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**HISTOPATHOLOGY RESULTS FOR ACUTE AND TWO-WEEK
INHALATION TOXICITY STUDIES WITH POLYALPHAOLEFIN
(PAO) FLUID IN RATS**

**Matthew D. Wegner
Armed Forces Research Institute of Medical Sciences
315/6 Rajvithi Rd, Bangkok 10400 THAILAND**

**Teresa R. Sterner
Henry M. Jackson Foundation
for the Advancement of Military Medicine
Molecular Mechanisms Branch
2728 Q St, Bldg 837
Wright-Patterson AFB OH 45433**

**Karen L. Mumy
Naval Medical Research Unit Dayton
2624 Q St, Bldg 851
Wright-Patterson AFB OH 45433**

**Brian A. Wong
Henry M. Jackson Foundation
for the Advancement of Military Medicine
Naval Medical Research Unit Dayton
2624 Q St, Bldg 851
Wright-Patterson AFB OH 45433**

**David R. Mattie
Human-Centered ISR Division
Molecular Mechanisms Branch**

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711TH HUMAN PERFORMANCE WING
AIRMAN SYSTEMS DIRECTORATE
HUMAN-CENTERED ISR DIVISION
MOLECULAR MECHANISMS BRANCH
WRIGHT-PATTERSON AFB OH 45433**

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DAVID R. MATTIE, Work Unit Manager
Molecular Mechanisms Branch
Airman Systems Directorate
711th Human Performance Wing
Air Force Research Laboratory

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LANCE N. DOVER, DR-III, DAF
Chief, Human-Centered ISR Division
Airman Systems Directorate
711th Human Performance Wing
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14. ABSTRACT Polyalphaolefin (PAO) fluids are being utilized as heat sink coolants for aircraft service air and electronic components. Available literature studies for PAO are inadequate to set exposure limits. An acute inhalation study examining the toxicity of PAO fluid in male and female Fischer 344 rats (0, 100, 500 and 1000 mg/m ³ aerosol for 6 hours) resulted in the 500 and 1000 mg/m ³ exposed rats displaying lethargy. Decreases in food and water consumption resulted in decreased body weights and skewed organ to body weight ratios in the 500 and 1000 mg/m ³ acute exposure groups. A 14-day recovery cohort indicated that these effects did not persist after 24 hours. A repeated inhalation study with 0, 20, 100 and 300 mg/m ³ PAO aerosol (6 hours/day, 5 days/week, 2 weeks) showed no significant effects. A preliminary no observable adverse effect concentration (NOAEC) was set at 300 mg/m ³ aerosol. Subsequently, histopathology results were received for the tissues collected (nasal airways, trachea, larynx, lungs, liver, kidneys, spleen, adrenals, brain and heart). Histopathology immediately post exposure for the acute study showed a dose response increase in severity (minimal to marked) of lesions in the posterior nasal cavities and lungs. Severity of lesions was reduced in the recovery cohort groups (normal to minimal). Histopathology for the two-week study showed lesions only in the posterior nasal cavities and lungs of the high dose group with minimal to mild severity scores. These results indicate that acute effects were short-lived and recoverable. The updated NOAEC for PAO aerosol is 100 mg/m ³ .					
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PREFACE

Support for the research described herein was provided through the Aerospace Toxicology Program in the Air Force Research Laboratory, 711th Human Performance Wing, Airman Systems Directorate, Bioeffects Division, Molecular Mechanisms Branch, 711 HPW/RHXJ (formerly Molecular Bioeffects Branch, 711 HPW/RHDJ) at Wright-Patterson Air Force Base (AFB) OH.

This research was conducted under cooperative agreement FA8650-15-2-6608 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) and Navy work unit number H1320 under the management of Naval Medical Research Unit - Dayton (NAMRU-D). The program manager for HJF was David R. Mattie, PhD (711 HPW/RHXJ), who was also the technical manager for this project. The technical manager for NAMRU-D was Karen L. Mumy, PhD.

The acute study without recovery was designed to be in compliance with the U.S. Environmental Protection Agency (EPA) guideline 870.1300, Acute Inhalation Toxicity (U.S. EPA, 1998a) and the Organisation for Economic Cooperation and Development (OECD) guideline, Test No. 403: Acute Inhalation Toxicity (OECD, 2009). The two-week study was designed to comply with OECD guideline, Test No. 412: Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (OECD, 1981). The neurotoxicity assays in this study follow U.S. EPA 870.6200 Neurotoxicity Screening Battery (U.S. EPA, 1998b).

This study was not performed in a Good Laboratory Practice (GLP) Standards certified laboratory, and therefore there is no certification of compliance with GLP regulations (40 CFR Part 792). However, this study was conducted with an effort to follow the intent and purpose of GLP requirements.

The protocol “Toxicity Studies in Rats (*Rattus norvegicus*) and in the Mouse (*Mus musculus*) for Polyalphaolefin (PAO) Fluid” was approved by the Wright-Patterson AFB Installation Animal Care and Use Committee (IACUC) as protocol number F-WA-2014-0154. The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). The study was performed in compliance with DODI 3216.1.

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

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1.0 SUMMARY

Formal occupational exposure limits (OELs) for polyalphaolefin (PAO) fluids have not yet been proposed. These fluids can be used as aircraft hydraulic fluid components and can be major constituents of heat sink coolant fluids for aircraft service air systems and electronics. These studies compared toxicity of a PAO fluid formulation (80 to 99.5 percent hydrogenated 1-decene dimer with a proprietary additive package (0.5 to 20 percent)) in male and female Fischer 344 rats by acute inhalation (aerosol exposures at 0, 100, 500 and 1000 mg/m³ for six hours) and two-week inhalation (0, 20, 100 and 300 mg/m³ for six hours per day, five days per week for two weeks). Food, water and body weights were monitored for all animals. Functional observational battery (FOB) and motor activity (MA) assessments were conducted on the animals following the six-hour acute exposure and two weeks after exposure in a recovery cohort. In the two-week study, FOB and MA measurements were conducted following the ninth and tenth six-hour exposures, respectively. Blood was collected during necropsy for clinical chemistry and hematology. Tissues were collected for histopathology following exposures and recovery: nasal airways (four levels), trachea, larynx, lungs (three levels), liver (two levels), both kidneys, spleen, both adrenal glands, brain and heart.

Histopathology results from the acute cohort immediately following exposure indicated a dose response increase in severity (minimal to mild) of lesions in the posterior nasal cavities and lungs. Severity of lesions was reduced in the 14-day recovery cohort, ranging from normal to minimal.

Histopathology for the two-week exposure showed lesions only in the posterior nasal cavities and lungs of the high exposure concentration group, with severity less than in the acute cohort intermediate and high concentration groups.

These results indicate that acute effects of PAO exposure are short-lived and recoverable. Short-term repeated exposure does not result in any cumulative effects except for minimal respiratory tract changes in the 300 mg/m³ group. Since shorter term operational exposure limits (OpELs) are needed to cover times that are more consistent with Air Force operations, Acute Exposure Guideline Level (AEGLs) were proposed for interim use by the Air Force. The data from the acute study were used to develop OpELs for the PAO coolant. Using the six-hour exposure for concentration and time, the no observed adverse effect concentration for histopathological effects seen in the respiratory tract (100 mg/m³), and uncertainty factors totaling 30, AEGL-1 values of 36, 36, 18, 4.5 and 1.7 mg/m³ were proposed for 10 and 30 minutes and one, four, and eight-hour exposures to this PAO formulation, respectively. These preliminary OpELs should be protective for inhalation effects from PAO aerosol exposures that could compromise Air Force missions and the health of the warfighter.

2.0 INTRODUCTION

PAO fluids are used as the chemical base stock in hydraulic fluids and are the primary component of heat sink coolants for high performance aircraft service air systems and electronics. In the F-22, the engine service air, after being cooled in heat sink units containing commercial PAO formulations, is used by the on board oxygen generator system (OBOGS) to provide oxygen to the pilot at altitude (Martin *et al.*, 2012).

PAOs have become a potential contaminant of concern for the U.S. Air Force (USAF). The PAO originating from the heat sink units has been found as a contaminant throughout the F-22 oxygen system in both ground and flight tests. Further, F-22 pilots have experienced unexplained physiological events during flight. Those incidences have been investigated as potentially having a neurological origin. Without sufficient data on contaminants such as PAOs, neurotoxicity of these fluids is an ongoing concern for the USAF (Martin *et al.*, 2012).

F-18 Navy Hornets and Marine Growlers also use OBOGS. These pilots have also experienced numerous unexplained physiological events (Freedberg, 2016; Myers, 2016). Therefore, there is cross-service interest in PAO toxicity information (John Krohn, personal communication).

Studies designed to facilitate the estimation of human OpELs for PAO formulations are necessary to assure safety and operational performance during exposure of pilots and maintainers of high performance aircraft. Acute and short-term tests are required due to the acute, repeat exposures of pilots and maintainers; these exposures differ from the common continuous eight-hour exposure investigated for a factory occupational setting. Preliminary evidence suggested acute (one to four hours) exposure to a PAO fluid caused lung irritation in rats (John Hinz, personal communication).

The studies described herein take into consideration the unique exposures and concerns for the USAF pilot. The acute study without recovery is based on the U.S. Environmental Protection Agency (EPA) guideline 870.1300, Acute Inhalation Toxicity (USEPA, 1998a) and the Organisation for Economic Cooperation and Development (OECD) guideline, Test No. 403: Acute Inhalation Toxicity (OECD, 2009). The acute study with recovery is a modification of the study design used by Mattie *et al.* (2012) to better address the acute exposure to the hydroprocessed esters and fatty acids - mixed fats jet fuel. The two-week study design is based on OECD guideline, Test No. 412: Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (OECD, 1981).

Formal occupational exposure limits (OELs) for polyalphaolefin (PAO) fluids have not been proposed. There are insufficient published literature studies to support proposing a DoD operational exposure limit (OpEL) from documented sources. An OpEL is simply defined as a data driven value calculated with the intent to protect the safety of Airmen when an OEL is unavailable. The data from these studies were used to develop OpELs for the PAO coolant (Mattie *et al.*, 2017).

2.1 Objective

The objective of this project was to conduct two inhalation studies with a PAO fluid meeting the Military Specification MIL-PRF-87252C for use in aircraft heat sink coolant systems. Data generated were utilized to propose OpELs for DoD use. To that end, an acute study was designed to investigate the inhalation toxicity of the PAO fluid by inhalation exposure at three concentrations (100, 500, and 1000 mg/m³), plus a zero clean air control level, to Fischer 344 rats (10 rats per sex per exposure group) for a single six-hour exposure. Endpoints included neurobehavioral testing, gross pathology, clinical chemistry, hematology, and histopathology. A second set of 80 animals was exposed at the same concentrations and then allowed recovery for two weeks prior to measurement of the same endpoints in order to permit evaluation of effects that may have persisted beyond the immediate exposure.

Results from the acute study were utilized to set exposure concentrations for the two-week study (six hours per day, ten exposures). Fischer 344 rats (10 rats per sex per exposure group) were exposed to 0, 20, 100, and 300 mg/m³ PAO fluid aerosol. Neurobehavioral testing was performed immediately following the ninth and tenth exposures, and necropsy, including general pathology, clinical chemistry, hematology and histopathology, occurred the following day.

Results from most endpoints from the two inhalation studies, including neurobehavioral testing, gross pathology, clinical chemistry and hematology, were reported in Mattie *et al.* (2017). This technical report fully discusses the histopathology results from both inhalation studies.

3.0 METHODS

Methods for both the acute and two-week studies are found in Mattie *et al.* (2017), including excerpts from the animal use protocol. This report concentrates on the histopathology methods employed.

3.1 Chemical

PAO fluids utilized in the environmental control systems of aircraft to cool the air for the OBOGS consist primarily of hydrogenated 1-decene dimers (Figure 1). The PAO formulation used was supplied by Radco Industries, Inc. (Batavia IL). The safety data sheet for this product can be found in Mattie *et al.* (2017). This product contains a 0.5 to 20 percent proprietary additive package. Use of a commercial name and product does not constitute endorsement of the product by the U.S. Air Force, U.S. Navy, or U.S. Army. This PAO formulation is currently in use aboard the F-22 and is believed to be representative of PAO coolants in this class that also meet MIL-PRF-87252C(1) specifications.

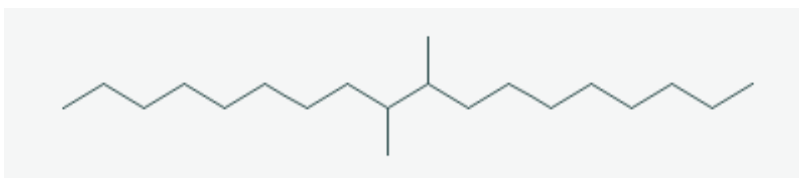


Figure 1: Hydrogenated 1-Decene Dimer

Chemical structure of main component in PAO fluids

3.2 Inhalation Exposures

In brief, Fischer 344 rats (Charles River Laboratories, International, Kingston NY) were exposed to aerosolized PAO fluid in acute and two-week studies. Rats were exposed in wire mesh cages in a glass and stainless steel whole-body exposure chamber (model H1000, Lab Products, Seaford DE) with a volume of 1-cubic meter. Four chambers were used, one for each exposure group. PAO fluid is non-volatile; the exposure atmosphere was in an aerosol form, with very little or no vapor. Concentrations in each of the four exposure chambers were measured using a 47 mm in-line gravimetric filter (In-Tox Products, Moriarty NM). An optical aerosol monitor was used to observe the stability of the concentration inside each of the chambers. This real-time aerosol monitor (DataRAM4, Thermo Scientific, subsidiary of Thermo Fisher Scientific, Waltham MA) measured aerosol concentration continuously and recorded a reading approximately every two minutes, which provided a near-instantaneous reading of the aerosol concentration and allowed for adjustments as necessary. Particle size measurements for the test atmosphere was taken once a day for the low, intermediate, and high exposure chambers. The

particle size distribution was measured using a seven stage cascade impactor (In-Tox Products). The uniformity of aerosol concentration was checked using the DataRam4. This real-time monitor was used to sample from nine locations (center and eight corner areas) after atmosphere generation had stabilized in each exposure chamber.

In the acute study, ten male and ten female rats per cohort were exposed to 0, 100, 500, or 1000 mg/m³ aerosolized PAO fluid for six hours. Animals were arranged in five replicates per exposure group to accommodate neurobehavioral testing and each exposure group had two cohorts. The acute cohort of each exposure group was tested in the functional observational battery (FOB) and motor activity (MA) assessments within two hours following exposure. All rats in this cohort were euthanized one day following exposure. The recovery cohort of each exposure group were run through the FOB and MA assessments 13 days following exposure and euthanized 14 days following exposure. All animals were euthanized in accordance with current American Veterinary Medical Association (AVMA) guidelines (AVMA, 2013).

In the two-week study, two replicates of 40 male or 40 female rats were exposed in order to accommodate neurobehavioral testing at the end of the exposure regimen. Groups of ten male and ten female rats per group were exposed to 0, 20, 100, or 300 mg/m³ aerosolized PAO fluid for six hours per day, five days per week for two weeks. FOB was assessed immediately following the 9th exposure and MA after the 10th exposure. Rats were euthanized in accordance with current AVMA guidelines (AVMA, 2013) one day following the 10th exposure.

3.3 Neurobehavioral Assessment

Neurobehavioral assessments are described in detail in the U.S. EPA Guideline 870.6200: Neurotoxicity Screening Battery (U.S. EPA, 1998b). The MA and FOB in this study are described in Mattie *et al.* (2017).

3.4 Necropsy, Clinical Chemistry and Hematology

Prior to scheduled necropsy, the rats were fasted overnight. Water was available *ad libitum*. The rats were weighed, anesthetized, and euthanized in accordance with current AVMA guidelines (AVMA, 2013).

Following gross necropsy, the rats were dissected and select tissues, including the brain, heart, both kidneys, both adrenal glands, liver (2 levels), spleen, lungs (3 levels), larynx and trachea, were processed, embedded, cut and mounted onto slides. The skull, saved for examination of the nasal tissues, and the slides were shipped to the pathology staff at 711 HPW/RHDV (Fort Sam Houston TX). There, the nasal cavities were sectioned according to Young (1981). Nasal cavity and lung sections are identified in Figure 2. All tissues from the acute and repeated exposure studies were read by a board certified pathologist.

Clinical chemistry and hematology details are described in Mattie *et al.* (2017).

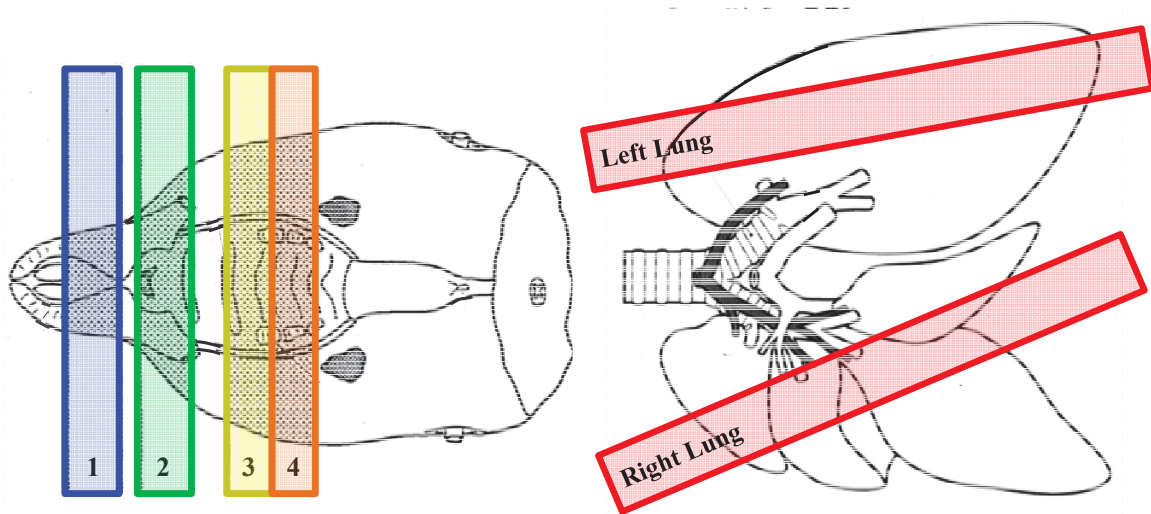


Figure 2: Nasal Cavity and Lung Tissue Sections used for Histopathology

Nasal cavity drawing (left) indicates nasal levels NL1 through NL4 (adapted from Young, 1981). Lung drawing (right) indicates sections taken from both right and left lungs (adapted from Nawata et al., 1997).

3.5 Statistical Analysis

As described by Mattie *et al.* (2017), statistical differences were assessed based on calculations between exposure groups, not replicates within exposure groups. Histopathological incidence data were compared using the appropriate statistical method, generally the Chi-Square test. Statistical analyses were performed using Sigma Plot software (Systat Software, Inc., San Jose CA). The probability value of less than 0.05 was used as the critical level of significance for each statistical test, except in the Chi-Square test, for which probability values of less than 0.001 and 0.01 were utilized.

4.0 ACUTE INHALATION STUDY RESULTS

4.1 Exposure Conditions

Complete exposure concentration information can be found in Mattie *et al.* (2017). Average group concentrations were within 2 percent of the target concentration. Replicate concentrations fell within 2.5 percent of the target concentrations. Aerosols were respirable (1.8 to 2.6 μm) and uniform between the replicates.

4.2 Overall Effects

4.2.1. Neurobehavioral Effects

In the acute study, MA and FOB measures in the acute cohort indicated significant neurobehavioral changes in the exposure groups as compared to the controls for both males and female rats. In the MA assessment, male and female animals exposed to either the intermediate or high concentration PAO were significantly less active than control animals in the total time travelled metric. Ambulatory beam breaks (a measure of motion including walking, head swings, etc.), and vertical beam breaks (rearing, a measure of exploratory behavior) were also significantly decreased among the intermediate and high concentration exposed animals, as compared to controls. For most FOB assessments, including cage side, open field and manipulation tests, no exposure group-related effects were reported for the males and female rats. Only the handling reactivity observations were different between exposure groups in the acute cohort. Both male and female rats exposed to the highest PAO concentration generally appeared more passive to handling than control animals. This effect was also observed in some of the males and females exposed to the intermediate concentration of PAO in the acute cohort.

The 14-day recovery cohort did not display significant differences between exposure groups in either neurobehavioral assay. Complete results for both cohorts are found in Mattie *et al.* (2017).

4.2.2. General Effects

Relative body weight change data were calculated using the exposure event as the reference time point (i.e., relative change is equal to the body weight on the day prior to exposure divided by the body weight on the exposure day). The weight ratio comparing post-exposure day 1 and the exposure day for the acute cohort showed a dose response decline. The ratio in the high concentration group animals was significantly smaller as compared to the ratio in both male and female control rats. The ratio was also smaller among the intermediate group animals, but was only significantly different than controls among the female of this group. Recovery cohort animals were assessed separately as they had not been fasted in preparation for necropsy at this time point. However, the results were similar. The weight ratio was smaller among both male and female rats in the high exposure group and among females in the intermediate exposure group on the day following exposure. By post-exposure day 14, the weight ratio data were not different between exposure groups.

Water consumption measured on the first post-exposure day was significantly decreased among the high-concentration males and females, and among the intermediate-concentration females. Water consumption measured on post-exposure day 2 indicated that water consumption was approximately the same as before exposure for all exposure groups.

Food consumption was significantly decreased on post-exposure day 1 among the high and intermediate concentration exposed males and females. Food measured on post-exposure day 2 was similar to the amount consumed before the exposure for all exposure groups.

Male and female rats in the high exposure group were shown to have increased relative brain to body weight ratios, as well as decreased liver to body weight and liver to brain weight ratios. Female rats in this exposure group also had decreased kidney to brain weights. Given that the recovery cohorts for both sexes indicated no differences between exposure groups, these acute changes are most likely due to the changes in body weight stemming from decreased food and water consumption among the high exposure group rats.

Blood samples were taken from the 14-day recovery cohort animals at the time of necropsy. There were no exposure group-related differences in any of the measured clinical chemistry parameters.

