpitt AR (2005) Bioactivation of the pulmonary toxicants naphthalene and 1-nitronaphthalene by rat CYP2F4. J Pharmacol Exp Ther 312:857-865.

Baldwin RM, Jewell WT, Fanucchi MV, Plopper CG, Buckpitt AR (2004) Comparison of pulmonary/nasal CYP2F expression levels in rodents and rhesus macaque. J Pharmacol Exp Ther 309:127-136.

Bruning T, Bartsch R, Bolt HM, Desel H, Drexler H, Gundert-Remy U, Hartwig A, Jackh R, Leibold E, Pallapies D, Rettenmeier AW, Schluter G, Stropp G, Sucker K, Triebig G, Westphal G, van Thriel C (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88:1855-1879.

Buckpitt A, Boland B, Isbell M, Morin D, Shultz M, Baldwin R, Chan K, Karlsson A, Lin C, Taff A, West J, Fanucchi M, Van Winkle L, Plopper C (2002) Naphthalene-induced respiratory tract toxicity: metabolic mechanisms of toxicity. Drug Metab Rev 34:791-820.

Korsak Z, Swiercz R, Rydzynski K (1995) Toxic effects of acute inhalation exposure to 1,2,4-trimethylbenzene (pseudocumene) in experimental animals. Int J Occup Med Environ Health 8:331-337.

Lanosa MJ, Willis DN, Jordt S, Morris JB (2010) Role of metabolic activation and the TRPA1 receptor in the sensory irritation response to styrene and naphthalene. Toxicol Sci 115:589-595.

Lewis DF, Ito Y, Lake BG (2009) Molecular modelling of CYP2F substrates: comparison of naphthalene metabolism by human, rat and mouse CYP2F subfamily enzymes. Drug Metabol Drug Interact 24:229-257.

Li L, Carratt S, Hartog M, Kovalchik N, Jia K, Wang Y, Zhang QY, Edwards P, Winkle LV, Ding X (2017) Human CYP2A13 and CYP2F1 Mediate Naphthalene Toxicity in the Lung and Nasal Mucosa of CYP2A13/2F1-Humanized Mice. Environ Health Perspect 125:067004.

Lindvall T (1985) Health effects of nitrogen dioxide and oxidants. Scand J Work Environ Health 11 Suppl 3:10-28.

Muller WJ, Schaeffer VH (1996) A strategy for the evaluation of sensory and pulmonary irritation due to chemical emissions from indoor sources. J Air Waste Manag Assoc 46:808-812.

Mumy KL (2019) NAVAIR/RCCA meeting, “Aircraft Chemicals of Concern”. In Patuxent River, MD, USA.

Nielsen GD, Yamagiwa M (1989) Structure-activity relationships of airway irritating aliphatic amines. Receptor activation mechanisms and predicted industrial exposure limits. Chem Biol Interact 71:223-244.

NIOSH (The National Institute for Occupational Safety and Health) (2021). United States Department of Labor: Occupation Safety and Health Administration: Nitrogen Dioxide. Accessed 08/17/2021. https://www.osha.gov/chemicaldata/21

NIST Mass Spec Data Center SES, director. NIST Chemistry WebBook, NIST Standard Reference Database Number 69. In. Gaithersburg, MD: National Institute of Standards and Technology.

Plopper CG, Suverkropp C, Morin D, Nishio S, Buckpitt A (1992) Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. J Pharmacol Exp Ther 261:353-363.

Ritchie GD, Still KR, Alexander WK, Nordholm AF, Wilson CL, Rossi J, 3rd, Mattie DR (2001) A review of the neurotoxicity risk of selected hydrocarbon fuels. J Toxicol Environ Health B Crit Rev 4:223-312.

Ruth JH (1986) Odor thresholds and irritation levels of several chemical substances: a review. Am Ind Hyg Assoc J 47:A142-151.

Smeets M, Dalton P (2002) Perceived odor and irritation of isopropanol: a comparison between naive controls and occupationally exposed workers. Int Arch Occup Environ Health 75:541-548.