Some hematology parameters were flagged as statistically significant. The basophil counts and percentages in the males from the high concentration exposure group were low compared with controls. The number and percent of eosinophils in the low and intermediate exposure group females were statistically different from each other.

The number of platelets in the high exposure group males was increased compared with low exposure group males. The intermediate exposure group females had significantly more platelets than the low or high concentration females. However, these values bracketed the control animals, and were not statistically different from control platelet numbers. Complete data on these general effects can be found in Mattie *et al.* (2017).

4.3 Histopathology

The incidence and severity of effects for nasal cavity sections (nasal levels NL3, NL4) and lungs are shown in Table 1 and Figure 3; photos depicting changes in these sections are found in Figures 4 to 40. No effects were seen in the first (NL1) and second (NL2) sections of the nasal cavities; therefore these results were not included in the table nor discussed below. In general, lesions in the nasal cavity epithelium were restricted to olfactory epithelium with relative sparing of the respiratory epithelium. Complete results can be found in Appendix A.

Table 1: Incidence and Severity for Tissue Sections from Rats Exposed to PAO Aerosol in Acute Study with Recovery Cohort

Tissue Section	Acute Cohort				Recovery Cohort			
	Control (0 mg/m ³)	Low (100 mg/m ³)	Intermediate (500 mg/m ³)	High (1000 mg/m ³)	Control (0 mg/m ³)	Low (100 mg/m ³)	Intermediate (500 mg/m ³)	High (1000 mg/m ³)
Incidence								
NL3	0	0	16/20 (80%)	18/20 (90%)	0	0	1/20 (5%)	0
NL4	0	10/20 (50%)	20/20 (100%)	20/20 (100%)	0	1/20 (5%)	11/19 (58%)	6/20 (30%)
Lung	0	2/20 (10%)	18/20 (90%)	18/20 (90%)	0	1/20 (5%)	4/20 (20%)	8/20 (40%)
Severity								
NL3	0	0	1.10 ± 0.16	1.00 ± 0.10	0	0	0.05 ± 0.05	0
NL4	0	0.90 ± 0.22	3.20 ± 0.14	3.10 ± 0.10	0	0.05 ± 0.05	0.95 ± 0.24	0.50 ± 0.18
Lung	0	0.11 ± 0.08	1.21 ± 0.12	1.47 ± 0.16	0	0.05 ± 0.05	0.20 ± 0.09	0.45 ± 0.14

Notes: Nasal Level (NL) 1 and 2 had no incidence of lesions and are not shown in the table. Incidence units = number of rats with effects/n (% number of rats with effects); Severity units = mean ± standard error of the mean; Severity ratings: 0 = Normal, 1 = Minimal/Rare, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe

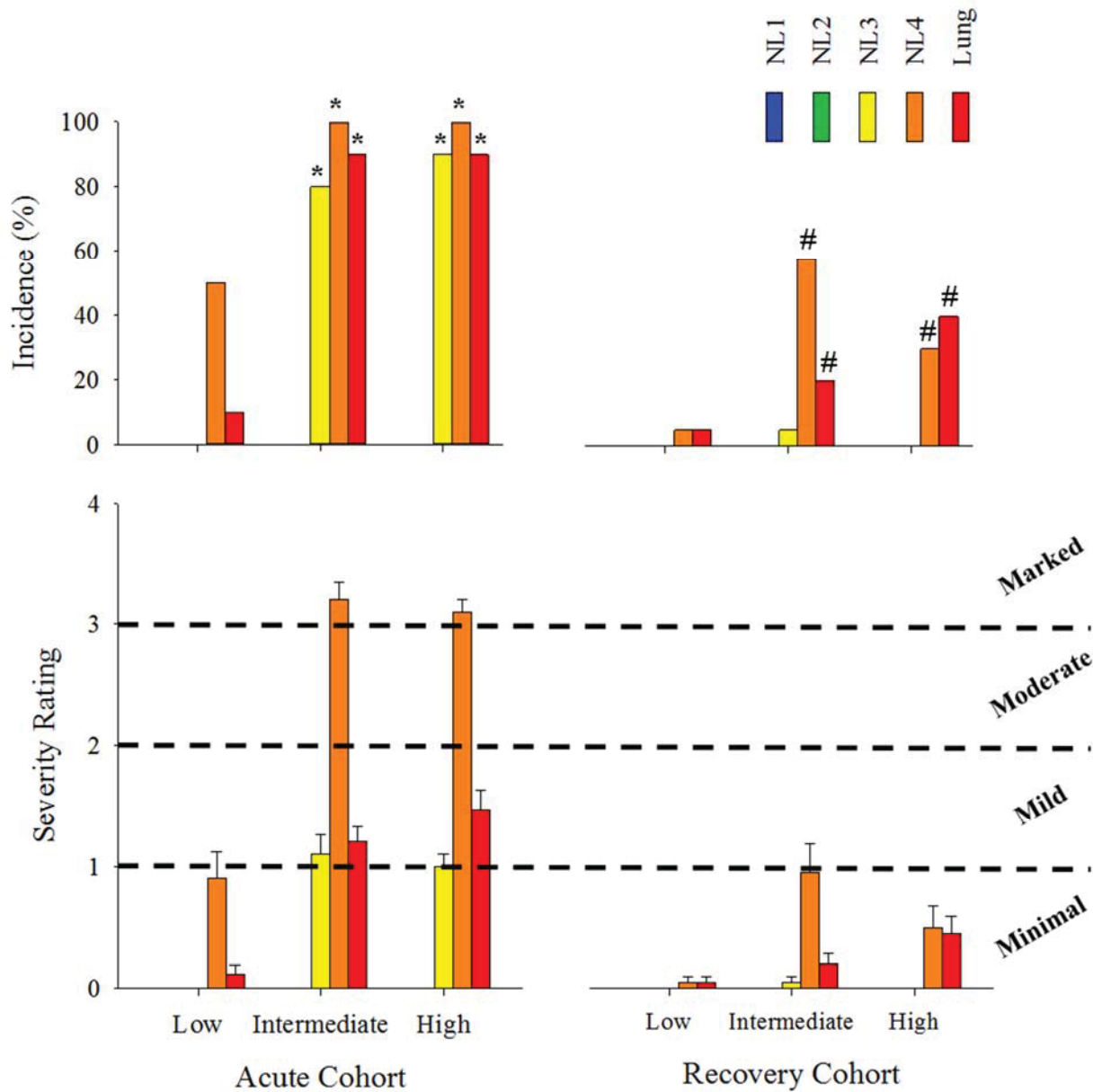


Figure 3: Histopathological Incidence and Severity of Nasal Tissues and Lungs for Male and Female Rats Combined

The drawings in Figure 2 indicate the nasal cavity tissue sections (NL1 through NL4) or lung tissue by corresponding color. No effects were seen in NL1 or NL2. Drawings were adapted from Young (1981) and Nawata et al. (1997), respectively. Significance markings indicate that exposure group and incidence are related ($p < 0.001$, # $p < 0.01$).*

4.3.1. Low Exposure Group - Nasal Cavities

In the nasal cavities of the acute cohort low exposure group rats, the only effects were seen in the most posterior section, NL4, as shown in Figure 5; control tissue from sham-exposed rat is shown in Figure 4 for comparison. Fifty percent of the rats presented with lesions that were, on average, minimal in severity (0.9 severity rating). However, in eight of the ten rats that displayed a lesion, the severity score was mild (severity = 2). Most lesions were classified as degeneration with necrosis (9 rats); a single rat developed only degeneration of the epithelium without necrotic evidence. Lesions were not limited to one site but were multifocal in all ten rats; lesions were more extensive in two of the rats with mild degeneration and necrosis. Two of the ten rats with lesions were rated as having only minimal multifocal degeneration and necrosis in the NL4 section of nasal cavities.

In the recovery cohort, the NL4 section of one rat was not available for analysis. Recovery was complete for 18 out of 19 rats. One rat still had minimal multifocal degeneration.

4.3.2. Low Exposure Group – Lungs

In the lungs of acute cohort low exposure group rats, two of 18 rats had mononuclear cell inflammation that was focal in one rat and multifocal in the other, with a resulting mean severity score of 0.11. Two additional rats in this exposure group (2 of 20) presented with multifocal hemorrhages consistent with euthanasia unrelated to PAO exposure.

In the recovery cohort, only one rat had an effect that was a single area of minimal ossification. As this was a focal effect in a recovery group, the only occurrence in the entire acute study, and is a noted background lesion in F344 rats (Renne *et al.*, 2009), the relevance to PAO exposure is questionable. There was no incidence of inflammation, hemorrhage, necrosis or degeneration in the lungs of the low exposure group recovery group rats.

4.3.3. Intermediate Exposure Group - Nasal Cavities. In acute cohort rats exposed to the intermediate concentration of PAO, effects were seen in both of the posterior sections of the nasal cavities, NL3 and NL4. In the NL3 section, the controls were normal (Figure 6) and multifocal degeneration with necrosis (Figure 7) was seen in 80 percent (16 out of 20) of the rats with a mean severity score of 1.1 (minimal). Individual severity scores were categorized as minimal in ten rats and mild in the other six rats.

In the NL4 section of nasal cavities, 100 percent of the rats were found to have multifocal degeneration with necrosis. The mean severity score was 3.2 or moderate (Figures 8 through 10). In 16 rats, the lesions were extensive. In 12 out of those 16 rats, edema was also present. In six out of the 16 rats with extensive multifocal effects, the severity score was a 4 (marked), while two rats had lesions of mild severity.

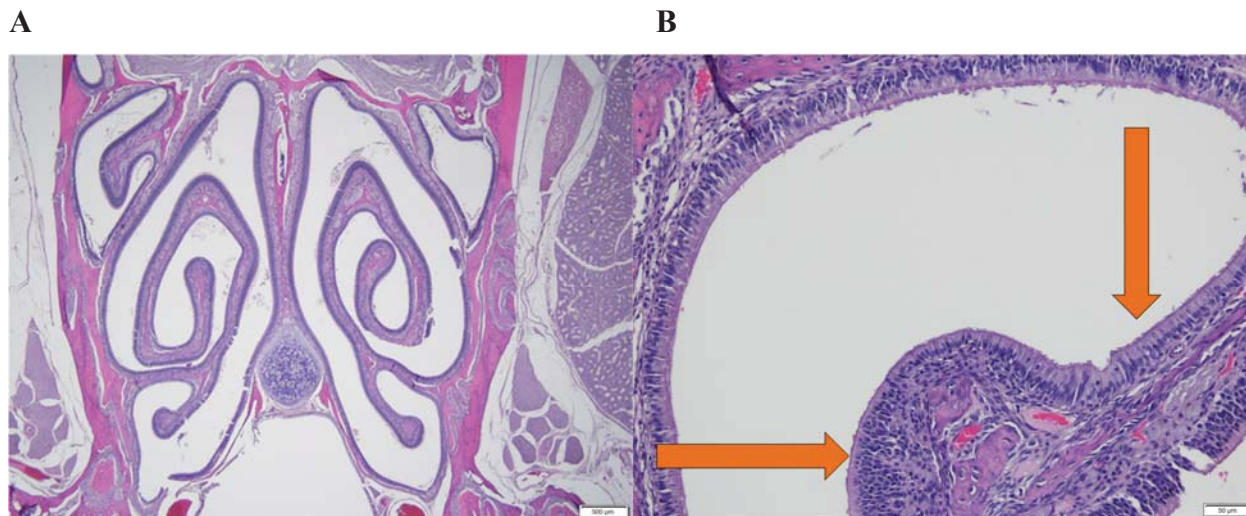


Figure 4: Normal Nasal Section NL4 from an Acute Study Control Group Rat Euthanized Two Weeks after Exposure

(A) This section is microscopically normal at the subgross view (scale bar (lower right corner) = 500 μ m). (B) A higher magnification of the section shows normal olfactory (horizontal arrow) and respiratory epithelium (vertical arrow) (scale bar = 50 μ m).

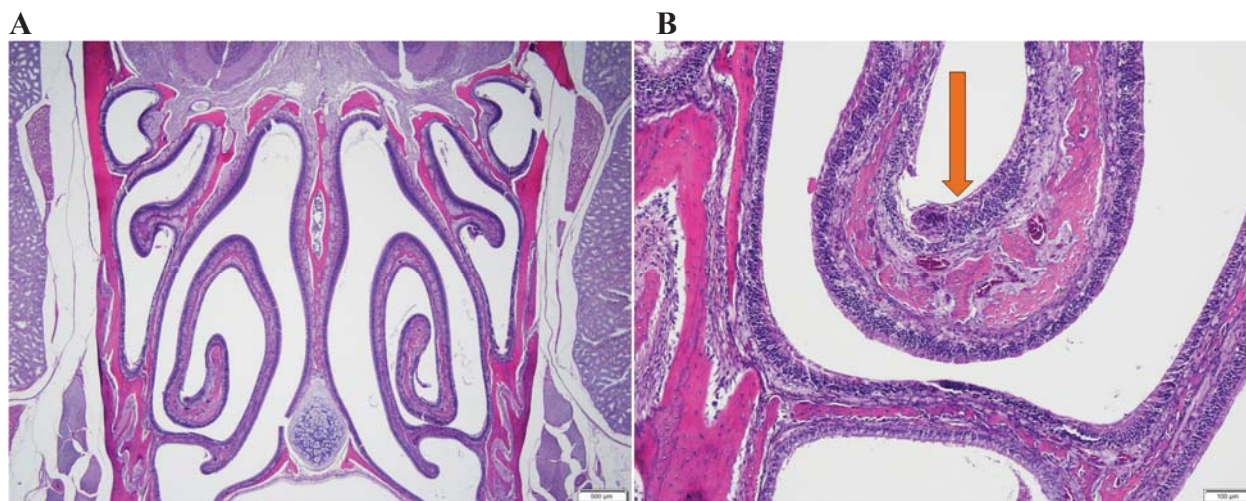


Figure 5: Nasal Section NL4 with Mild Lesions from an Acute Study Low Exposure Group Rat Euthanized One Day after Exposure

(A) This section is microscopically normal at the subgross view (scale bar = 500 μ m). (B) There is mild, early degeneration and necrosis of the olfactory epithelium (arrow); the respiratory epithelium is microscopically normal and unaffected (scale bar = 100 μ m).

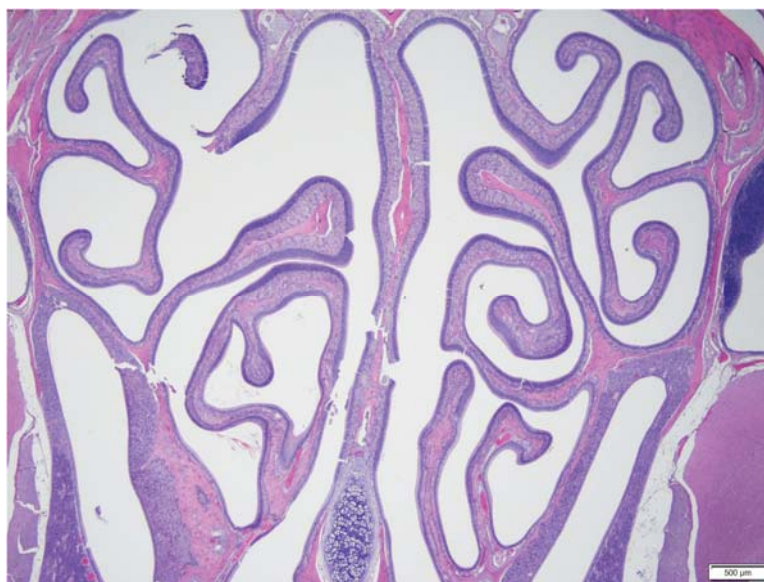


Figure 6: Normal Nasal Section NL3 from an Acute Study Control Group Rat Euthanized Two Weeks after Exposure

Scale bar = 500 μ m

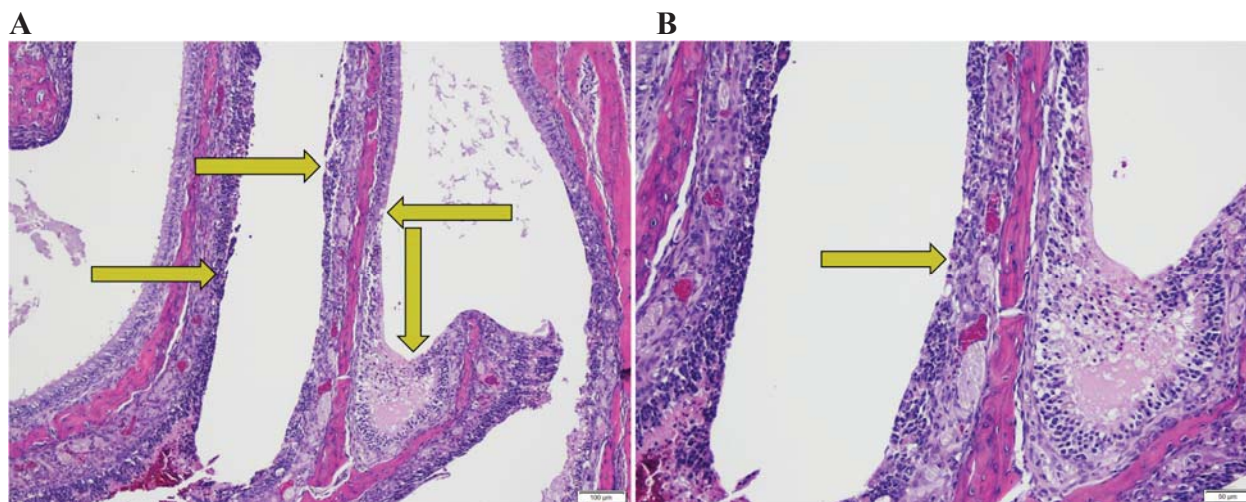


Figure 7: Nasal Section NL3 with Mild Lesions from an Acute Study Intermediate Exposure Group Rat One Day after Exposure

(A) There is mild multifocal degeneration, necrosis, and loss of the olfactory epithelium (horizontal arrows) and exudation of serous fluid admixed with cellular debris (vertical arrow). The respiratory epithelium appears unaffected and microscopically normal as seen in the left and the upper right of the image (scale bar = 100 μ m). (B) Higher magnification showing mild multifocal degeneration and necrosis of the olfactory epithelium. There is a focally extensive area of loss of the epithelium (arrow) with exudation of serous fluid admixed with necrotic cellular debris on the opposing side of the turbinate (scale bar = 50 μ m).

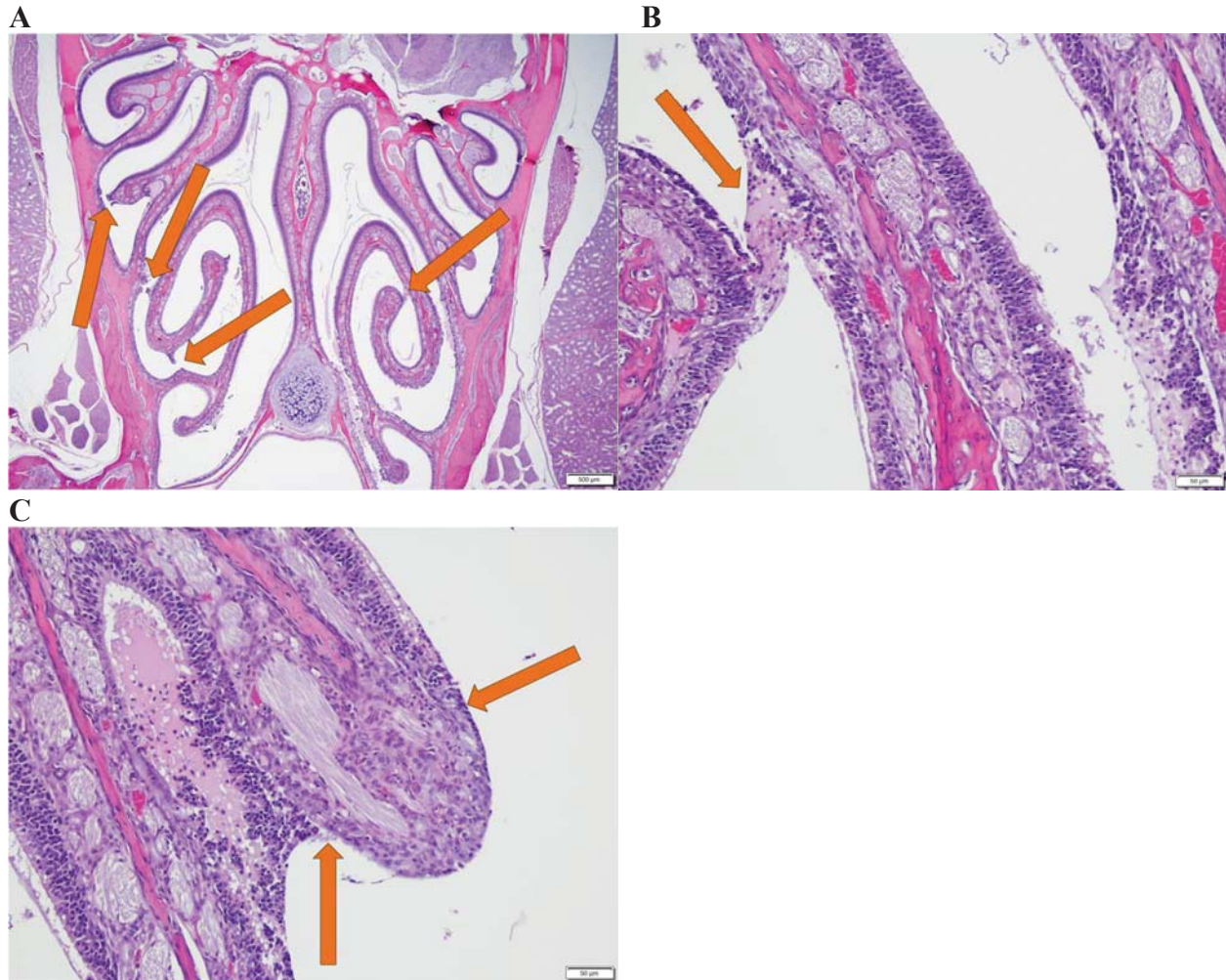


Figure 8: Nasal Section NL4 with Mild Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized One Day after Exposure

(A) There is mild, multifocal, degeneration and necrosis of the olfactory epithelium. There are several areas of adhesion between epithelium covering ethmoturbinates (synchia; arrows) associated with necrosis and loss of epithelium and a serofibrinous exudate (scale bar = 500 μ m). (B) Higher magnification of Fig. 8a showing a synchia (arrow). There is necrosis, degeneration, and loss of olfactory epithelium, which is covered by a serofibrinous exudate and cellular debris (scale bar = 50 μ m). (C) Higher magnification showing an area of ulceration between the arrows at the end of the turbinate where there is complete loss of epithelium and only the basement membrane and lamina propria remain. There are few inflammatory cells in the lamina propria. Adjacent to the ulcerated area there is necrosis, degeneration, and loss of the olfactory epithelium which is overlain by serofibrinous exudate and cellular debris (scale bar = 50 μ m).

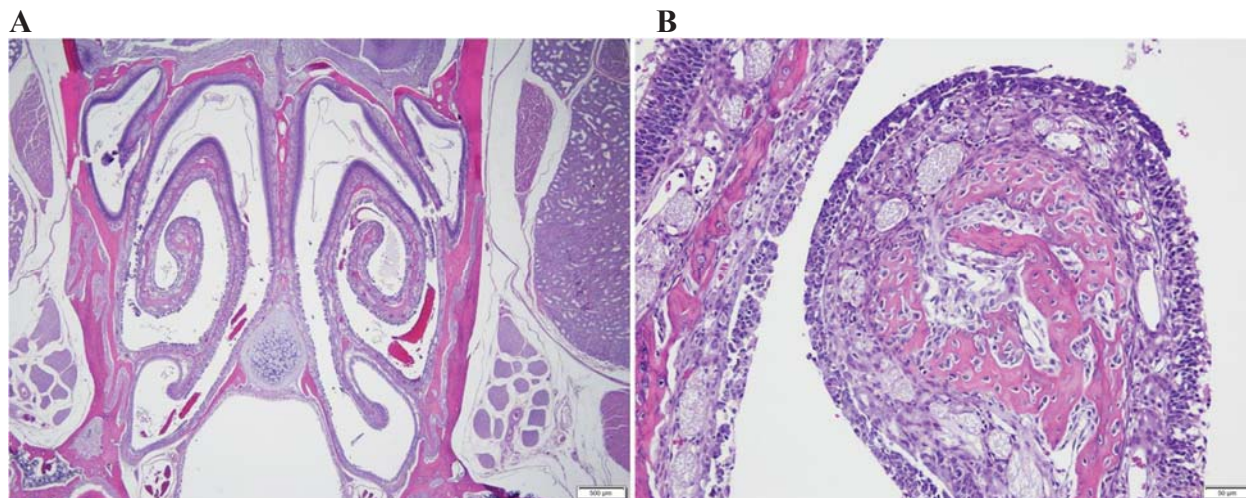


Figure 9: Nasal Section NL4 with Moderate Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized One Day after Exposure

(A) There is moderate, multifocal degeneration, necrosis, and loss of the olfactory epithelium. The hemorrhage is likely artifact from euthanasia (scale bar = 500 μ m). (B) Higher magnification showing epithelial degeneration, necrosis, and detachment from the basement membrane. There are moderate numbers of inflammatory cells infiltrating the lamina propria (scale bar = 50 μ m).

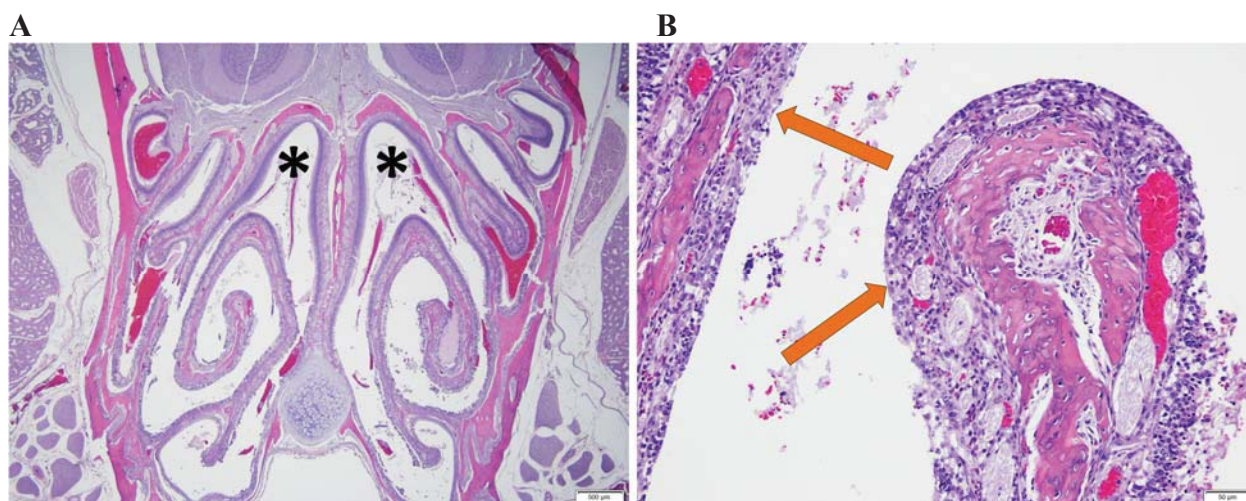


Figure 10: Nasal Section NL4 with Marked Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized One Day after Exposure

(A) There is marked, multifocally extensive to almost diffuse, degeneration, necrosis, and loss of the olfactory epithelium (OE). The OE at the dorsal aspect of the nasal passage appears microscopically normal (asterisks). The hemorrhage could be artifact from euthanasia (scale bar = 500 μ m). (B) There is multifocal complete loss of the OE (ulceration; arrows) with occasional replacement by necrotic debris and inflammatory cells. There is edema in the lamina propria as well as moderate numbers of inflammatory cells (scale bar = 50 μ m).

Among the recovery cohort intermediate exposure rats, recovery in the NL3 section was complete in 19 out of 20 rats. However, one rat still had minimal multifocal degeneration with necrosis.

In contrast, in the NL4 section, recovery was seen in only eight out of 19 rats; one NL4 section was not available for analysis. Severity of lesions ranged from extensive multifocal degeneration, necrosis, edema, and neutrophilic inflammation that was moderate (severity = 3) in two rats (Figure 11), to minimal multifocal neutrophilic inflammation in five rats. In between were two rats with multifocal degeneration (1 mild, 1 moderate; Figures 12 and 13, respectively); one rat with mild multifocal neutrophilic inflammation, degeneration and regeneration; and one rat with mild multifocal neutrophilic inflammation, degeneration and necrotic debris. Mean lesion severity was 0.95.

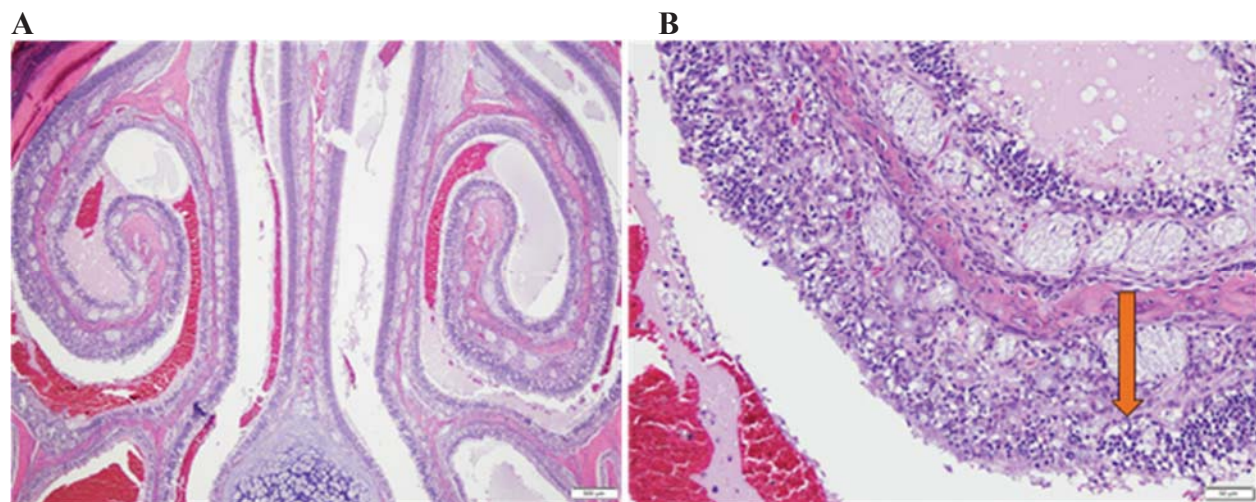


Figure 11: Nasal Section NL4 with Moderate Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized Two Weeks after Exposure

(A) There is moderate, multifocally extensive epithelial degeneration and necrosis of the olfactory epithelium (scale bar = 500 μ m). (B) Higher magnification showing swelling and detachment of the cells from the basement membrane (arrow) and disruption and loss of the cilia. There are many neutrophils and lymphocytes infiltrating the lamina propria. The hemorrhage could be due to euthanasia (scale bar = 50 μ m).

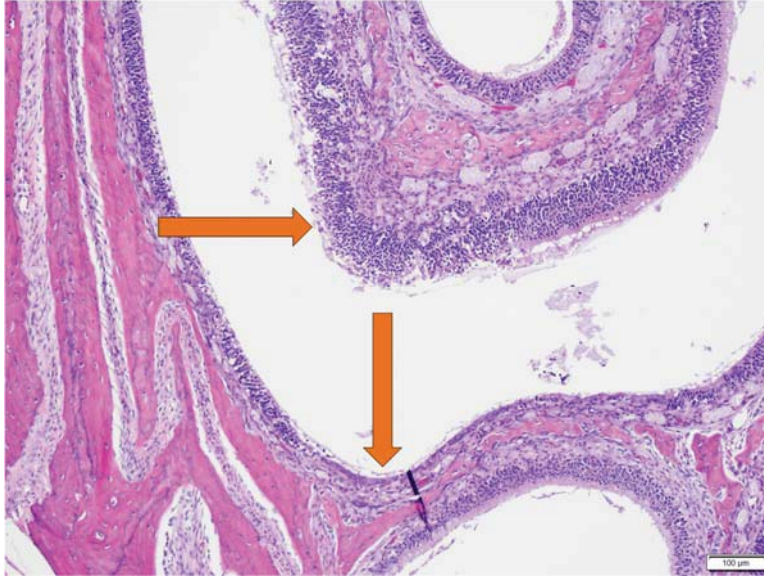


Figure 12: Nasal Section NL4 with Mild Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized Two Weeks after Exposure

There are multifocal mild areas of ongoing cellular degeneration (horizontal arrow) and epithelial regeneration (vertical arrow) with a single layer of flattened epithelium covering an area of prior OE necrosis and loss. There are moderate numbers of inflammatory cells within the lamina propria, especially in the area of ongoing degeneration (scale bar = 100 μm).

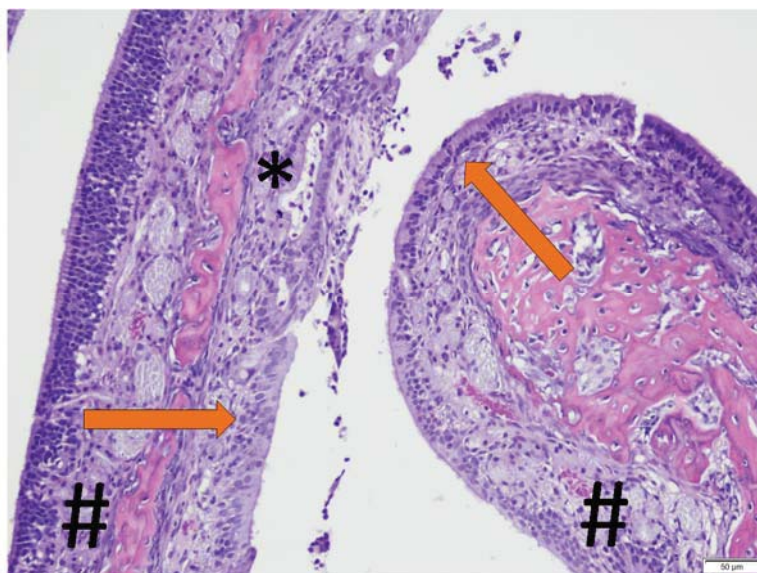


Figure 13: Nasal Section NL4 with Moderate Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized Two Weeks after Exposure

There is ongoing moderate degeneration, necrosis, and repair of the OE. The submucosal glands contain scant necrotic debris and the overlying OE is completely lost (). There is regeneration of the OE (#); the OE in the upper left of the image appears microscopically normal indicating it is either completely repaired or was not affected. There is multifocal metaplasia from OE to respiratory epithelium (arrows) (scale bar = 50 μm).*

4.3.4 Intermediate Exposure Group – Lungs

Following the acute intermediate PAO exposure, 18 out of 20 rats (90 percent) showed exposure related lesions while sham-exposed control rats were normal (Figure 14). The overall mean severity score was 1.21, which is essentially a minimal response (Figure 15). However, five of the 18 rats had individual severity scores of 2, which indicates a mild effect (Figure 16). All 18 rats had multifocal inflammation; 11 rats were classified with neutrophilic inflammation, six rats with mononuclear inflammation, and one rat with both neutrophilic and mononuclear. For the five rats with the mild severity score of 2, three had neutrophilic inflammation and two had mononuclear inflammation. A single rat of the 20 showed normal tissue. The remaining rat in this exposure group presented with multifocal hemorrhage consistent with euthanasia protocol; the lesion is unrelated to PAO exposure.

In the recovery cohort, 16 out of 20 rats had lungs that were fully recovered. The four rats that were not yet recovered displayed multifocal minimal neutrophilic inflammation as shown in Figure 17. As a result, the overall group mean severity score was 0.2.

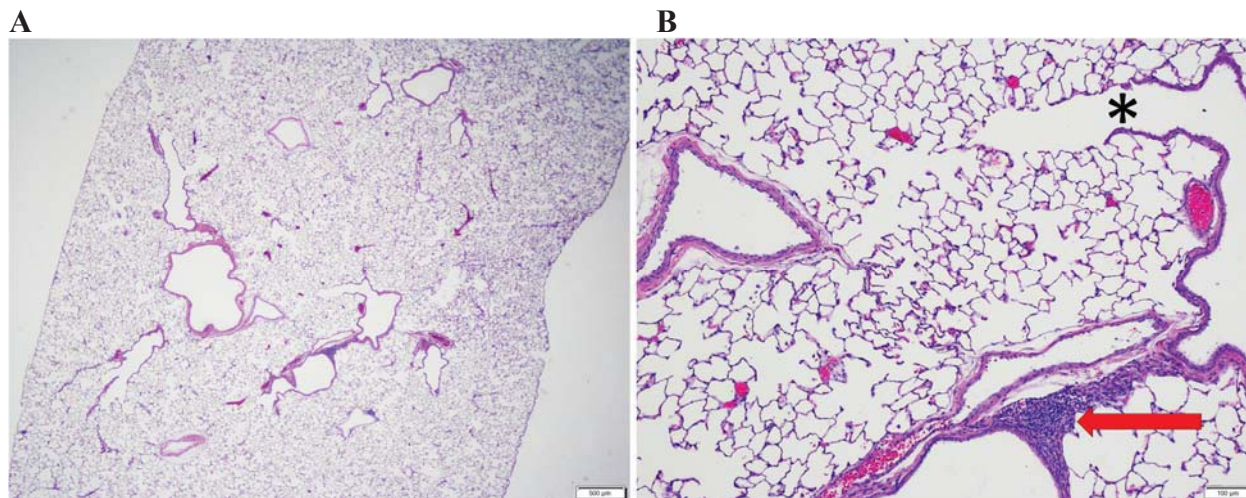


Figure 14: Normal Lung Section from an Acute Study Control Group Rat Euthanized Two Weeks after Exposure

(A) This lung is microscopically normal (scale bar = 500 μ m). (B) Higher magnification showing the transition from terminal bronchiole to respiratory bronchiole at the top right (*). Arrow denotes normal bronchial associated lymphoid tissue (BALT). There are a few normal pulmonary alveolar macrophages (PAMs) in the alveolar spaces (scale bar = 100 μ m).

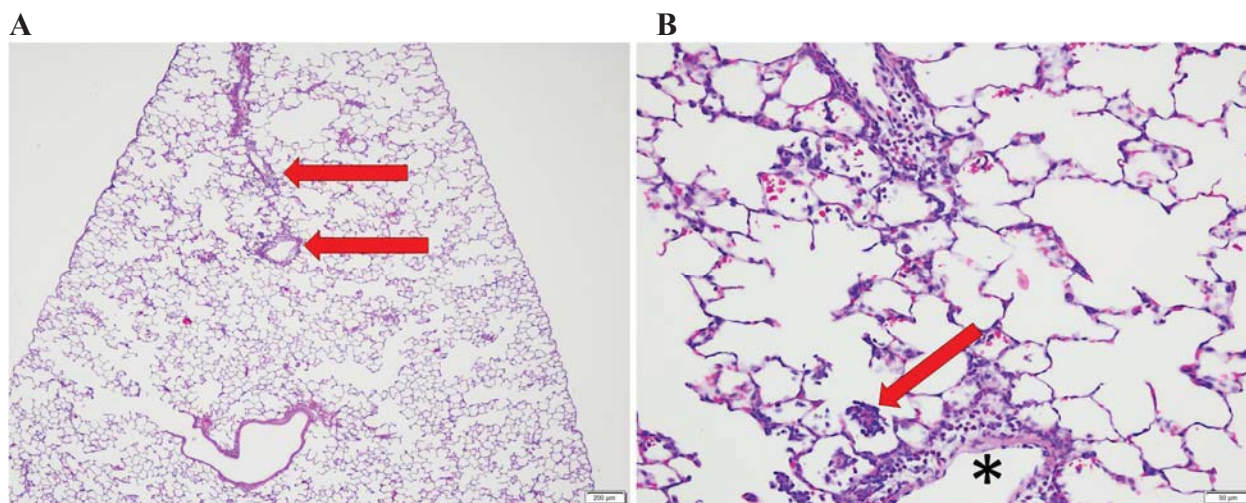


Figure 15: Lung Section with Minimal Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized One Day after Exposure

(A) There are minimal, multifocal perivascular accumulations of inflammatory cells (arrows) (scale bar = 200 μ m). (B) Higher magnification showing perivascular neutrophil infiltration. Some alveoli contain accumulations of neutrophils mixed with lymphocytes, macrophages, and scant cellular debris (arrow). Asterisk denotes blood vessel lumen (scale bar = 50 μ m).

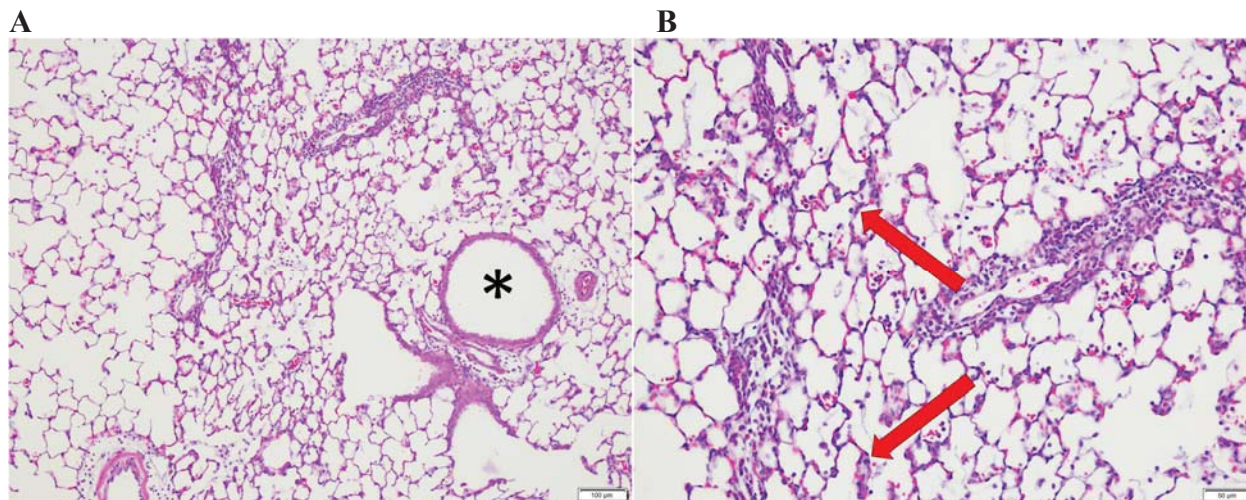


Figure 16: Lung Section with Mild Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized One Day after Exposure

(A) There is mild, multifocal perivascular inflammatory cell infiltration. Alveoli in the right half of the photomicrograph occasionally contain a cellular infiltrate and alveolar septa are thickened. Bronchioles appear to be normal and unaffected () (scale bar = 100 μ m). (B) Higher magnification showing perivascular neutrophils. Some alveoli contain accumulations of neutrophils admixed with lymphocytes, macrophages, and scant light blue, wispy mucinous debris. Alveolar septa are thickened by infiltrates of neutrophils and lymphocytes (arrows) (scale bar = 50 μ m).*

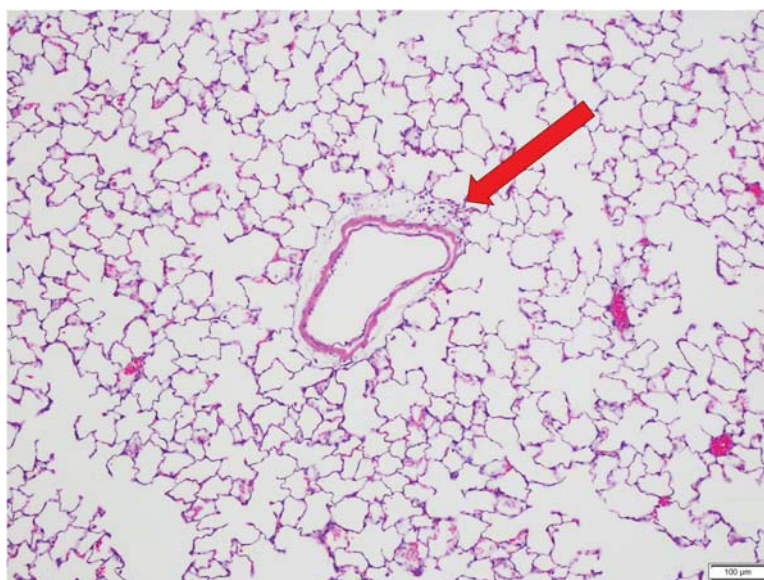


Figure 17: Lung Section with Minimal Lesions from an Acute Study Intermediate Exposure Group Rat Euthanized Two Weeks after Exposure

There are minimal, multifocal perivascular accumulations of inflammatory cells (arrow) (scale bar = 100 μ m).

4.3.5. High Exposure Group - Nasal Cavities

There was no microscopic evidence of damage in the rostral sections of NL1 and NL2 as shown in Figures 18 through 21. In the NL3 sections of the high exposure acute cohort rats, only two rats out of 20 had normal epithelium, while 13 out of 20 rats developed minimal (11 rats) to mild (2 rats, Figure 22) multifocal necrosis (90 percent incidence total). The overall severity score was 1.0, which is considered minimal. One rat had a focal area of mild neutrophilic inflammation and necrosis, while four rats showed mild neutrophilic inflammation and multifocal necrosis.

In the most posterior section (NL4), multifocal neutrophilic inflammation and necrosis were found in seven of 20 rats, ranging from mild (one rat, Figure 23) to moderate (six rats, Figure 24) severity. Additionally, eight rats developed moderate, multifocal necrosis; it was considered extensive in half of these rats. Four rats had extensive multifocal neutrophilic inflammation plus necrosis that was moderate in two rats, but marked (severity = 4) in the other two (Figure 25). One rat had marked (severity = 4) multifocal neutrophilic inflammation, necrosis and edema. In summation, all 20 rats presented with a lesion of some type, with a mean severity score of 3.10.

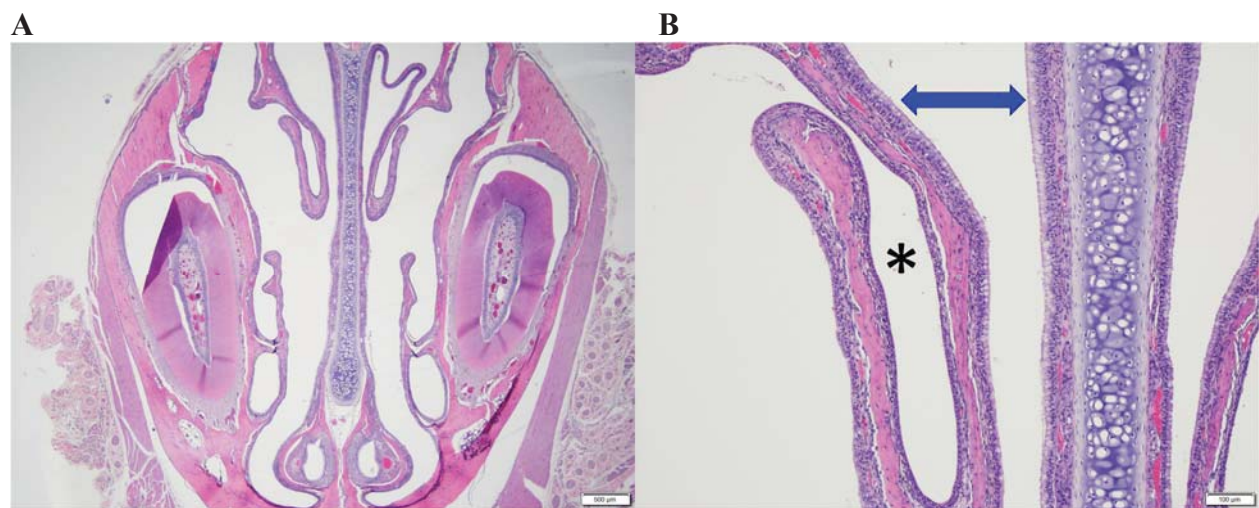


Figure 18: Normal Nasal Section NL1 from an Acute Study Control Group Rat Euthanized One Day after Exposure

(A) This section is microscopically normal (scale bar = 500 µm). (B) Higher magnification of NL1 from showing normal respiratory epithelium (double-headed arrow) and transitional epithelium on either side of the asterisk (100 µm).

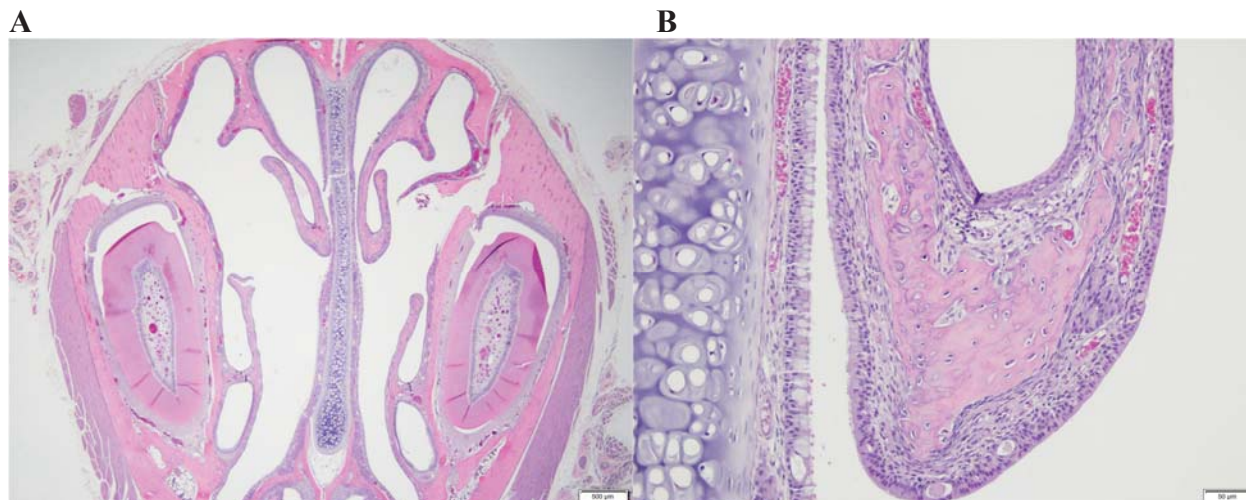


Figure 19: Normal Nasal Section NL1 from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) This section is microscopically normal (scale bar = 500 μ m). (B) Respiratory and transitional epithelium are both unaffected (scale bar = 50 μ m).

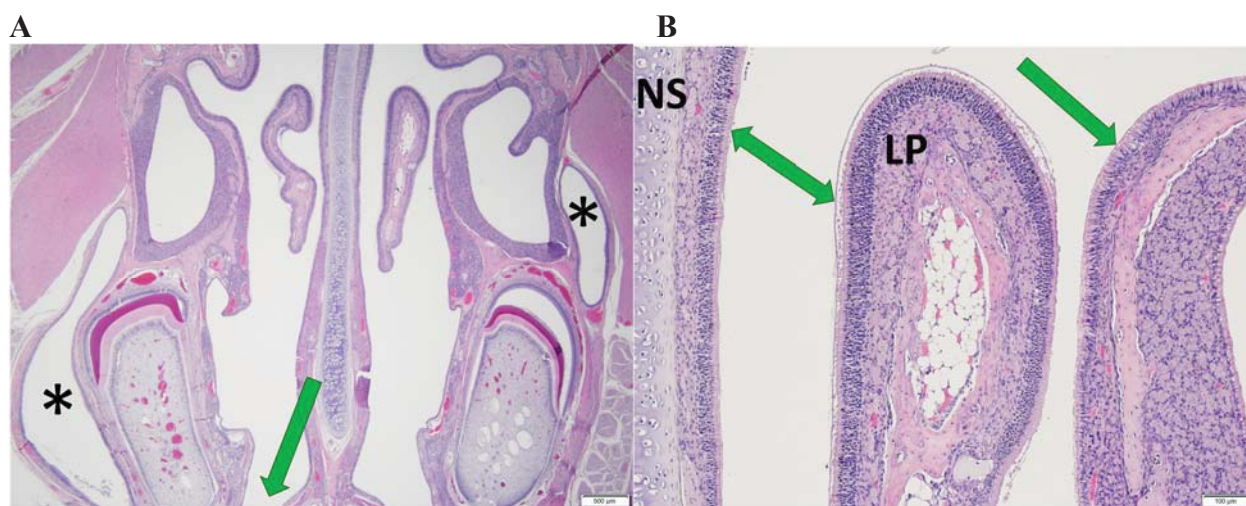


Figure 20: Normal Nasal Section NL2 from an Acute Study Control Group Rat Euthanized One Day after Exposure

(A) This section is microscopically normal. The nasopalatine ducts (arrow) and nasolacrimal ducts (*) are shown (scale bar = 500 μ m). (B) Higher magnification of Fig. 22a showing multi-layered olfactory epithelium (double-headed arrow) with cilia lying parallel to the cell surface contrasted with the ciliated pseudocolumnar respiratory epithelium (single-headed arrow). NS=nasal septum; LP=lamina propria (scale bar = 100 μ m).

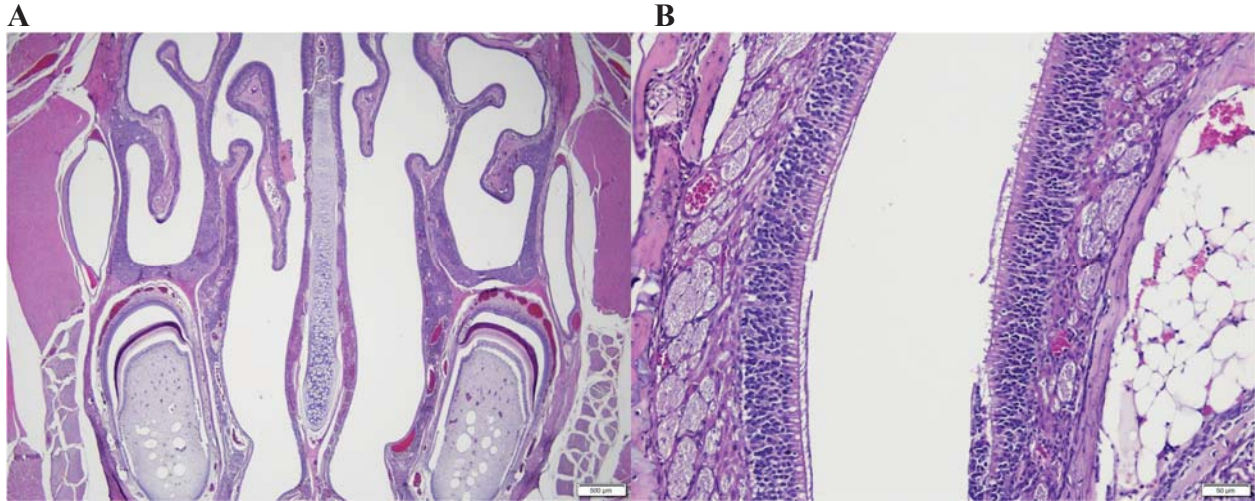


Figure 21: Normal Nasal Section NL2 from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) This section is microscopically normal (scale bar = 500 μ m). (B) The olfactory epithelium is unaffected at this level (scale bar = 50 μ m).

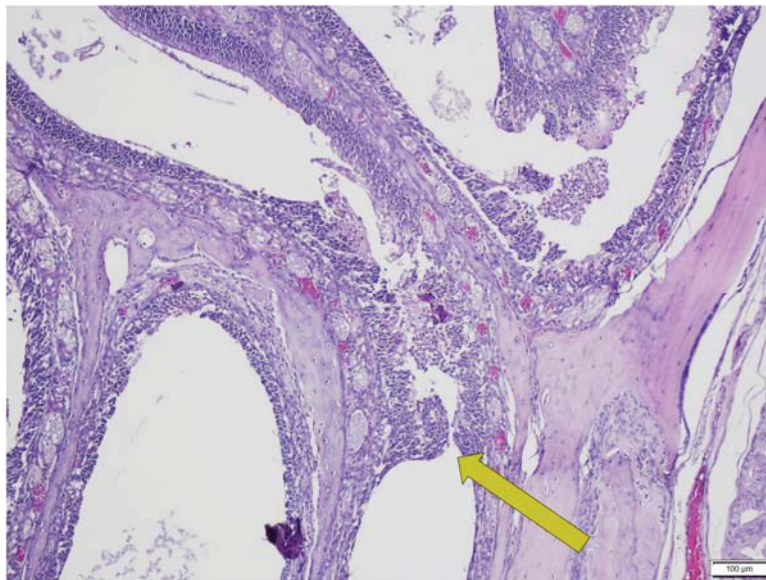
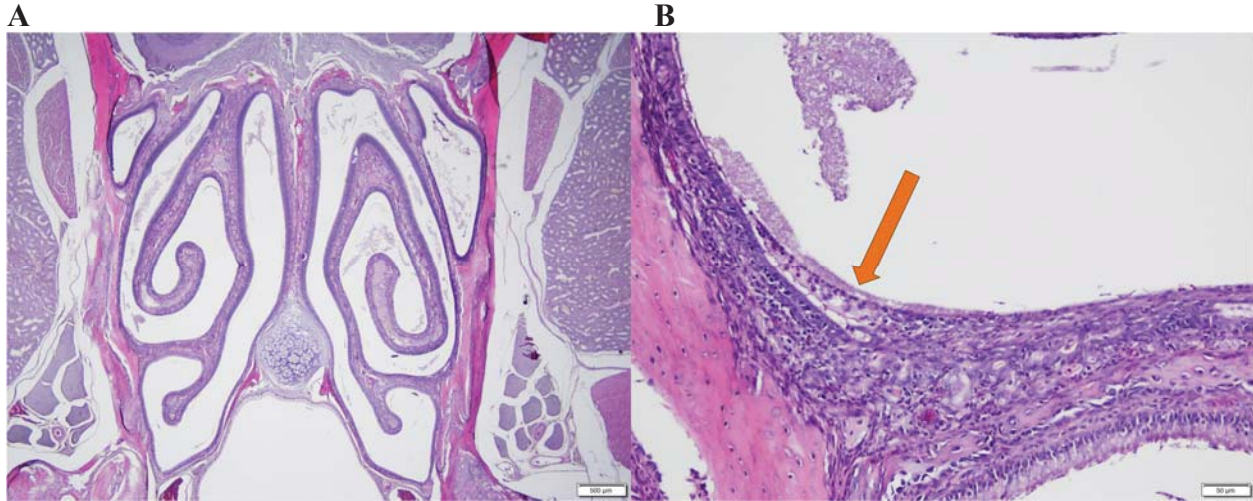


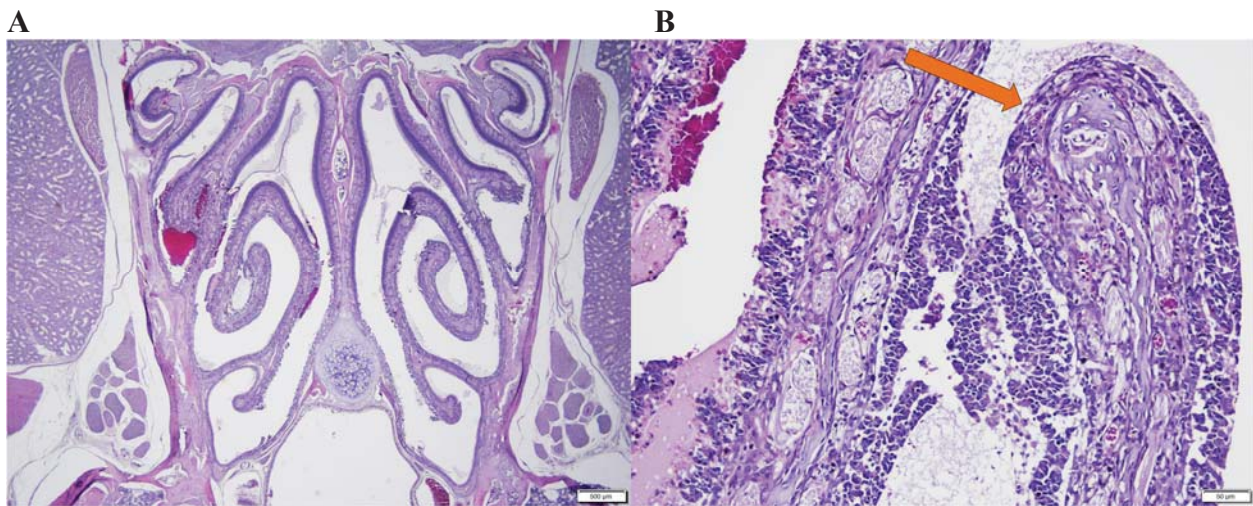
Figure 22: Nasal Section NL3 with Mild Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

There is mild, multifocal degeneration, necrosis, and loss of the olfactory epithelium and exudation of serous fluid admixed with cellular debris. There is likely an adhesion between two of the epithelial surfaces (synechia; arrow) (scale bar = 100 μ m).



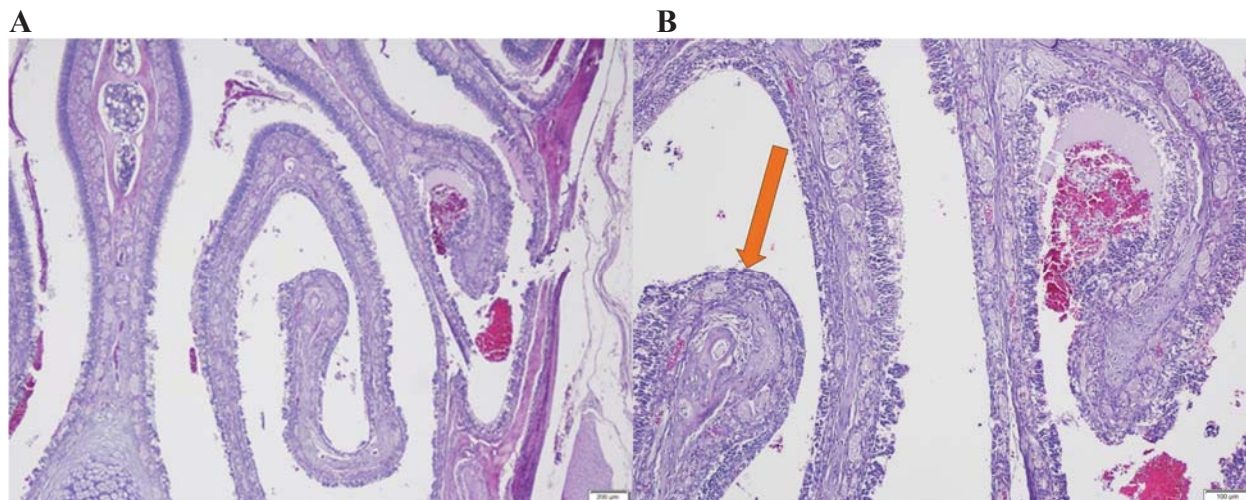
Figures 23: Nasal Section NL4 with Mild Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) There is mild multifocal necrosis and degeneration of the OE (scale bar = 500 μm). (B) Higher magnification showing mild degeneration and necrosis of the OE (arrow) and a focal area of almost complete loss of the OE (erosion) which is covered with necrotic cellular debris, serous fluid, and few inflammatory cells (scale bar = 50 μm).



Figures 24: Nasal Section NL4 with Moderate Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) There is a moderate, multifocally extensive lesion consisting of necrosis, degeneration, and loss of the OE (scale bar = 500 μm). (B) Higher magnification shows the lesion varies from cell swelling and dissociation to complete loss of OE (arrow). Note the serous exudate admixed with necrotic cellular debris and exfoliated cells in the lower left of the image (scale bar = 50 μm).



Figures 25: Nasal Section NL4 with Marked Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) There is marked, diffuse necrosis, degeneration, and loss of the OE (scale bar = 200 μ m). (B) Higher magnification in shows swelling of cells, detachment from adjacent cells and the basement membrane, multifocal loss of the OE (erosion; arrow), exudation of serous fluid, and necrotic cellular debris in the airway (scale bar = 100 μ m).

In the recovery cohort, there was 100 percent recovery in the NL3 tissues (Figure 26). In the NL4 tissues, there was only 70 percent recovery; six rats did not return to normal. Four rats still showed necrotic debris of mild severity; three were multifocal and one was only focal. Two of the multifocal rats also showed regeneration, which is indicative of recovery. The remaining rat exhibited multifocal necrotic debris with degeneration still occurring in some locations (Figure 27). The rat with the focal effect showed regeneration, but still had indications of neutrophilic inflammation; recovery had begun, but adverse effects were still present. Finally, one of the six affected rats showed minimal multifocal regeneration indicating recovery, while the remaining animals still had multifocal minimal necrosis and degeneration.

4.3.6 High Exposure Group – Lungs

Among the acute cohort high exposure group, one rat had normal lungs while 17 out of 20 rats had neutrophilic inflammation ranging from minimal (10 rats, Figure 28) to mild (7 rats, Figure 29). Of the remaining two rats out of the 20, one had mild, mixed multifocal inflammation (both mononuclear and neutrophilic), while the other rat had a severity score of moderate (severity = 3) with focally extensive neutrophilic inflammation, necrosis, and edema (Figure 30). The mean severity score was 1.45 (mild).

In the recovery cohort, there were 12 out of 20 rats (60 percent) that had recovered completely after two weeks. Of the eight rats with lesions (40 percent incidence), four still showed minimal (severity = 1) multifocal neutrophilic inflammation (Figure 31). The other four had

granulomatous lesions; two were minimal and multifocal and two were focal with one minimal (Figure 32) and the other mild severity ratings. Overall, the mean severity score was 0.45 (minimal).

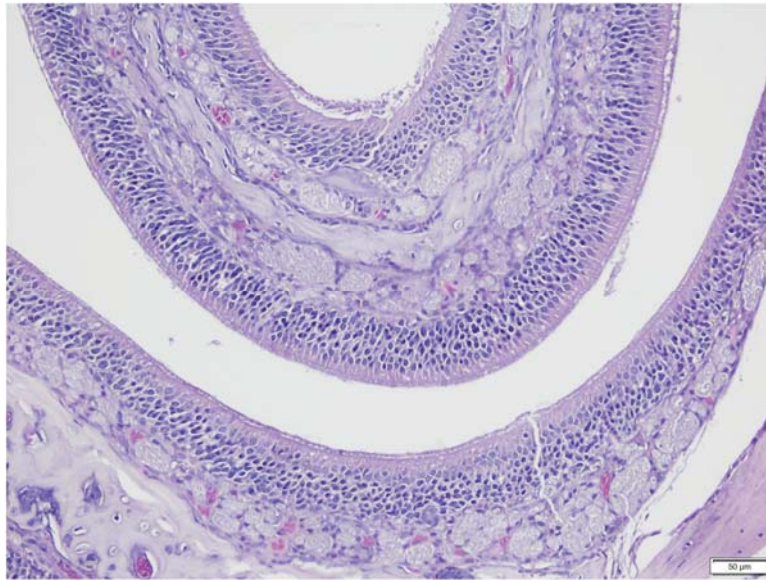


Figure 26: Normal Nasal Section NL3 in an Acute Study High Exposure Group Rat Euthanized Two Weeks after Exposure

Based on this image, it cannot be determined whether the olfactory epithelium was damaged and repaired or was not affected at all (scale bar = 50 μm).

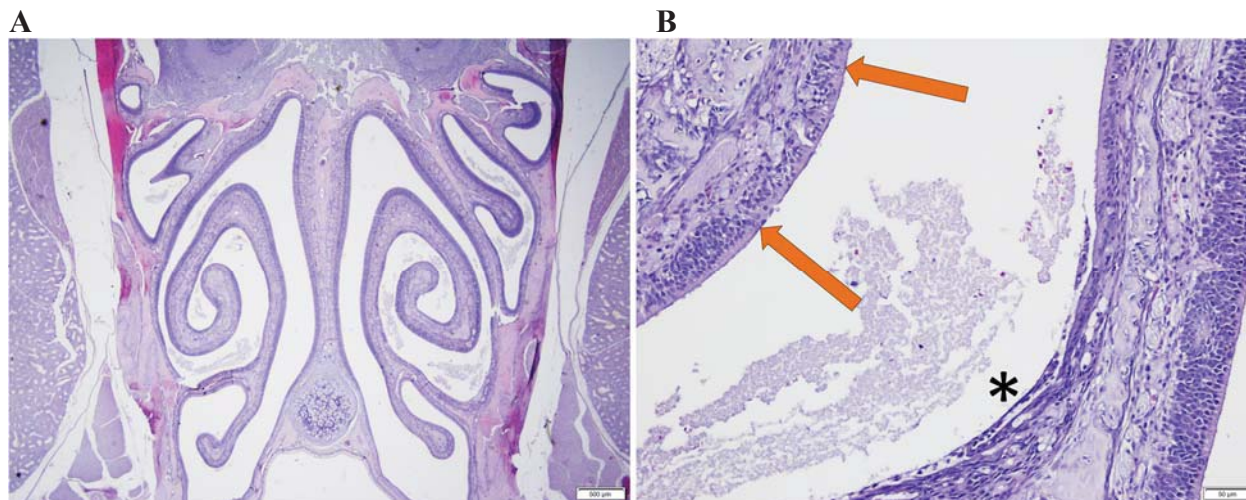


Figure 27: Nasal Section NL4 with Minimal Lesions in an Acute Study High Exposure Group Rat Euthanized Two Weeks after Exposure

(A) There is minimal, multifocal necrosis, degeneration, and loss of the olfactory epithelium (scale bar = 500 μ m).
 (B) Between the arrows there is focal thinning and disorganization of the OE, suggesting regeneration. There is a focal loss of the OE and infiltration by inflammatory cells in the area denoted at the asterisk (scale bar = 50 μ m).

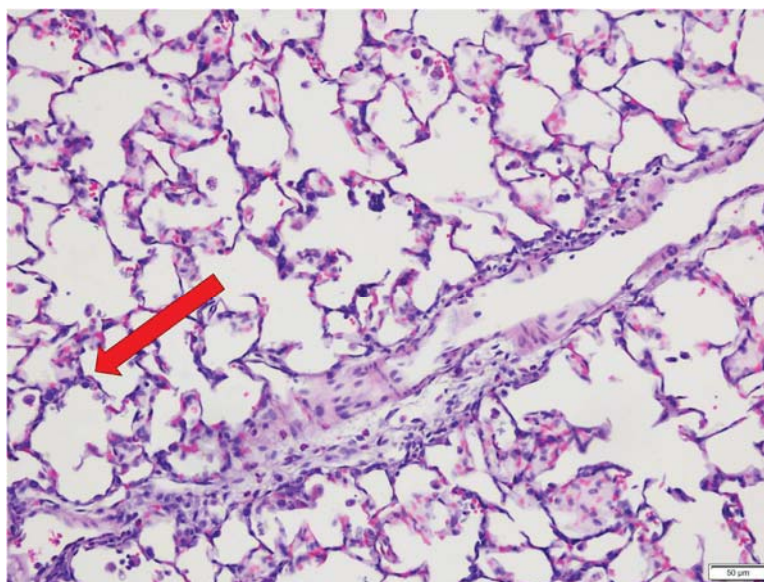


Figure 28: Lung Section with Minimal Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

There is minimal perivascular inflammatory cell accumulation composed predominately of neutrophils with fewer lymphocytes. Alveolar septa are thickened by lymphocytes and macrophages (arrow) and alveoli contain few inflammatory cells consisting of neutrophils, PAMs, lymphocytes, and plasma cells (scale bar = 50 μ m).

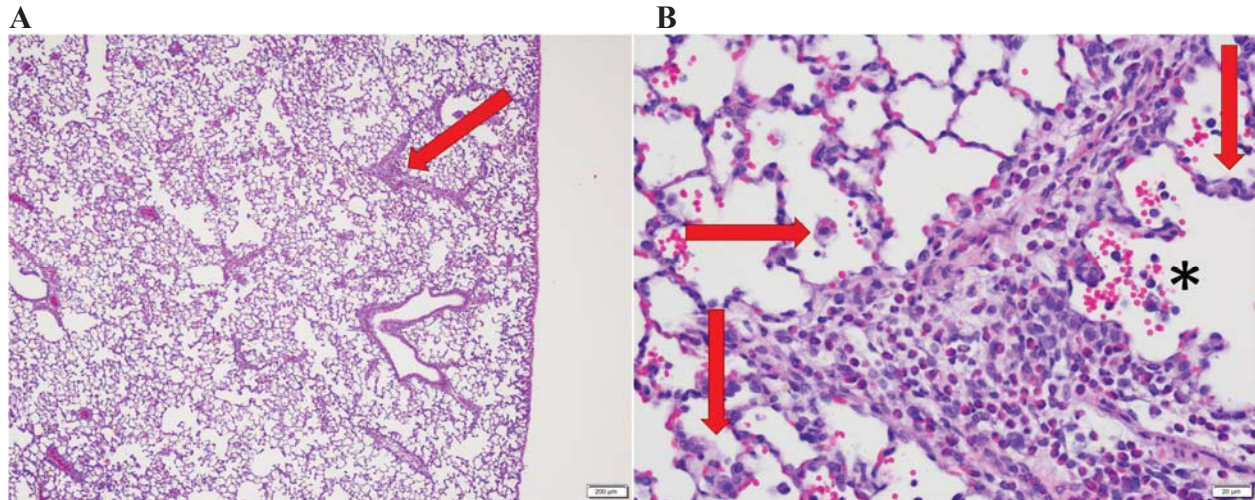


Figure 29: Lung Section with Mild Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

(A) There is mild accumulation of perivascular inflammatory cells (arrow) and multifocally extensive thickening of alveolar septa. Bronchioles appear to be normal and unaffected (scale bar = 200 μ m). (B) Higher magnification of shows the perivascular cellular infiltrate is composed of neutrophils, lymphocytes, plasma cells, and macrophages. There is minimal alveolar hemorrhage as demonstrated by extravasated erythrocytes and erythrophagocytosis by PAMs (horizontal arrow). Alveoli occasionally contain few lymphocytes and plasma cells () admixed with scant necrotic cellular debris. Alveolar septa are expanded by lymphocytes, plasma cells, macrophages, and neutrophils (vertical arrows) (scale bar = 20 μ m).*

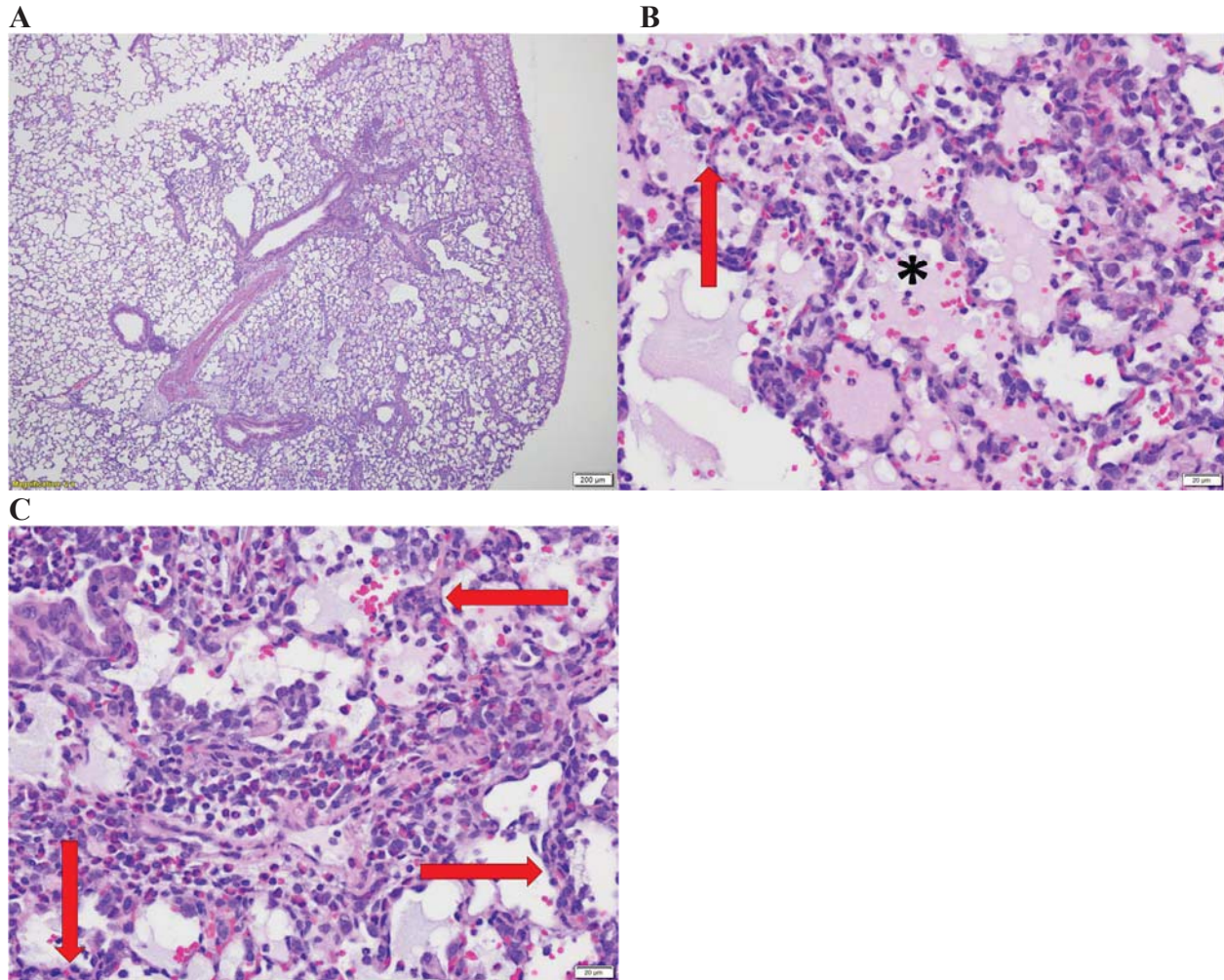


Figure 30: Lung Section with Moderate Lesions from an Acute Study High Exposure Group Rat Euthanized One Day after Exposure

This rat had the most severe lung lesion of any of the rats examined. (A) There are moderate numbers of perivascular inflammatory cells. There is multifocally extensive thickening of alveolar septa and edema and exudation of fluid into the alveoli. Bronchioles appear to be normal and unaffected (scale bar = 200 μ m). (B) Higher magnification showing moderate numbers of alveolar inflammatory cells (PAMs, viable and degenerate neutrophils, lymphocytes, plasma cells, eosinophils), minimal hemorrhage, pulmonary edema, and cellular debris (). There are occasional necrotic type I pneumocytes lining the alveoli (arrow) (scale bar = 20 μ m). (C) Higher magnification shows moderate, dense perivascular accumulations of neutrophils, eosinophils, macrophages, lymphocytes, and plasma cells. Alveolar inflammatory cells consist of PAMs, viable and degenerate neutrophils, lymphocytes, plasma cells and eosinophils admixed with minimal hemorrhage, edema fluid, and cellular debris. There is multifocally extensive thickening of alveolar septa (arrows) (scale bar = 20 μ m).*

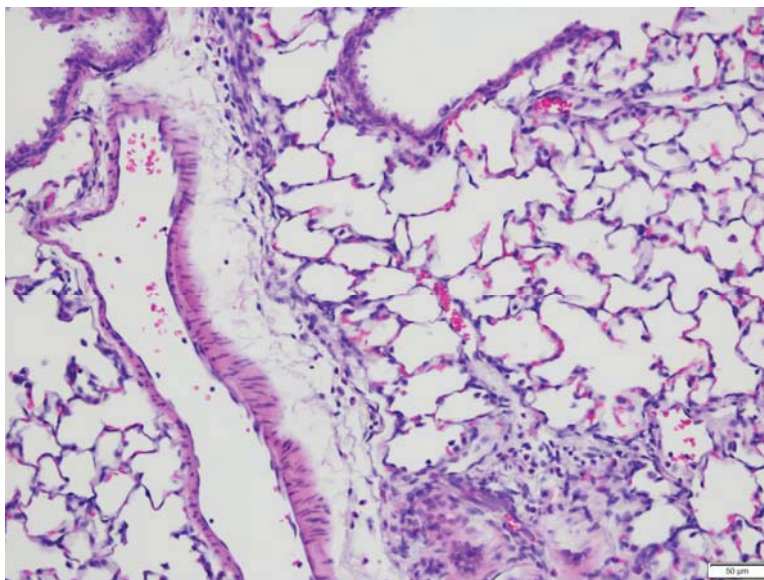


Figure 31: Lung Section with Minimal Lesions from an Acute Study High Exposure Group Rat Euthanized Two Weeks after Exposure

There are minimal perivascular accumulations of inflammatory cells dominated by neutrophils (scale bar = 50 μ m).

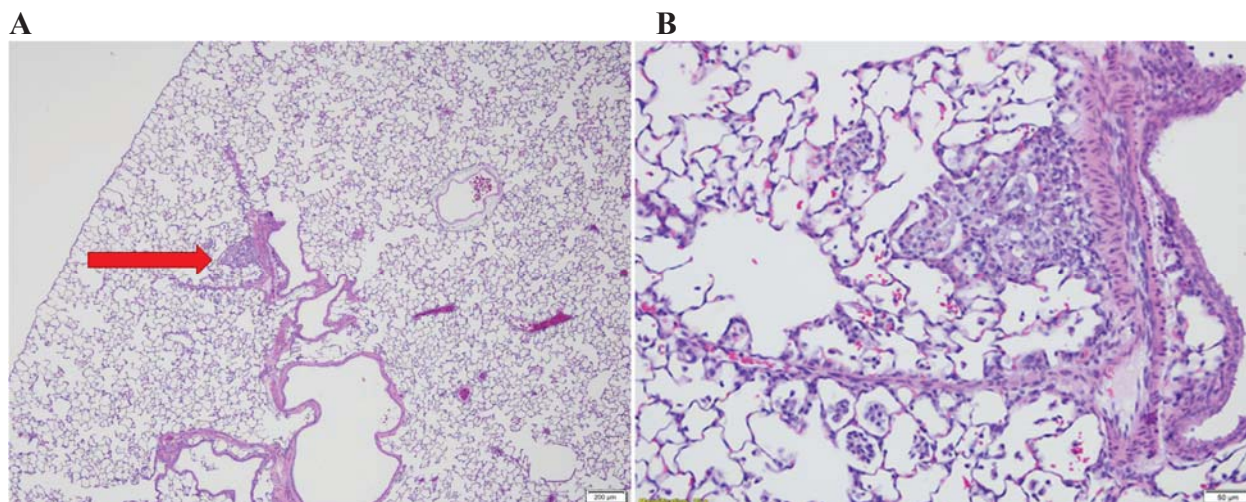


Figure 32: Lung Section with Minimal Lesions from an Acute Study High Exposure Group Rat Euthanized Two Weeks after Exposure

(A) There is a focus of minimal granulomatous alveolar inflammation (arrow) and rare perivascular inflammatory cells (scale bar = 200 μ m). (B) The inflammatory cells are predominately macrophages with fewer lymphocytes, plasma cells, and neutrophils. Alveolar septa in this area are mildly thickened by inflammatory cells consisting of lymphocytes, plasma cells and macrophages (scale bar = 50 μ m).

5.0 TWO-WEEK INHALATION STUDY RESULTS

5.1 Exposure Conditions

Average PAO aerosol exposures were found to be within 2 percent of the targeted concentrations. Aerosols measured within the respirable range (1.3 to 2.6 μm) and were uniform between exposure days. Detailed exposure condition information can be found in Mattie *et al.* (2017).

5.2 Overall Effects

5.2.1. Neurobehavioral Effects

For all MA and FOB measures, no significant differences between exposure groups were reported for either the males or the females. Complete neurobehavioral results can be found in Mattie *et al.* (2017).

5.2.2. General Effects

Animals were weighed before each exposure, and when analyzed statistically, there was no significant difference between group mean body weights when compared on the same day. Animals from all groups gained weight throughout the exposure regimen. Animals were necropsied on the day following the last exposure and neurobehavioral tests. Animals were fasted after approximately 8 to 10 PM of the evening prior to necropsy and show corresponding weight loss at the final weighing. There was no statistically significant difference among exposure groups for water and food consumed in the same daily period.

No statistically significant effects on relative organ weights were found between exposure groups in the two-week study. Female rats in the intermediate exposure group were found to have a significantly higher left kidney to brain weight ratio; this was considered an aberration and not an effect of exposure to PAO.

Clinical chemistry parameters were measured in blood samples taken at necropsy. There were no exposure group-related differences in any of the measured clinical chemistry parameters in males or females with one exception. The aspartate aminotransferase (AST) levels among the females exposed at the lowest concentration were significantly higher than controls. No elevation of AST was seen in the intermediate or high concentration exposed females; no dose-response effect is evident.

Hematology parameters were measured in blood samples taken at necropsy. Some statistically significant changes were noted. Male rats showed a dose-dependent increase in percent neutrophils (Table 2). A significant increase in mean platelet volume occurred in the intermediate group male rats. Neither the low or high exposure group male values were different from control values.

Female rats also showed sporadic but significant changes in mean platelet volume, number of platelets, and total white blood cell counts (due to decreased lymphocyte counts). These changes were not dose-dependent. Statistically significant dose-dependent increases in percent neutrophils and decreases in percent lymphocytes were also observed (Table 3). Complete data for these results are found in Mattie *et al.* (2017).

Table 2: Hematology Results in Two-Week Inhalation Study Male Rats

Exposure Group	Control (0 mg/m ³)		Low (20 mg/m ³)		Intermediate (100 mg/m ³)		High (300 mg/m ³)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
White blood cells (# x 10 ³ /μL)	4.42	2.22	4.10	1.51	5.55	1.83	5.32	2.15
Neutrophils (# x 10 ³ /μL)	0.90	0.55	0.81	0.33	1.38	0.69	1.35	0.68
Lymphocytes (# x 10 ³ /μL)	3.20	1.54	3.03	1.12	3.75	1.06	3.63	1.33
Monocytes (# x 10 ³ /μL)	0.29	0.20	0.24	0.12	0.36	0.17	0.31	0.23
Eosinophils (# x 10 ³ /μL)	0.02	0.03	0.01	0.01	0.04	0.08	0.02	0.02
Basophils (# x 10 ³ /μL)	0.01	0.02	0.00	0.00	0.02	0.05	0.01	0.02
Neutrophils (%)	18.50	5.28	19.68	2.42	23.57*	6.23	24.10*	7.22
Lymphocytes (%)	74.74	7.13	74.04	4.04	69.13	7.75	70.23	9.30
Monocytes (%)	6.08	2.52	5.88	1.91	6.36	2.41	5.08	2.47
Eosinophils (%)	0.41	0.64	0.32	0.37	0.58	1.11	0.40	0.43
Basophils (%)	0.28	0.37	0.08	0.09	0.36	0.68	0.20	0.32
Red blood cells (# x 10 ⁶ /μL)	8.36	1.89	9.09	0.68	9.32	0.44	8.77	1.39
Hemoglobin (g/dL)	13.83	3.40	15.11	1.28	15.58	0.70	14.71	2.39
Hematocrit (%)	45.24	10.37	49.41	3.81	50.96	3.21	47.74	7.31
Mean corpuscular volume (fL)	54.12	1.30	54.38	0.66	54.69	2.01	54.54	0.94
Mean corpuscular hemoglobin (pg)	16.44	0.67	16.62	0.53	16.73	0.45	16.76	0.45
Mean corpuscular hemoglobin concentration (g/dL)	30.38	1.31	30.60	1.00	30.63	1.25	30.74	1.11
Red blood cell distribution width (%)	17.03	0.94	17.27	0.52	17.31	0.72	16.90	0.61
Platelets (# x 10 ³ /μL)	613	292	437	351	349	338	674	335
Mean Platelet Volume (fL)	6.75	1.17	7.64	1.58	8.41*	1.70	7.06	1.77

Notes: *Different from control ($p < 0.05$); dL: deciliter; fL: femtoliter; pg: pictogram; SD: standard deviation

Table 3: Hematology Results in Two-Week Inhalation Study Female Rats

Exposure Group	Control (0 mg/m ³)		Low (20 mg/m ³)		Intermediate (100 mg/m ³)		High (300 mg/m ³)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
White blood cells (# x 10 ³ /μL)	4.98	1.66	4.36	2.10	3.74*	0.64	5.31	1.50
Neutrophils (# x 10 ³ /μL)	0.72	0.32	0.74	0.43	0.68	0.17	1.06	0.37
Lymphocytes (# x 10 ³ /μL)	4.04	1.37	3.40	1.54	2.85*	0.45	4.02	1.13
Monocytes (# x 10 ³ /μL)	0.21	0.11	0.21	0.18	0.19	0.11	0.21	0.10
Eosinophils (# x 10 ³ /μL)	0.012	0.010	0.009	0.013	0.011	0.019	0.012	0.015
Basophils (# x 10 ³ /μL)	0.005	0.007	0.006	0.011	0.007	0.013	0.007	0.010
Neutrophils (%)	14.16	4.12	16.79*	2.47	17.94*	3.09	19.92*	3.19
Lymphocytes (%)	81.18	4.49	78.66	3.77	76.78*	4.76	75.85*	3.79
Monocytes (%)	4.34	1.78	4.12	2.17	4.87	2.57	3.83	1.82
Eosinophils (%)	0.219	0.178	0.248	0.380	0.257	0.363	0.246	0.234
Basophils (%)	0.091	0.103	0.178	0.371	0.153	0.259	0.155	0.166
Red blood cells (# x 10 ⁶ /μL)	9.02	0.43	8.96	0.55	9.02	0.41	9.31	0.47
Hemoglobin (g/dL)	15.02	0.48	14.80	1.12	14.84	0.75	15.40	0.76
Hematocrit (%)	51.58	2.66	51.62	3.36	51.41	2.46	52.89	2.93
Mean corpuscular volume (fL)	57.19	0.58	57.59	1.10	57.03	1.22	56.83	0.56
Mean corpuscular hemoglobin (pg)	16.67	0.53	16.49	0.49	16.47	0.48	16.55	0.43
Mean corpuscular hemoglobin concentration (g/dL)	29.14	1.02	28.67	0.97	28.88	0.99	29.15	0.81
Red blood cell distribution width (%)	15.86	0.57	15.94	0.67	15.86	0.57	16.10	0.54
Platelets (# x 10 ³ /μL)	535	307	255*	293	629	276	419	255
Mean Platelet Volume (fL)	6.46	1.94	8.16*	1.84	6.46	1.03	6.63	2.16

Notes: *Different from control ($p < 0.05$); dL: deciliter; fL: femtoliter; pg: picogram; SD: standard deviation

5.3 Histopathology

The incidence and severity of effects for nasal cavity sections (NL3 and NL4) and lungs are shown in Table 4 and Figure 33. The descriptions of effects are described below. No effects were seen in the first (NL1), second (NL2) and third (NL3) sections of the nasal cavities (Figure 34) and are therefore not discussed below. To remain consistent with Section 4.7, NL1 and NL2 are not shown in Table 4. Complete histopathology tissue review results are found in Appendix B.

Table 4: Incidence and Severity Ratings for Tissue Sections from Rats Exposed to PAO Aerosol in Two-Week Inhalation Study

Tissue Section	Control (0 mg/m ³)	Low (20 mg/m ³)	Intermediate (100 mg/m ³)	High (300 mg/m ³)
Incidence				
NL3	0	0	0	0
NL4	0	0	0	12/20 (60%)
Lung	0	0	1/20 (5%)	19/20 (95%)
Severity				
NL3	0	0	0	0
NL4	0	0	0	0.71 ± 0.13
Lung	0	0	0.05 ± 0.05	1.25 ± 0.12

Notes: Nasal Level (NL) 1 and 2 had no incidence of lesions and are not shown in the table. Incidence units = number of rats with effects/n (% number of rats with effects); Severity units = mean ± standard error of the mean; Severity ratings: 0 = Normal, 1 = Minimal/Rare, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe

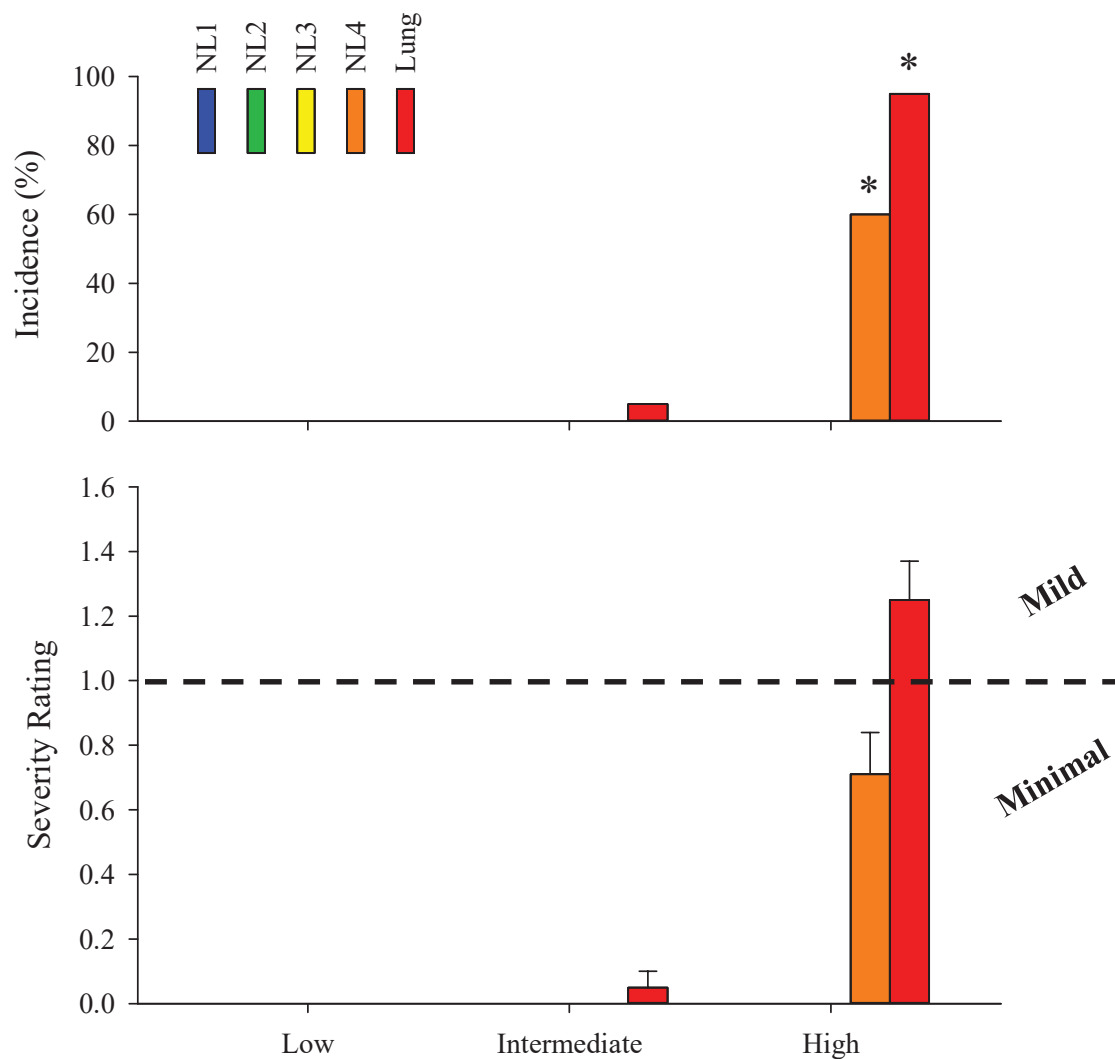


Figure 33: Histopathological Incidence and Significance of Regional Nasal Tissues and Lung for Male and Female Rats Combined

Severity ranking is noted at the right of the graph. The drawings in Figure 2 indicate the nasal cavity tissue section (NL1 through NL4) or lung tissue by color. No effects were seen in NL1, NL2 or NL3 (Figure 34). Significance markings indicate that exposure group and incidence are related (* $p < 0.001$).

Column A

Column B



Figure 34: Normal Nasal Sections from Two-Week Study Control and High Exposure Group Rats

(Column A) Control group rat nasal sections NL1 (top row), NL2 (middle row), and NL3 (bottom row) (scale bars = 500 μ m). (Column B) High exposure group rat nasal sections NL1 (top row), NL2 (middle row), and NL3 (bottom row) (scale bars = 500 μ m). All high exposure group rats were microscopically normal in these nasal sections.

5.7.1. Intermediate Exposure Group

In the intermediate exposure group after two weeks of exposure to PAO, only one rat had an effect; the tissues of 19 rats were normal. In the single rat with an effect, a peri-mortem focal area of hemorrhage was found in a lung. As this lesion was not seen in any of the other lungs in the two-week study and as this lesion can be induced during euthanasia, it was considered unrelated to the PAO exposure.

5.7.2. High Exposure Group - Nasal Cavities

The NL4 section in high exposure group rats was the only nasal cavity region to have an effect due to exposure to PAO. Epithelial changes were typically restricted to olfactory epithelium with relative sparing of the respiratory epithelium. Lesions occurred in 12 out of 20 rats (60 percent incidences) with a mean severity score of 0.71 (minimal). Five out of 12 rats displayed minimal multifocal neutrophilic inflammation with loss of epithelium and necrotic debris (Figures 35 and 36). Four of the 12 showed minimal multifocal neutrophilic inflammation, but one also had necrotic debris, one had single cell necrosis, one had loss of epithelium, and one had simple inflammation. Two of the remaining three rats of 12 affected showed a single minimal multifocal effect, one with necrotic debris and one with loss of epithelium. Only a single rat had mild multifocal loss of epithelium with edema.

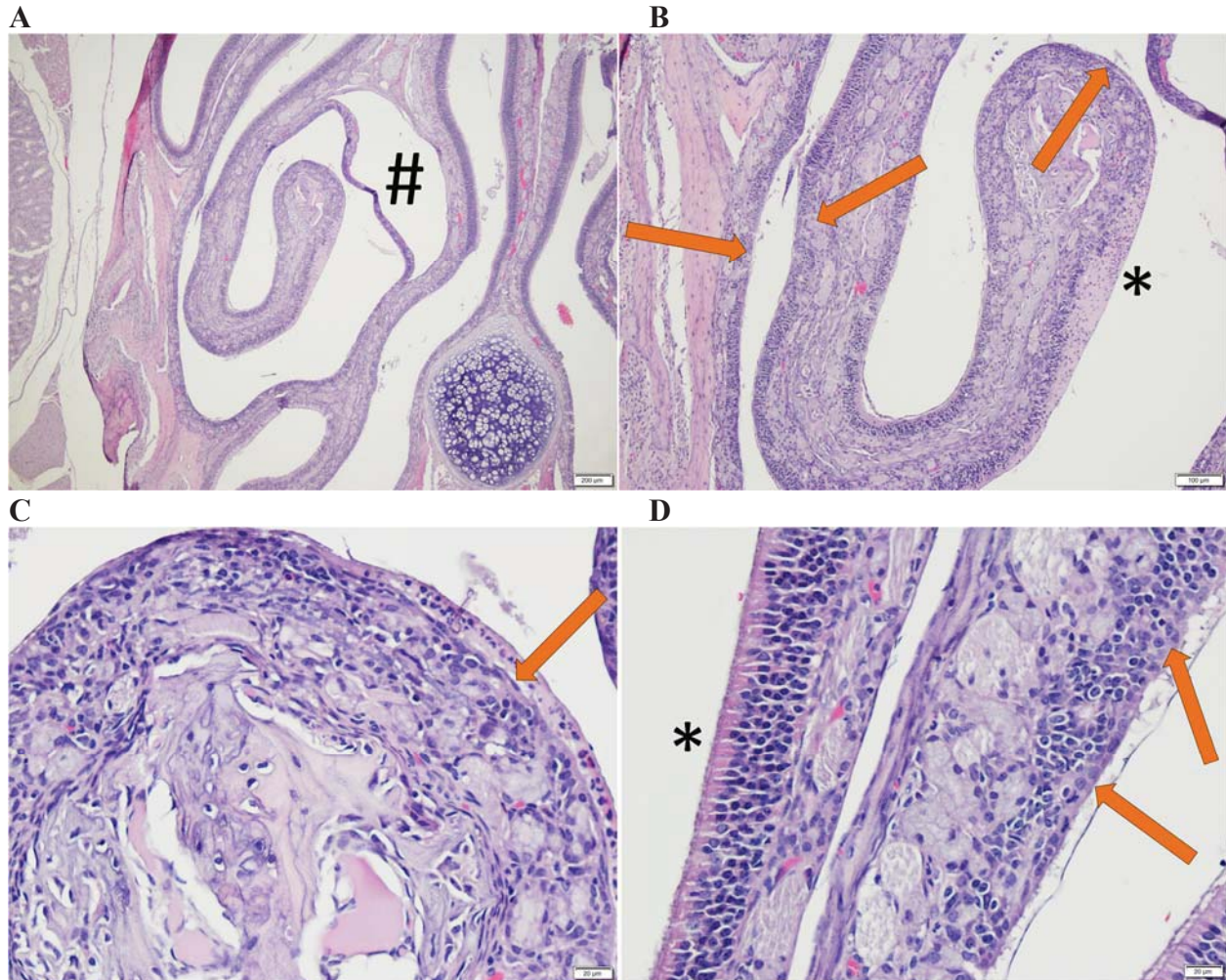


Figure 35: Nasal Section NL4 with Minimal Lesions from a Two-Week Study High Exposure Group Rat

There is minimal, multifocal degeneration, necrosis, and loss of the OE. The RE is microscopically normal. (A) The area indicated by the # is artificial separation of the OE that occurred during tissue processing and is not related to experimental manipulation (scale bar = 200 μ m). (B) There is focally extensive OE necrosis, degeneration, and loss. There are multifocal erosions of the OE (arrows). There is also a focally extensive area of necrosis overlain by necrotic debris, inflammatory cells, and serous exudate () (scale bar = 100 μ m). (C) High magnification of the erosion in Figure 36b showing a single layer of flattened epithelium covering the basement membrane (arrow). This area is covered by necrotic cell debris, viable and degenerate neutrophils, and serous exudate. There are also few neutrophils, lymphocytes, plasma cells, and macrophages infiltrating the lamina propria (scale bar = 20 μ m). (D) The OE on the left is microscopically normal (*). The OE on the right is reduced in thickness and there is disorganization to the cells present indicating a regenerative effort. The immature cells are plump with large nuclei, have abundant pale blue cytoplasm, and lack cilia (arrows) (scale bar = 20 μ m).*

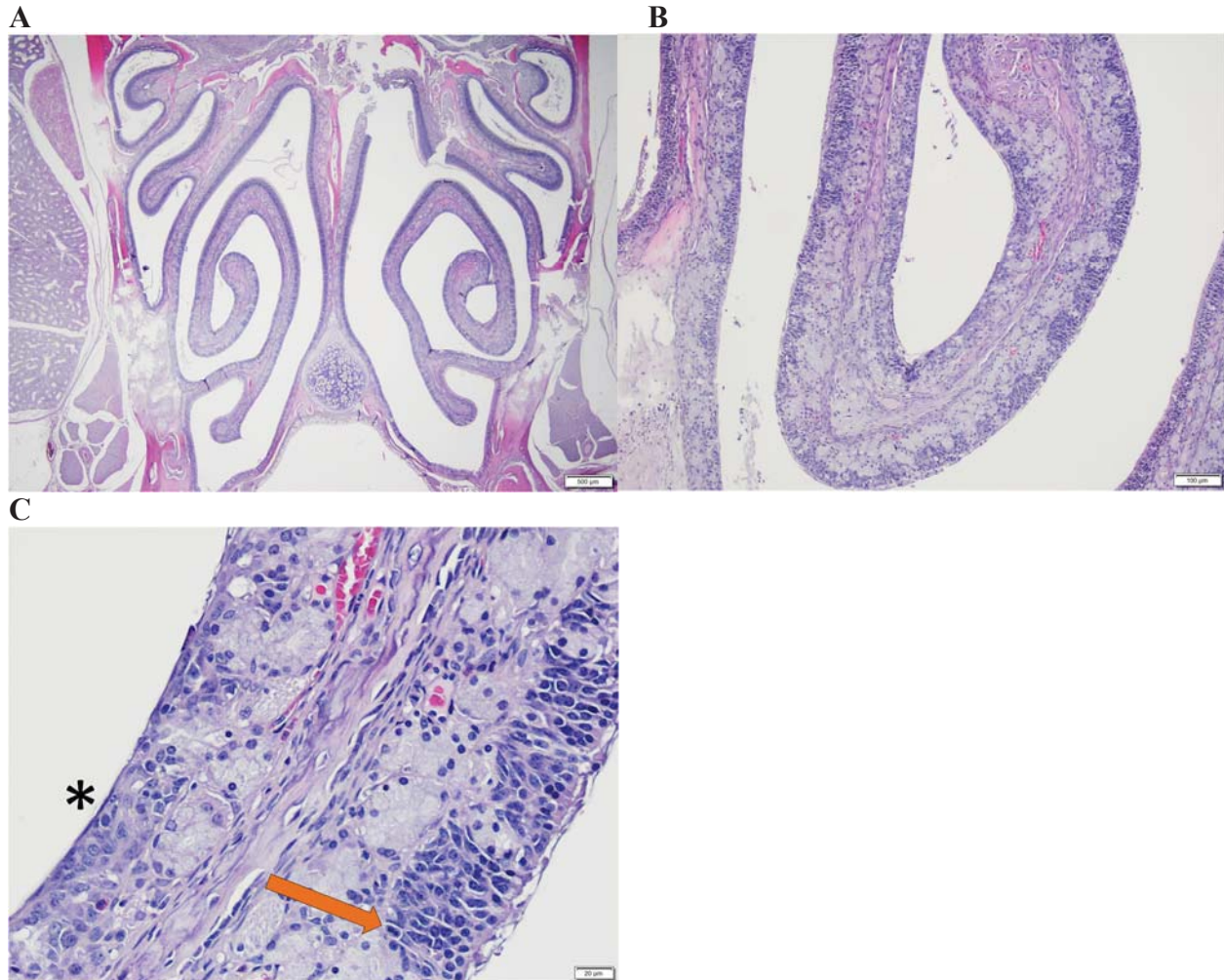


Figure 36: Nasal Section NL4 with Minimal Lesions from a Two-Week Study High Exposure Group Rat

(A) There is minimal, multifocal degeneration, necrosis, and loss of the OE (scale bar = 500 μ m). (B) There is multifocal, mild loss of the OE and some attempts at epithelial regeneration (scale bar = 100 μ m). (C) Regeneration is present as evidenced by plump cells with large nuclei and abundant lightly basophilic cytoplasm (*), mitotic figures (arrow), and basal cell hyperplasia. The surface of the regenerating OE is covered with a single layer of flattened epithelium (scale bar = 20 μ m).

5.7.3. High Exposure Group – Lungs

In the lungs of the high exposure group rats, the overall occurrence of lesions was 95 percent as a single rat out of 20 had normal lungs. The overall severity was 1.25 as six rats had a score of mild (severity = 2, Figures 37 and 38), while the other 13 a minimal effect (severity = 1, Figure 39). All of the effects were multifocal with the primary lesion being mild or minimal mixed minimal neutrophilic and mononuclear inflammation in 16 of the 19 rats. One rat showed

minimal neutrophilic inflammation without other findings. Two rats exhibited mild mixed neutrophilic and mononuclear inflammation with necrotic debris and single cell death.

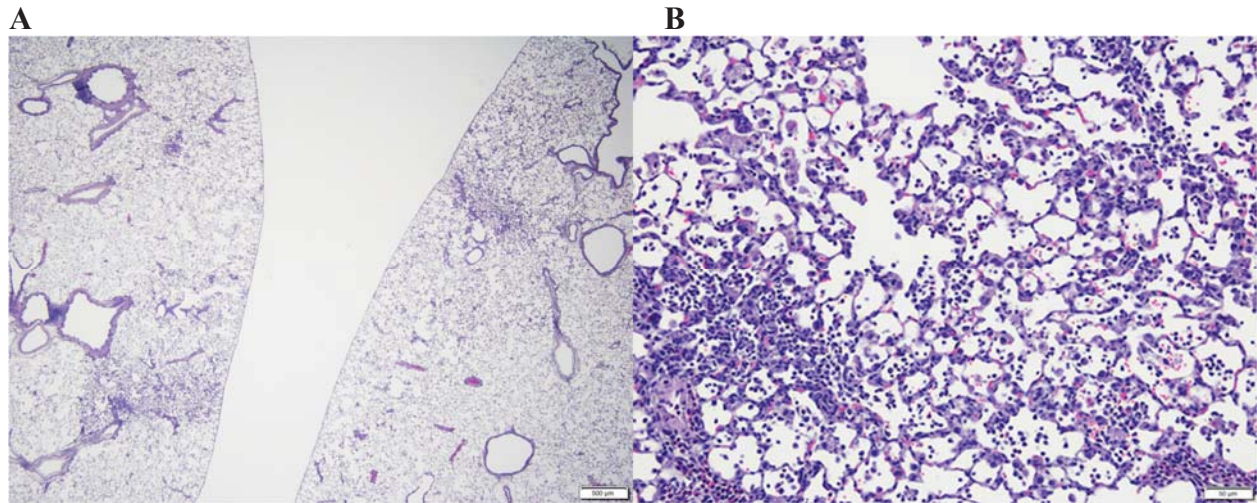


Figure 37: Lung Section with Mild Lesions from a Two-Week Study High Exposure Group Rat

(A) There are mild, multifocal areas of inflammation (scale bar = 500 µm). (B) Higher magnification shows mild accumulations of pulmonary alveolar macrophages (PAMs), neutrophils, lymphocytes, plasma cells, and necrotic cellular debris within the alveolar spaces. Alveolar septa are multifocally thickened by inflammatory cells and edema. There are moderate perivascular neutrophilic infiltrates (scale bar = 50 µm).

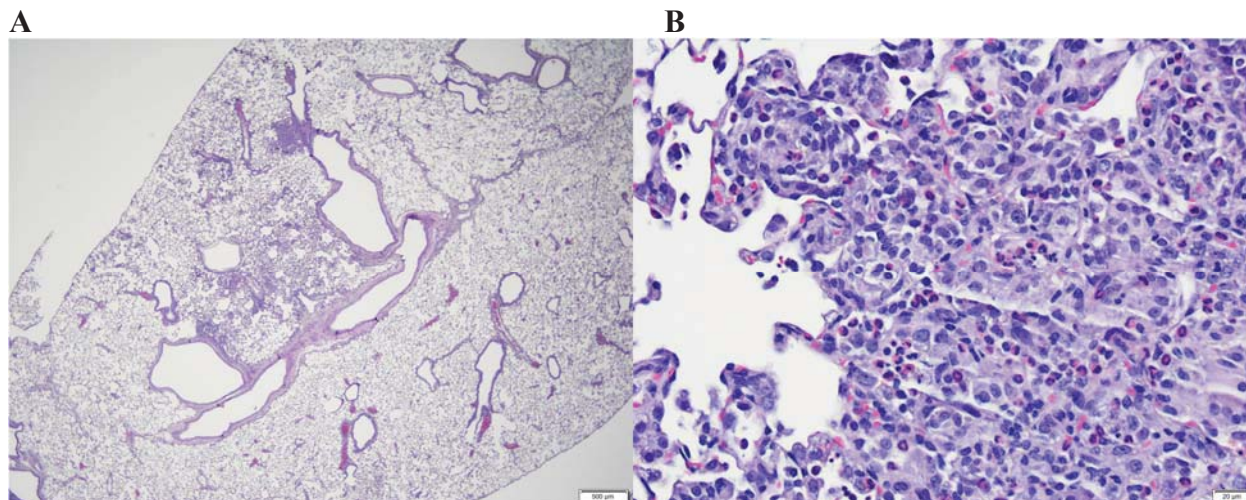


Figure 38: Lung Section with Mild Lesions from a Two-Week Study High Exposure Group Rat

(A) There are mild, multifocal areas of inflammation composed of a focal area of dense inflammation as well as multiple foci of less dense inflammation (scale bar = 500 μ m). (B) Higher magnification shows the dense focus of inflammation. The alveoli are filled with micro-granulomas characterized by a core of degenerate neutrophils surrounded by macrophages, epithelioid macrophages, lymphocytes, and plasma cells. Septa in this area are also thickened with inflammatory cells (scale bar = 20 μ m).

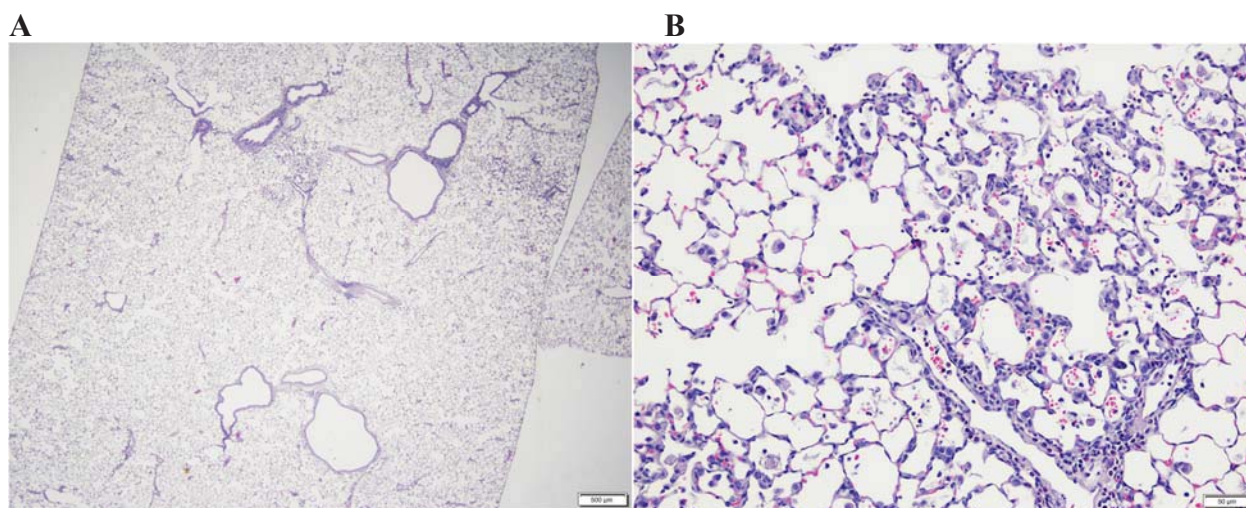


Figure 39: Lung Section with Mild Lesions from a Two-Week Study High Exposure Group Rat

(A) There are minimal, multifocal areas of inflammation (scale bar = 500 μ m). (B) Higher magnification shows accumulations of pulmonary alveolar macrophages (PAMs), neutrophils, lymphocytes, and cellular debris within the alveoli. Alveolar septa are multifocally thickened by inflammatory cells and edema. There are perivascular neutrophilic infiltrates (scale bar = 50 μ m).

6.0 DISCUSSION

6.1 Acute Study Discussion

The acute PAO inhalation study successfully served as a pilot study and range finder for the two-week study. The male and female Fischer 344 rats were exposed in five replicates for their single six-hour acute exposures to the PAO fluid aerosol. The exposures in each replicate were uniform. Each replicate concentration fell within 2.5 percent of the study average concentration and the study average concentrations for each exposure level were within 2 percent of the target concentration. Particle size distribution measurements of the exposure atmospheres showed that the PAO aerosol was respirable, and generally uniform from among the exposures.

The neurobehavioral tests conducted immediately following the acute exposure showed that rats, both males and females, appeared to be more passive after exposure to the high concentration PAO, and to a lesser extent the intermediate concentration PAO. The handling test of the FOB and the total distance traveled and ambulatory beam breaks measurements of the motor activity provided evidence of increased passivity. These effects were not observed in the animals held for 14 days post-exposure.

Body weight data were analyzed using the exposure event as the reference time point. The weight ratio of the first post-exposure day weight divided by the exposure day weight showed a dose-response in severity. The largest weight ratio decrease was seen in the high concentration exposure group for both the acute and recovery cohorts; this change was significant in both males and females (8 and 10 percent, respectively). A smaller change for the intermediate group of both cohorts was also seen in both males and females, but was statistically significant only in females. This clear dose-dependent effect on body weight at high concentrations of PAO fluid aerosol did not persist in the recovery cohort. All recovery cohort animals lost a comparable proportion of weight on the necropsy day due to overnight fasting in preparation for this procedure.

In order to determine whether this body weight effect was related to food or water consumption, the amount of food or water consumed relative to the day of exposure was calculated. Concurrent with weight loss, food and water consumption was significantly reduced in the groups of animals that lost weight. Animals in the high- and intermediate-concentration exposure groups ate less and drank less during the time period immediately following exposure to PAO. On the following day, the consumption rates for food and water appeared to go back to normal. Exposure to PAO may have made food and water temporarily unpalatable, resulting in lower consumption. Alternatively, the food and water consumption may be linked to the neurobehavioral observations that the high and intermediate concentration exposed animals were more passive in both the FOB and MA tests. Thus, animals may have eaten and drank less because they were sedated or otherwise less inclined to move. The effect of the exposure at high and intermediate PAO concentration was to decrease the food and water intake, leading to the decreased weight gain. Those effects diminished after a day, and the animals' food and water consumption appeared to return to normal two days after exposure.

Relative organ weight ratios for the acute cohort were also affected by the decreased food and water intake. Organ to body weight and organ to brain weight ratios were not different in the exposed groups as compared to the control group among the recovery cohort, indicating that this effect is secondary to the body weight effect discussed above.

Clinical chemistry and hematology results did not show trends related to a biological response due to inhalation of PAO. The number of basophils in the high concentration exposure group of males was low compared with control values. A dose-response trend is not clear due to the very low number of basophils observed. The percent basophils metric indicates that a dose-response may be seen in the data. However, this is confounded by the fact that all of these values fall within historical data from healthy control animals of similar age (Charles River Laboratories International, Inc., 2012). Therefore, there is probably no biological significance to this finding.

The acute exposure of rats to the PAO at the levels in this study led to animals that were more passive and explored less than controls or rats in lower exposure groups. In turn, the animals exposed to the highest PAO concentration (1000 mg/m³) ate and drank less and had significantly lower weight gain during the 24-hour period following exposure. Those effects appeared to diminish by the next day, when food and water consumption returned to the same levels as controls.

All three exposure groups of PAO caused effects in the nasal cavities and lungs of male and female rats exposed to the PAO aerosol. No effects were seen in the most anterior sections (NL1 and NL2) of the nasal cavities. Effects included degeneration and necrosis indicating cell death due to the acute aerosol exposure. The incidence and severity of the cellular toxicity increased in a dose-response manner progressing to inflammation that was seen in multiple areas of the nasal cavities and lungs. In the NL4 section of the nasal cavities, the cellular changes appeared to peak at the intermediate exposure concentration and remain relatively the same for the high exposure group.

In the lungs, there was an observed result leading to suggestion of increased severity from the intermediate to the high exposure, but the increase was due to just one rat with a higher severity score. Although recovery was occurring in all of the nasal cavities and lungs affected, it was not 100 percent after two weeks. However, in the low exposure group (both impacted tissues) and the intermediate exposure group NL3 tissue, recovery was not 100 percent due to effects remaining in one rat. The intermediate and high exposure group NL4 nasal cavity sections and the lungs had the slowest recovery, with only 42 to 80 percent recovery as evaluated by incidence; however, recovery was reduced to minimal effects as evaluated by severity rating. Acute exposure to PAO aerosol for six hours caused significant toxicity in the nasal cavities and lungs of male and female rats. Dose-dependent recovery occurred, but it was not 100 percent after two weeks.

The effects seen in the acute study allowed appropriate exposure concentrations to be set for the two-week study. Because of the neurological effects at the high and intermediate concentrations, target concentrations for the two-week (ten-day) exposure were reduced to 20, 100, and 300 mg/m³ PAO.

6.2 Two-Week Study Discussion

Two-week study target concentrations were set to provide a similar exposure on a cumulative concentration multiplied by time basis. Male and Female Fischer 344 rats were exposed to the PAO fluid aerosol for six hours per day, five days per week over two weeks for a total of ten exposure days. The intermediate exposure concentration of 100 mg/m³ for the two-week study was set such that five days of exposure would match one day of exposure at 500 mg/m³ in the acute exposure, and 10 days of exposure would match one day of exposure at 1000 mg/m³ for the high concentration in the acute exposure. Also, five days of exposure at 300 mg/m³ at the high concentration for the two week study would exceed one day of exposure at 1000 mg/m³ in the acute study. The low concentration target of 20 mg/m³ for the two week study over five days would match the single day low concentration of 100 mg/m³ for the acute study.

Animals were exposed in two replicates in order to accommodate neurobehavioral testing at the end of the exposure regimen. Particle size distribution measurements of the exposure atmospheres showed that the PAO aerosol was respirable, and generally uniform among the exposures. The aerosol concentration measurements demonstrated that the exposure concentration was uniform among the exposures and that the measured concentration was within two percent of the target concentration. Therefore, there were no notable differences in the exposure atmosphere among the two replicate exposures.

The neurobehavioral tests showed no significant effects in either male or female rats after the last two days of exposure. The body weights of the animals did not show any exposure-related effects. Food and water consumption did not show any exposure-related effects, nor did the relative organ weights at necropsy. This contrasts with neurobehavioral effects, body weight loss, and altered organ to body weight and organ to brain weight ratios seen after a single acute exposure to high concentrations of PAO fluid aerosol.

The two-week inhalation study results with PAO fluid aerosol indicated that the substantial neurobehavioral and weight loss effects seen immediately following the acute study were not observed with repeated exposures at lower concentrations. The highest exposure group in this study, 300 mg/m³, then serves as the no observed adverse effect level for neurobehavioral changes.

Clinical chemistry results did not show any trends that are indicators of a significant biological response due to inhalation of PAO fluid aerosol. Hematology results, however, were not as clearly interpretable (Tables 2 and 3). Male rats showed a dose-dependent increase in percent neutrophils; however, percentages for all exposure groups including controls were lower than the reference range (reference mean: 37.25 percent, range: 24.66 to 52.65 percent) published by the animal provider (Charles River Laboratories International, Inc., 2012). As the highest exposure group percent neutrophils number approaches the normal low, the increase is not considered biologically significant. Female rats also showed statistically significant dose-dependent increases in percent neutrophils and corresponding decreases in percent lymphocytes. Similar to the situation in males, the neutrophils in the control, low, and intermediate female rats were lower than the reference range (reference mean: 32.13 percent, range: 19.12 to 47.33 percent, Charles River Laboratories International, Inc., 2012). Only the increased high exposure group

female percentages fell within the reference range. All groups of females had percent lymphocyte numbers higher than the reference range (reference mean: 59.13 percent, range: 41.61 to 74.18 percent, Charles River Laboratories International, Inc., 2012); the decreasing trend in this percentage approaches normal based on data from 210 rats of the same strain and supply. Although these trends are of questionable biological significance, they could also be considered evidence of a mild inflammatory response that complements the histopathological findings.

Histopathology results showed repeated dose effects in the NL4 sections of nasal cavities and lungs of the 300 mg/m³ exposure group. In the 20 and 100 mg/m³ exposure groups, there were no treatment related effects in any tissue examined, including the nasal and lung target tissues. The NL4 section of nasal cavities in the high exposure group had cell loss, necrosis and inflammation that were minimal in all but one rat (mild); incidence of lesions was only 60 percent overall. Based on the nasal results in the acute study, recovery would be expected in these rats following repeated exposures.

A single lung effect in the intermediate exposure group was identified as a peri-mortem focal hemorrhage; as this type of effect can be associated with euthanasia, it is not considered to be related to PAO exposure. In the high exposure group, all rats but one showed exposure-related effects. In the affected multifocal areas, alveolar septa were expanded by inflammatory cells and alveoli containing neutrophils, macrophages, scant fibrin and necrotic debris; these signs of inflammation are consistent with the hematology results discussed above. Again recovery is expected given the minimal to mild severity of these lesions seen in the lungs and based on the lung recovery results in the acute study. The respiratory no observable adverse effect concentration (NOAEC) for the PAO aerosol would be 100 mg/m³ for the purpose of OpEL calculation.

6.3 Histopathology Results Used to Draft OpELs

Military exposures to PAO fluids do not span eight hours a day for five days a week, as is assumed for a civilian industrial setting. Shorter term OpELs are needed to cover times that are more consistent with Air Force operations. Acute Exposure Guideline Level (AEGL) guidelines provide a potential way to propose OpELs for military exposures that are not expected to occur frequently. AEGLs are calculated for emergency exposure periods of 10 and 30 minutes and one, four, and eight hours. There are three AEGL levels based on the severity of response to a chemical. AEGL-1 is an airborne concentration of a substance to which the general public, including susceptible individuals, could experience “notable discomfort, irritation, or certain asymptomatic non-sensory effects [that] are not disabling and are transient and reversible upon cessation of exposure” (NRC, 2001). Only this first level (AEGL-1) applies to the tested PAO fluid due to the transient neurobehavioral effects and irritation based inhalation effects observed in the study herein.

The complete derivation of the OpELs are detailed in Mattie *et al.* (2017). In brief, a point of departure (POD) value required for AEGL-type calculations was determined from study data using both the acute and two-week repeated exposure studies featured in this report. Using the acute study data, the POD was determined to be the six-hour NOAEC (100 mg/m³ aerosol). In

the two-week study, exposure-related respiratory irritation was not seen in the 100 mg/m³ group. In concurrence with the acute study, 100 mg PAO/m³ aerosol was found to be the NOAEC for this study.

Dosimetric adjustments were performed using the Multi-Path Particle Dosimetry (MPPD) Model (v 3.04; Applied Research Associates, Inc., Albuquerque NM). MPPD software simulations indicated that deposition of the PAO aerosol to which the rats were exposed would occur primarily in the nasal cavities of rats and humans. A regional deposited dose ratio of 0.23 was calculated by normalizing the ratio of animal to human fractional depositions to respiratory surface area and minute volume.

A composite uncertainty factor of 10 was chosen based on the use of an interspecies uncertainty factor of 1 and an intraspecies uncertainty factor of 10. Interspecies uncertainty was reduced to unity due to the use of dosimetric adjustments and the reasoning that the PAO irritation mode of action is unlikely to differ significantly between species. The intraspecies uncertainty factor was kept at 10 due to the lack of human data for PAO.

Therefore, when applying the uncertainty factors and dosimetric adjustments to the POD, a six-hour AEGL-1 value of 2.3 mg/m³ aerosol was calculated. Extrapolation to different exposure durations is based on the premise of ($C^n \times t = k$), where C is concentration, t is time, k is the toxic load. The value of n can be assumed to equal one if the endpoint for the AEGL does not produce lethality and is reversible (NRC, 2001). A k value of 828 mg-minutes/m³ was calculated. The proposed values for eight-hour, four-hour, one-hour, 30-minute, and 10-minute AEGL-based OpELs are 1.7, 3.5, 14, 28, and 28 mg/m³ aerosol, respectively (Table 5). Per the NRC guidance, when extrapolating using four- to eight-hour values, the 10-minute AEGL-1 is assigned the same value as the 30-minute level. Therefore, the 10-minute value for this PAO aerosol is 28 mg/m³.

Table 5: Proposed OpEL Values for PAO Fluid Aerosol

Time Point	Proposed OpEL (aerosol, mg/m ³)
10 minutes	28
30 minutes	28
1 hour	14
4 hours	3.5
8 hours	1.7

7.0 CONCLUSION

Acute effects of the current PAO fluid aerosol are short-lived and recoverable. Short-term repeated exposure did not result in any cumulative effects except for minimal changes in the 300 mg/m³ exposure group. Since shorter term exposure levels are needed to cover times that are more consistent with Air Force operations, AEGL guidance was used to calculate military OpELs. Using the six-hour exposure for concentration and time, and the no observed adverse effect concentration for histopathological effects seen in the respiratory tract (100 mg/m³) from both the acute and two-week studies, OpEL values of 28, 28, 14, 3.5, and 1.7 mg/m³ were proposed for 10 and 30 minutes and one, four, and eight hours, respectively. These preliminary values should be protective for the inhalation effects described and shown in this report that could compromise the mission and the health of the warfighter.

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APPENDIX A: ACUTE EXPOSURE STUDY HISTOPATHOLOGY GRADES

Appendix Key

Severity Ratings	
0	Normal Tissue
1	Minimal/Rare
2	Mild
3	Moderate
4	Marked
5	Severe
Description Key	
BT	basophilic tubules
D	diffuse
DG	degeneration
ED	edema
ENT	essentially normal tissue
F	focal
FE	focal, extensive
G	granulomatous
HM	hemorrhage
IM	inflammation (mononuclear)
IN	inflammation (neutrophilic)
IX	inflammation (mixed)
MF	multifocal
MFE	multifocal, extensive
N	necrosis
ND	necrotic debris
Neph	nephropathy (non-specific)
NP	not processed
O	ossification
RG	regeneration
TD	tubular degeneration (nephropathy)
Abbreviations	
SD	standard deviation
SEM	standard error of the mean

Control, Acute Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150255	1	0		0		0		0		0	
150256	2	0		0		0		0		0	
150257	3	0		0		0		0		0	
150258	4	0		0		0		0		0	
150263	9	0		0		0		0		0	
150264	10	0		0		0		0		0	
150265	11	0		0		0		0		0	
150266	12	0		0		0		0		0	
150271	17	0		0		0		0		0	
150272	18	0		0		0		0		0	
150273	19	0		0		0		0		0	
150274	20	0		0		0		0		0	
150279	25	0		0		0		0		0	
150280	26	0		0		0		0		0	
150281	27	0		0		0		0		0	
150282	28	0		0		0		0		0	
150287	33	0		0		0		0		0	
150288	34	0		0		0		0		0	
150289	35	0		0		0		0		0	
150290	36	0		0		0		0		0	
Mean		0		0		0		0		0	

Animal ID	Fore & Mid-Brain,						Adrenal Glands,		
	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues
1	NP	ENT	ENT	ENT	ENT	IM/F/R	ENT	ENT	cerebellum, thyroid
2	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
3	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
4	NP	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
9	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
10	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	cerebellum
11	ENT	ENT	ENT	ENT	ENT	IM/MF/R	ENT	ENT	thyroid
12	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
17	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
18	NP	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
19	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
20	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	cerebellum, thyroid
25	NP	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
26	NP	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
27	NP	ENT	ENT	ENT	ENT	ENT	BT/TD/MF/R	ENT	thyroid
28	NP	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
33	ENT	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
34	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
35	ENT	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid
36	ENT	NP	ENT	ENT	ENT	ENT	Neph/MF/R	ENT	thyroid

Control, Recovery Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150259	5	0		0		0		0		0	
150260	6	0		0		0		0		0	
150261	7	0		0		0		0		0	
150262	8	0		0		0		0		0	
150267	13	0		0		0		0		0	
150268	14	0		0		0		0		0	
150269	15	0		0		0		0		0	
150270	16	0		0		0		0		0	
150275	21	0		0		0		0		0	
150276	22	0		0		0		0		0	
150277	23	0		0		0		0		0	
150278	24	0		0		0		0		0	
150283	29	0		0		0		0		0	
150284	30	0		0		0		0		0	
150285	31	0		0		0		0		0	
150286	32	0		0		0		0		0	
150291	37	0		0		0		0		0	
150292	38	0		0		0		0		0	
150293	39	0		0		0		0		0	
150294	40	0		0		0		0		0	
Mean		0		0		0		0		0	

Animal ID	Fore & Mid-Brain,						Adrenal Glands,		
	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues
5	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
6	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
7	ENT	ENT	ENT	IX/F/R	ENT	ENT	BT/MF/R	ENT	thyroid
8	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
13	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
14	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
15	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
16	ENT	NP	ENT	ENT	ENT	IM/MF/R	ENT	ENT	thyroid
21	ENT	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	-
22	ENT	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	-
23	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
24	ENT	ENT	ENT	ENT	ENT	IM/MF/R	ENT	ENT	-
29	ENT	NP	ENT	IM/N/F/R	ENT	ENT	BT/MF/R	ENT	thyroid
30	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
31	NP	NP	ENT	IM/F/R	ENT	ENT	BT/MF/R	ENT	thyroid
32	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
37	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
38	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
39	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
40	ENT	NP	ENT	ENT	ENT	ENT	IM/F/R	ENT	thyroid

100 mg/m3, Acute Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150295	41	0	ENT	0		0		0		2	DG/MF
150296	42	1*	HM/MF	0		0		0		0	ENT
150297	43	1	IM/F	0		0		0		2	DG/N/MF
150298	44	0	ENT	0		0		0		2	DG/N/MF
150303	49	0	ENT	0		0		0		2	DG/N/MFE
150304	50	0	ENT	0		0		0		2	DG/N/MFE
150305	51	0	ENT	0		0		0		0	ENT
150306	52	0	ENT	0		0		0		0	ENT
150311	57	0	ENT	0		0		0		2	DG/N/MF
150312	58	0	ENT	0		0		0		2	DG/N/MF
150313	59	0	ENT	0		0		0		0	ENT
150314	60	0	ENT	0		0		0		0	ENT
150319	65	0	ENT	0		0		0		0	ENT
150320	66	0	ENT	0		0		0		1	DG/N/MF
150321	67	0	ENT	0		0		0		0	ENT
150322	68	1	IM/MF	0		0		0		0	ENT
150327	73	0	ENT	0		0		0		2	DG/N/MF
150328	74	1*	HM/MF	0		0		0		1	DG/N/MF
150329	75	0	ENT	0		0		0		0	ENT
150330	76	0	ENT	0		0		0		0	ENT
Mean		0.11		0		0		0		0.90	
SD		0.32								0.97	
SEM		0.08								0.22	
Lesions		2								10	
N		20								20	
Incidence, %		10								50	
*Multifocal hemorrhage due to euthanasia and not test compound administration; Severity score not included in mean as occurrence is unrelated to compound											

Fore & Mid-Brain,											Adrenal Glands,
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues	
150295	41	ENT	NP	ENT	ENT	ENT	IM/MF/R	ENT	ENT	thyroid	
150296	42	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150297	43	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150298	44	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150303	49	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150304	50	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid, adrenal gland	
150305	51	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	adrenal gland	
150306	52	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid, adrenal gland	
150311	57	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	adrenal gland	
150312	58	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150313	59	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150314	60	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150319	65	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150320	66	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150321	67	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150322	68	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	adrenal gland	
150327	73	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150328	74	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150329	75	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150330	76	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	

100 mg/m3, Recovery Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150299	45	0		0		0		0		0	
150300	46	0		0		0		0		0	
150301	47	0		0		0		0		0	
150302	48	0		0		0		0		0	
150307	53	0		0		0		0		0	
150308	54	0		0		0		0		0	
150309	55	0		0		0		0		0	
150310	56	0		0		0		0		1	DG/MF
150315	61	0		0		0		0		0	
150316	62	0		0		0		0		0	
150317	63	0		0		0		0		0	
150318	64	0		0		0		0		0	
150323	69	0		0		0		0		0	
150324	70	0		0		0		0		0	
150325	71	0		0		0		0		0	
150326	72	0		0		0		0		0	
150331	77	0		0		0		0		0	
150332	78	0		0		0		0		0	
150333	79	1	O/F*	0		0		0		0	
150334	80	0		0		0		0		0	
Mean		0.05		0		0		0		0.05	
SD		0.22								0.22	
SEM		0.05								0.05	
Lesions		1								1	
N		20								20	
Incidence, %		5								5	
* Pulmonary osseous metaplasia (background tension)											

Fore & Mid-Brain,										
Adrenal Glands,										
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues
150299	45	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150300	46	ENT	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	-
150301	47	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150302	48	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150307	53	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150308	54	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150309	55	ENT	ENT	ENT	IM/F/R	ENT	ENT	ENT	ENT	thyroid, adrenal gland
150310	56	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150315	61	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150316	62	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150317	63	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150318	64	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150323	69	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150324	70	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150325	71	ENT	ENT	ENT	ENT	ENT	ENT	IM/F/R	ENT	thyroid
150326	72	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	cerebellum, thyroid
150331	77	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150332	78	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid, adrenal gland
150333	79	ENT	NP	ENT	N/IM/F/R	ENT	ENT	ENT	ENT	thyroid
150334	80	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid

500 mg/m3, Acute Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150335	81	1	IN/MF	0		0		2	N/DG/MF	3	N/DG/MF
150336	82	2	IN/MF	0		0		0	ENT	3	N/DG/MF
150337	83	1*	HM/MFE	0		0		0	ENT	3	N/DG/MF
150338	84	1	IN/MF	0		0		2	N/DG/MF	3	N/DG/MF
150343	89	1	IN/MF	0		0		1	N/DG/MF	3	N/DG/MFE
150344	90	2	IN/MF	0		0		1	N/DG/MF	3	N/DG/MFE
150345	91	1	IM/MF	0		0		1	N/DG/MF	2	N/DG/MFE
150346	92	2	IN/MF	0		0		1	N/DG/MF	3	N/DG/MFE
150351	97	0	ENT	0		0		1	N/DG/MF	3	N/DG/ED/MFE
150352	98	1	IN/MF	0		0		2	N/DG/MF	4	N/DG/ED/MFE
150353	99	1	IM/MF	0		0		2	N/DG/MF	4	N/DG/ED/MFE
150354	100	2	IM/MF	0		0		1	N/DG/MF	4	N/DG/ED/MFE
150359	105	2	IM/MF	0		0		1	N/DG/MF	3	N/DG/ED/MFE
150360	106	1	IN/MF	0		0		1	N/DG/MF	4	N/DG/ED/MFE
150361	107	1	IN/MF	0		0		0	ENT	3	N/DG/ED/MFE
150362	108	1	IM/MF	0		0		1	N/DG/MF	3	N/DG/ED/MFE
150367	113	1	IM/MF	0		0		1	N/DG/MF	3	N/DG/ED/MFE
150368	114	1	IN/IM/MF	0		0		0	ENT	2	N/DG/ED/MFE
150369	115	1	IN/MF	0		0		2	N/DG/MF	4	N/DG/ED/MFE
150370	116	1	IN/MF	0		0		2	N/DG/MF	4	N/DG/ED/MFE
Mean		1.21		0		0		1.10		3.20	
SD		0.54						0.72		0.62	
SEM		0.12						0.16		0.14	
Lesions		18						16		20	
N		20						20		20	
Incidence, %		90						80		100	
*Multifocal extensive hemorrhage due to euthanasia and not test compound administration; Severity score not included in mean as occurrence is unrelated to compound											

Fore & Mid-Brain,											Adrenal Glands,
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues	
150335	81	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150336	82	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150337	83	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid, spleen	
150338	84	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150343	89	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150344	90	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150345	91	ENT	ENT	ENT	ENT	ENT	ENT	BT/F/R	ENT	thyroid	
150346	92	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150351	97	NP	ENT	ENT	ENT	ENT	ENT	IM/F/R	ENT	-	
150352	98	ENT	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid	
150353	99	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	-	
150354	100	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	-	
150359	105	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150360	106	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150361	107	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150362	108	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150367	113	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150368	114	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150369	115	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150370	116	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	

500 mg/m3, Recovery Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150339	85	0	ENT	0		0		1	N/DG/MF	2	IN/DG/ND/MF
150340	86	0	ENT	0		0		0	ENT	1	IN/MF
150341	87	1	IN/MF	0		0		0	ENT	1	IN/MF
150342	88	1	IN/MF	0		0		0	ENT	-	NP
150347	93	0	ENT	0		0		0	ENT	0	ENT
150348	94	0	ENT	0		0		0	ENT	1	IN/MF
150349	95	0	ENT	0		0		0	ENT	0	ENT
150350	96	1	IN/MF	0		0		0	ENT	1	IN/MF
150355	101	0	ENT	0		0		0	ENT	0	ENT
150356	102	0	ENT	0		0		0	ENT	0	ENT
150357	103	0	ENT	0		0		0	ENT	2	DG/MF
150358	104	0	ENT	0		0		0	ENT	0	ENT
150363	109	0	ENT	0		0		0	ENT	1	IN/MF
150364	110	0	ENT	0		0		0	ENT	1	DG/MF
150365	111	0	ENT	0		0		0	ENT	2	DG/RG/IN/MF
150366	112	0	ENT	0		0		0	ENT	0	ENT
150371	117	0	ENT	0		0		0	ENT	3	N/DG/ED/IN/MFE
150372	118	0	ENT	0		0		0	ENT	0	ENT
150373	119	1	IN/MF	0		0		0	ENT	0	ENT
150374	120	0	ENT	0		0		0	ENT	3	N/DG/ED/IN/MFE
Mean		0.20		0		0		0.05		0.95	
SD		0.41						0.22		1.03	
SEM		0.09						0.05		0.24	
Lesions		4						1		11	
N		20						20		19	
Incidence, %		20						5		58	

Fore & Mid-Brain,											
Adrenal Glands,											
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues	
150339	85	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150340	86	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150341	87	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150342	88	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150347	93	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150348	94	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150349	95	NP	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	-	
150350	96	NP	ENT	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid	
150355	101	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150356	102	NP	NP	ENT	ENT	ENT	ENT	IM/BT/MF/R	ENT	-	
150357	103	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150358	104	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150363	109	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150364	110	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150365	111	NP	NP	ENT	ENT	ENT	ENT	BT/MF/R	ENT	thyroid	
150366	112	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150371	117	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150372	118	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150373	119	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150374	120	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	

1000 mg/m3, Acute Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150375	121	0	ENT	0		0		1	N/IN/MF	3	N/IN/MF
150376	122	1	IN/MF	0		0		1	N/IN/MF	3	N/IN/MF
150377	123	1	IN/MF	0		0		1	N/IN/MF	3	N/IN/MF
150378	124	1*	IN/MF	0		0		1	N/IN/MF	2	N/IN/MF
150383	129	2	IN/MF	0		0		0	ENT	3	N/IN/MF
150384	130	2	IN/MF	0		0		0	ENT	4	N/IN/ED/MF
150385	131	2	IN/MF	0		0		1	N/IN/F	3	N/IN/MF
150386	132	2	IX/MF	0		0		1	N/MF	3	N/IN/MF
150391	137	1	IN/MF	0		0		1	N/MF	3	N/MF
150392	138	1	IN/MF	0		0		2	N/MF	3	N/MF
150393	139	3	IN/N/ED/FE	0		0		1	N/MF	3	N/MF
150394	140	2	IN/MF	0		0		2	N/MF	3	N/MF
150399	145	2	IN/MF	0		0		1	N/MF	4	N/IN/MFE
150400	146	1	IN/MF	0		0		1	N/MF	4	N/IN/MFE
150401	147	1	IN/MF	0		0		1	N/MF	3	N/IN/MFE
150402	148	2	IN/MF	0		0		1	N/MF	3	N/IN/MFE
150407	153	1	IN/MF	0		0		1	N/MF	3	N/MFE
150408	154	2	IN/MF	0		0		1	N/MF	3	N/MFE
150409	155	1	IN/MF	0		0		1	N/MF	3	N/MFE
150410	156	1	IN/MF	0		0		1	N/MF	3	N/MFE
Mean		1.47		0		0		1		3.1	
SD		0.70						0.46		0.45	
SEM		0.16						0.10		0.10	
Lesions		18						18		20	
N		20						20		20	
Incidence, %		90						90		100	
* Pulmonary inflammation is background lesion											

Fore & Mid-Brain,											
Adrenal Glands,											
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues	
150375	121	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150376	122	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150377	123	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150378	124	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150383	129	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150384	130	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150385	131	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150386	132	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150391	137	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150392	138	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	adrenal gland	
150393	139	ENT	ENT	ENT	ENT	ENT	IM/MF/R	ENT	ENT	-	
150394	140	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150399	145	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150400	146	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150401	147	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150402	148	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150407	153	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	
150408	154	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150409	155	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid	
150410	156	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-	

1000 mg/m3, Recovery Cohort											
Animal											
Accession #	ID	Lungs	Description	NL1	Description	NL2	Description	NL3	Description	NL4	Description
150379	125	1	G/MF	0		0		0		1	N/DG/MF
150380	126	1	G/MF	0		0		0		0	ENT
150381	127	0	ENT	0		0		0		0	ENT
150382	128	1	G/F	0		0		0		0	ENT
150387	133	0	ENT	0		0		0		2	ND/IN/RG/F
150388	134	0	ENT	0		0		0		0	ENT
150389	135	2	G/F	0		0		0		2	ND/DG/MF
150390	136	0	ENT	0		0		0		0	ENT
150395	141	1	IN/MF	0		0		0		2	ND/RG/MF
150396	142	1	IN/MF	0		0		0		2	ND/RG/MF
150397	143	1	IN/MF	0		0		0		0	ENT
150398	144	1	IN/MF	0		0		0		0	ENT
150403	149	0	ENT	0		0		0		0	ENT
150404	150	0	ENT	0		0		0		0	ENT
150405	151	0	ENT	0		0		0		1	RG/MF
150406	152	0	ENT	0		0		0		0	ENT
150411	157	0	ENT	0		0		0		0	ENT
150412	158	0	ENT	0		0		0		0	ENT
150413	159	0	ENT	0		0		0		0	ENT
150414	160	0	ENT	0		0		0		0	ENT
Mean		0.45		0		0		0		0.5	
SD		0.60								0.83	
SEM		0.14								0.18	
Lesions		8								6	
N		20								20	
Incidence, %		40								30	

Fore & Mid-Brain,										
Adrenal Glands,										
Accession #	Animal ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid	Missing Tissues
150379	125	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150380	126	NP	ENT	ENT	ENT	ENT	IM/MF/R	ENT	ENT	-
150381	127	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150382	128	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150387	133	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150388	134	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150389	135	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150390	136	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150395	141	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150396	142	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150397	143	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150398	144	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150403	149	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150404	150	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150405	151	ENT	ENT	ENT	IM/N/F/R	ENT	ENT	ENT	ENT	thyroid
150406	152	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150411	157	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
150412	158	NP	NP	ENT	ENT	ENT	IM/MF/R	ENT	ENT	-
150413	159	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
150414	160	ENT	ENT	ENT	ENT	ENT	IM/MF/R	ENT	ENT	-

APPENDIX B: REPEATED EXPOSURE STUDY HISTOPATHOLOGY GRADES

Appendix Key

Severity Ratings	
0	Normal Tissue
1	Minimal/Rare
2	Mild
3	Moderate
4	Marked
5	Severe
Description Key	
BT	basophilic tubules
ED	edema
EL	epithelial loss
ENT	essentially normal tissue
F	focal
HM	hemorrhage
IM	inflammation (mononuclear)
IN	inflammation (neutrophilic)
MF	multifocal
MFE	multifocal, extensive
ND	necrotic debris
NP	not processed
SCN	single cell necrosis
Abbreviations	
SD	standard deviation
SEM	standard error of the mean

Control Group											
Animal											
Accession #	ID	NL1	Description	NL2	Description	NL3	Description	NL4	Description	Lungs	Description
160035	1	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160036	2	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160037	3	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160038	4	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160039	5	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160040	6	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160041	69	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160042	28	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160043	9	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160044	10	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160045	11	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160046	12	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160047	13	-	NP	-	NP	0	ENT	0	ENT	0	ENT
160048	14	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160049	15	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160050	16	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160051	17	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160052	18	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160053	19	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160054	20	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
Mean		0		0		0		0		0	

Animal									
ID		Fore & Mid Brain,				Adrenal Glands,			Missing
		Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid Gland
1	NP	ENT		ENT	ENT	ENT	ENT	ENT	-
2	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
3	ENT	NP		ENT	ENT	ENT	ENT	ENT	thyroid
4	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
5	ENT	ENT		ENT	ENT	ENT	ENT	ENT	thyroid
6	NP	NP		ENT	ENT	ENT	ENT	ENT	-
69	NP	ENT		ENT	ENT	ENT	ENT	ENT	-
28	ENT	NP		ENT	ENT	ENT	ENT	ENT	thyroid
9	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
10	ENT	ENT		ENT	ENT	ENT	ENT	ENT	thyroid
11	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
12	NP	ENT		ENT	ENT	ENT	ENT	ENT	-
13	ENT	ENT		ENT	ENT	ENT	ENT	ENT	thyroid
14	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
15	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
16	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
17	ENT	ENT		ENT	ENT	ENT	ENT	ENT	thyroid
18	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
19	ENT	ENT		ENT	ENT	ENT	ENT	ENT	-
20	ENT	NP		ENT	ENT	ENT	ENT	ENT	-

20 mg/m3 Exposure Group											
Animal											
Accession #	ID	NL1	Description	NL2	Description	NL3	Description	NL4	Description	Lungs	Description
160055	21	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160056	22	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160057	23	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160058	24	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160059	25	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160060	26	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160061	27	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160062	58	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160063	29	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160064	30	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160065	73	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160066	32	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160067	33	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160068	34	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160069	35	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160070	36	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160071	37	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160072	68	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160073	39	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160074	40	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
Mean		0		0		0		0		0	

Animal									
		Fore & Mid Brain,						Adrenal Glands,	
ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid Gland	Missing
21	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
22	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
23	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
24	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
25	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
26	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
27	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
58	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
29	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
30	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
73	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
32	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
33	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
34	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
35	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
36	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
37	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
68	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
39	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
40	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-

100 mg/m3 Exposure Group											
Animal											
Accession #	ID	NL1	Description	NL2	Description	NL3	Description	NL4	Description	Lungs	Description
160075	41	0	ENT	0	ENT	0	ENT	0	ENT	1*	HM/F
160076	42	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160077	43	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160078	44	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160079	45	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160080	46	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160081	47	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160082	48	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160083	49	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160084	50	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160085	51	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160086	52	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160087	53	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160088	54	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160089	55	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160090	56	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160091	57	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160092	8	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160093	59	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160094	60	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
Mean		0		0		0		0		0	
*Multifocal hemorrhage due to euthanasia and not test compound administration; Severity score not included in mean											
as occurrence is unrelated to compound.											

Animal									
		Fore & Mid Brain,						Adrenal Glands,	
ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid Gland	Missing
41	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
42	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
43	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
44	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
45	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
46	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
47	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
48	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
49	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
50	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
51	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
52	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
53	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
54	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
55	NP	NP	ENT	ENT	ENT	ENT	ENT	ENT	-
56	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
57	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	spleen
8	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
59	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
60	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid

300 mg/m3 Exposure Group											
Animal											
Accession #	ID	NL1 Description	NL2 Description	NL3 Description	NL4 Description	Lungs	Description				
160095	61	0	ENT	0	ENT	0	ENT	0	ENT	1	IN/IM/MF
160096	62	0	ENT	0	ENT	0	ENT	1	ND/IN/MF	1	IN/IM/MF
160097	63	0	ENT	0	ENT	0	ENT	0	ENT	1	IN/IM/MF
160098	64	0	ENT	0	ENT	0	ENT	0	ENT	2	IN/IM/MF
160099	65	0	ENT	0	ENT	0	ENT	1	ND/MF	1	IN/IM/MF
160100	66	0	ENT	0	ENT	0	ENT	1	ND/IN/EL/MF	2	IN/IM/MF
160101	67	0	ENT	0	ENT	0	ENT	1	EL/MF	1	IN/IM/MF
160102	38	0	ENT	0	ENT	0	ENT	1	ND/EL/IN/MF	1	IN/IM/MF
160103	7	0	ENT	0	ENT	0	ENT	1	ND/EL/IN/MF	1	IN/IM/MF
160104	70	0	ENT	0	ENT	0	ENT	1	ND/EL/IN/MF	2	IN/IM/MF
160105	71	0	ENT	0	ENT	0	ENT	1	SCN/IN/MF	2	IN/IM/MF
160106	72	0	ENT	0	ENT	0	ENT	0	ENT	2	IN/IM/ND/SCD/MFE
160107	31	0	ENT	0	ENT	0	ENT	0	ENT	1	IN/IM/MF
160108	74	0	ENT	0	ENT	0	ENT	1	ND/EL/IN/MF	2	IN/IM/ND/SCD/MFE
160109	75	0	ENT	0	ENT	0	ENT	1	IN/MF	1	IN/MF
160110	76	0	ENT	0	ENT	0	ENT	0	ENT	1	IN/IM/MF
160111	77	0	ENT	0	ENT	0	ENT	2	ED/EL/MF	1	IN/IM/MF
160112	78	0	ENT	0	ENT	0	ENT	0	ENT	1	IN/IM/MF
160113	79	0	ENT	0	ENT	0	ENT	0	ENT	0	ENT
160114	80	0	ENT	0	ENT	0	ENT	1	EL/IN/MF	1	IN/IM/MF
Mean		0		0		0		0.65		1.25	
SD								0.59		0.55	
SEM								0.13		0.12	
Lesions								12		19	
N								20		20	
Incidence, %								60		95	

Animal	Fore & Mid Brain,							Adrenal Glands,	Missing
ID	Larynx	Trachea	Cerebellum	Heart	Esophagus	Liver	Kidneys	Spleen, Thyroid Gland	Tissues
61	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
62	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
63	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
64	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
65	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
66	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
67	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
38	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
7	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
70	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
71	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
72	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
31	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
74	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
75	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
76	ENT	ENT	ENT	ENT	ENT	ENT	BT/F/I	ENT	thyroid
77	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
78	ENT	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-
79	ENT	NP	ENT	ENT	ENT	ENT	ENT	ENT	thyroid
80	NP	ENT	ENT	ENT	ENT	ENT	ENT	ENT	-

LIST OF ACRONYMS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AEGL	Acute Exposure Guideline Level
AFB	Air Force Base
AST	aspartate aminotransferase
AVMA	American Veterinary Medical Association
BALT	bronchial associated lymphoid tissue
DTIC	Defense Technical Information Center
EPA	Environmental Protection Agency
FOB	functional observational battery
GLP	Good Laboratory Practices
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
IACUC	Installation Animal Care and Use Committee
MA	motor activity
MPPD	Multi-Path Particle Dosimetry
NAMRU-D	Naval Medical Research Unit – Dayton
NL	nasal level
NOAEC	no observable adverse effect concentration
OBOGS	on board oxygen generator system
OE	olfactory epithelium
OECD	Organisation for Economic Cooperation and Development
OEL	occupational exposure limit
OpEL	operational exposure limit
ORISE	Oak Ridge Institute for Science and Education
PAM	pulmonary alveolar macrophages
PAO	polyalphaolefin
POD	point of departure
USAF	U.S. Air Force